SCCS/1685/25
Final version

Scientific Committee on Consumer Safety

SCCS

SCIENTIFIC ADVICE

on Cannabidiol (CBD)

(CAS/EC No. 13956-29- 1/ 689-176-3)

used in cosmetic products

The SCCS adopted this document in plenary meeting on 26 March 2026

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This Scientific Advice has been subject to a commenting period of min eight weeks after its initial publication (from 19 November 2025 to 21 January 2026, due to holiday period). Comments received during this period were considered by the SCCS. Main changes occurred in SCCS comment under 3.2.1., section 3.4.3.3, 3.4.4.2, SCCS comment 3.4.5.1., SCCS comment 3.4.5.2., section 3.4.9., SCCS comment in section 3.5, discussion section, and SCCS response to question 2. A Preamble has also been added.

All Declarations of Working Group members are available on the following webpage:
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1. ABSTRACT

The SCCS concludes the following:

1. *Taking under consideration the information/data submitted via the respective call for data, the SCCS is requested:*

   (a) to assess the maximum concentration of Cannabidiol that is considered safe when used in cosmetic products

   Based on the limited available data, the SCCS considers CBD safe when used at concentrations up to 0.19% in dermal cosmetic products and oral cosmetic products – whether used separately or in combination.

   (b) to identify the maximum safe level of Delta- 9-tetrahydrocannabinol (THC) present as a contaminant in Cannabidiol preparations

   The SCCS considers the presence of THC impurities as safe at concentrations up to 0.00025% in dermal and oral cosmetic products – whether used separately or in combination.

   The SCCS acknowledges that the current evaluation may have some limitations because of the paucity of data/information received from a few respondents to the Commission's Call for data. These limitations can be addressed as and when adequate data / information can be made available by interested Applicants.

2. *Does the SCCS have any further scientific concerns with regard to the use of CBD and the possible non-intended presence at trace levels of other cannabinoids, including THC, in cosmetic products?*

   This assessment is based only on the safety of pure CBD.
   This Scientific Advice does not consider the use of CBD in cosmetic products that may lead to exposure of the end-user's lungs by inhalation.

Keywords: SCCS, scientific advice, Cannabidiol, CBD, CAS No. 13956-29- 1, EC 689-176-3, Regulation 1223/2009

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Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems, which may pose an actual or potential threat.
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SCCS

The Committee shall provide Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

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Table of Contents

ACKNOWLEDGMENTS 2
1. ABSTRACT 3
2. MANDATE FROM THE EUROPEAN COMMISSION 7
3. SCIENTIFIC ADVICE 9
3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS 9
3.1.1 Chemical identity 9
3.1.2 Physical form 10
3.1.3 Molecular weight 10
3.1.4 Purity, composition and substance codes 10
3.1.5 Impurities / accompanying contaminants 13
3.1.6 Solubility 14
3.1.7 Partition coefficient (Log Pow) 15
3.1.8 Additional physical and chemical specifications 15
3.1.9 Homogeneity and Stability 15
3.2 TOXICOKINETICS 17
3.2.1 Dermal / percutaneous absorption 17
3.2.2 Other studies on toxicokinetics 20
3.3 EXPOSURE ASSESSMENT 26
3.3.1 Function and uses 26
3.4 TOXICOLOGICAL EVALUATION 26
3.4.1. Irritation and corrosivity 26
3.4.2 Skin sensitisation 28
3.4.3 Acute toxicity 30
3.4.4 Repeated dose toxicity 31
3.4.5 Reproductive toxicity 37
3.4.6 Mutagenicity / genotoxicity 40
3.4.7 Carcinogenicity 41
3.4.8 Photo-induced toxicity 43
3.4.9 Human data 43
3.4.10 Special investigations 43
3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS) 44
3.6 DISCUSSION 50
4. CONCLUSION 52
5. MINORITY OPINION 52
6. REFERENCES 53
7. GLOSSARY OF TERMS 55
8. LIST OF ABBREVIATIONS 55
9. ANNEX A 56
Mutagenicity / genotoxicity *in vitro* 56
Mutagenicity / genotoxicity in vivo 85

ANNEX B. Summary of results of the Ames tests on different CBD materials from different producers 87
10. ANNEX C 96

2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Cannabidiol (CBD) is one of the approximately hundred naturally occurring cannabinoids found in Cannabis plants and may account for up to 40 % of the plant's extract. However, there is no definition of CBD in the Union law applicable to the area of cosmetic products. According to World Health Organisation (WHO) Expert Committee on Drug Dependence (ECDD), CBD as '*a 21-carbon terpenophenolic compound which is formed following decarboxylation from a cannabidiolic acid precursor, although it can also be produced synthetically*'[1]. In addition, CBD is considered a non-psychoactive cannabinoid that exhibits no effects indicative of any abuse or dependence potential[2] . Moreover, ECDD stated that CBD has been found to have relatively low toxicity, stressing nonetheless that not all potential effects have been explored.
Furthermore, there is a high volume of clinical research on cannabidiol including studies related to anxiety, cognition, movement disorders and pain, but there is still insufficient evidence that CBD is effective for these conditions. It is important to note that most EU countries allow, or are considering allowing, the medical use of cannabinoids (including CBD) in some form under specified conditions.

'Cannabidiol' (CBD) is also the INCI name of '2-[(6R)-3-methyl-6-prop-1-en-2-ylcyclohex-2- en-1-yl]-5-pentylbenzene-1,3-diol' (CAS/EC No. 13956-29-1/ 689-176-3), which is included in the European database for information on cosmetic substances and ingredients (CosIng) with the reported functions of 'skin conditioning', 'skin protecting', 'antioxidant', 'anti-sebum', etc.

Currently, CBD as such is not regulated under Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products[3] (hereunder referred to as the 'Cosmetics Regulation'). However, entry 306 of Annex II to the Cosmetics Regulation prohibits 'Narcotics, natural and synthetic: All substances listed in Tables I and II of the Single Convention on narcotic drugs signed in New York on 30 March 1961' for use in cosmetic products.

On 19 November 2020, the Court of Justice of the EU (CJEU) delivered a judgment in Case C-663/18[4] concerning the legal status of cannabidiol. In the judgement, the CJEU concluded that CBD at stake in the main proceedings, should not be considered as a drug under the UN Single Convention on Narcotic Drugs of 1961. However, the CJEU added that a legislation limiting the marketing of CBD could be appropriate for securing the attainment of the objective of protecting public health as long as does not go beyond what is necessary for that purpose[5] , adding that '*A correct application of the precautionary principle presupposes, first, identification of the potentially negative consequences for health of the proposed use of the substance at issue and, second, a comprehensive assessment of the risk to health based on the most reliable scientific data available and the most recent results of international research*'[6]. In light of the CJEU ruling and the increasing number of cosmetic products reported to contain CBD, Member State authorities, as well as civil society organisations have expressed their support to assess the safety of CBD and the possible non-intended presence at trace levels of other cannabinoids, including THC.

Moreover, the European Food Safety Authority (EFSA) has not been able to pronounce itself on the safety of CBD and its qualification as novel food due to knowledge gaps. In particular, on 26 April 2022, EFSA stated: '*The effect of CBD on liver, gastrointestinal tract, endocrine system, nervous system and on psychological function needs to be clarified. Studies in animals show*

[1] CANNABIDIOL (CBD), Critical Review Report, Expert Committee on Drug Dependence, Fortieth Meeting Geneva, 4-7 June 2018, 4-7 June 2018, CANNABIDIOL (CBD) (who.int) CANNABIDIOL (CBD) Critical Review Report Expert Committee on Drug Dependence Fortieth Meeting
[2] https://www.who.int/medicines/access/controlled-substances/5.2_CBD.pdf
[3] EUR-Lex - 02009R1223-20180801 - EN - EUR-Lex
[4] Case C-663/18, B S and C A, ECLI:EU:C:2020:938
[5] Paragraph 96.
[6] Paragraph 91.

significant reproductive toxicity, and the extent to which this occurs in humans generally and in women of child-bearing age specifically needs to be assessed. Considering the significant uncertainties and data gaps, the Panel concludes that the safety of CBD as a Novel Food cannot currently be established'[7].

Considering the very limited available information regarding the safety of CBD in cosmetic products, and to enable the SCCS to perform a safety assessment, a call for data[8] to collect relevant scientific information was launched from 1 June 2023 to 30 September 2024. In view of this, the Commission, requests the SCCS to assess the safety of Cannabidiol in cosmetic products.

Terms of reference

(1) Taking under consideration the information/data submitted via the respective call for data, the SCCS is requested:

> *(a) to assess the maximum concentration of Cannabidiol that is considered safe when used in cosmetic products*
>
> *(b) to identify the maximum safe level of Delta-9-tetrahydrocannabinol (THC) present as a contaminant in Cannabidiol preparations*

(2) Does the SCCS have any further scientific concerns with regard to the use of CBD and the possible non-intended presence at trace levels of other cannabinoids, including THC, in cosmetic products?

[7] Statement on safety of cannabidiol as a novel food: data gaps and uncertainties | EFSA
[8] Call for data on ingredients used in cosmetic products - European Commission

3. SCIENTIFIC ADVICE

Preamble
As explained in the background section of the mandate, a call for data to collect relevant scientific information was launched by the EU Commission between 2023 and 2024.
A limited number of respondents supplied relevant information in response to that Call.
This **Scientific Advice to the Commission** is therefore based only on the limited amount of data/information received as well as other information available to the SCCS at that time.
These limitations can be addressed as and when adequate data / information can be made available by interested Applicants. Therefore, this **Scientific Advice** does not preclude the submission by interested parties of dossier(s) to support the safety of the use of CBD in their cosmetic products.

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Cannabidiol

3.1.1.2 Chemical names

2-[(1R,6R)-3-Methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol

IUPAC:
2-[(1R,6R)-3-methyl-6-prop-1-en-2-yl)cyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol

Ref.: CMH SCCS CBD dossier

1,3-Benzenediol, 2-(3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl)-5-pentyl-, (1R-trans)-

Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/Cannabidiol

(-)-trans-2-para-Metha-1,8-dien-3-yl-5-pentylresorcinol
2-[(1R,6R)-6-Isopropenyl-3-methylcyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol
Ref. https://echa.europa.eu/el/substance-information/-/substanceinfo/100.215.986
and https://pubchem.ncbi.nlm.nih.gov/compound/Cannabidiol

3.1.1.3 Trade names and abbreviations

Cannabidiol / CBD

(-)-Cannabidiol
(-)-trans-Cannabidiol
Epidyolex

Ref.: CBD_Call for data_ Final.docx (EIHA)

Epidiolex®

Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/Cannabidiol

Kanabidiol

Ref.: https://chem.echa.europa.eu/100.215.986/identity

3.1.1.4 CAS / EC number

CAS: 13956-29-1

EC: 689-176-3

3.1.1.5 Structural formula

3.1.1.6 Empirical formula

$C_{21}H_{30}O_2$

3.1.2 Physical form

Physical Form	Colourless solid
Appearance	White to yellowish powde

Naturally occurring CBD has the absolute stereo conformation (-)-trans

3.1.3 Molecular weight

314.5 g/mol or 314.5 Da

3.1.4 Purity, composition and substance codes

The characteristics of the synthetic production of CBD are largely consistent across different suppliers, as they follow similar routes of synthesis. The primary starting materials used to produce synthetic CBD are typically olivetol and menthadienol. For the purpose of this submission, the respondent 1 have used a representative synthetic CBD supplier; however, all suppliers operate in a similar manner regarding the synthesis process. The use of these key starting materials ensures that the synthetic CBD produced achieves a purity at least 97%-102% purity, aligning with industry standards across various manufacturers. CBD proposed for use in cosmetic products is well characterised chemically by using the below techniques:

- IR spectroscopy
- ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy
- ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy

- Mass Spectrometry
- UV Spectroscopy

INFRARED (IR) SPECTRA: The IR spectra for cannabidiol was collected on a Nicolet iS10 FT-IR spectrometer using an ATR attachment. A summary of the significant bands is listed in Table 1 and the bands are consistent with the functional groups in CBD.

Table 1. Assignment of principle absorption bands for Cannabidiol (CBD)

Wave number (cm-1)	Functional group assignment
3522, 3411	-OH (stretch)
3074, 3032	$=CH_2$, $=CH$
2965, 2926, 2871, 2855, 2829, 2728, 2669	Aliphatic (CH_3, CH_2, CH)
1799	=CH2 (bending)
1644 - 1514	Aromatic ring (stretch)

Ref: CMH SCCS CBD dossier

^{1}H -NMR Spectroscopy:
The proton nuclear magnetic resonance (^{1}H NMR) spectrum of cannabidiol was determined in deuterated chloroform ($CDCl_3$) at 25 °C on a Bruker 600 MHz spectrometer. The ^{1}H NMR spectrum of cannabidiol is provided by the respondent 1 and the observed proton chemical shifts are consistent with the structure of cannabidiol. **Cannabidiol ^{13}C Nuclear Magnetic Resonance (13C NMR) Spectroscopy:**

The Carbon Nuclear Magnetic Resonance (^{13}CMR**)** for cannabidiol was determined in deuterated chloroform ($CDCl_3$) at 25°C on a Bruker 600 MHz instrument.

MASS SPECTROMETRY:
The LC-MS spectrum of cannabidiol was collected on a Waters Quattro Premier XE Mass Spectrometer using electrospray ionization (ESI). The spectrum is shown in Figure 1**.** The mass/charge ratio of 315.2 $[M+H]^+$ is consistent with the expected mass of cannabidiol (314.47 g/mol, exact mass 314.22 g/mol).

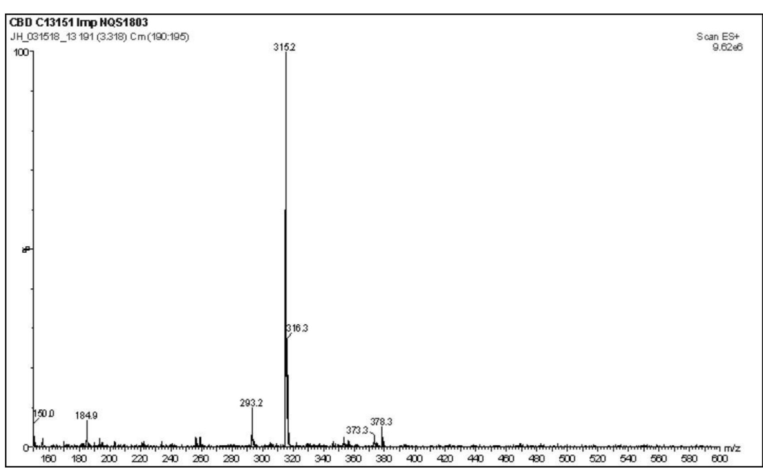

Figure 1 Mass Spectrum of Cannabidiol (CBD)

Ref: CMH SCCS CBD dossier

HPLC Purity determination:

The purity of synthetic CBD batches is determined by means of a validated test method utilising Agilent HPLC systems. The Certificates of Analysis (CoA) representative of synthetically derived CBD of 97%- 102% purity, representative of the molecule being evaluated within this dossier for cosmetic use, are provided by the respondent 1 and as listed in **Table 2** below including the CBD used in the toxicological testing reference (Batch no. 19124053).

The Purity of each batch was determined via validated HPLC-UV method. It can be readily seen that the CBD Assay is consistently proving a purity level well within the required specification and for the most part >99%. A typical reference standard was used in HPLC-UV detection for determination of CBD purity

Table 2. Batches for which Certificates of Analysis are provided along with HPLC purity determination

Batch no.	HPLC Assay (%w/w)	Specification
19124053	100.3%	97%-102%
19444041	100.8%	97-102%
20454047	99.8%	97%-102%
20454050	100.1%	97%-102%

Table 3. Representative certificate of Analysis (Batch 19124053)

Test	Specification	Result
Appearance	White to slightly beige (or slightly yellowish brown / slightly yellow / slightly brown) crystalline powder	Pass
Identification by IR	Corresponds to reference	Corresponds to reference
Identification by HPLC	Corresponds to reference	Corresponds to reference
Assay by HPLC (on dried basis)	97.0% to 102.0% w/w	100.3%
Chromatographic Purity		
Olivetol	NMT 0.15 %	< 0.03%
4-Monobromo-cannabidiol	NMT 0.15 %	< 0.03%
Δ9-Tetrahydrocannabinol	NMT 0.10 %	< 0.02%, ND
Individual unspecified impurity	NMT 0.10 %	< 0.03%
Sum of impurities	NMT 1.0 %	< 0.05%
Water Content (Coulometer)	NMT 0.5%	< 0.1%
Residual Solvents (ROS)		
Isopropanol	NMT 5000 ppm	< 500 ppm
n-Heptane	NMT 5000 ppm	< 500 ppm
Dichloromethane	NMT 600 ppm	< 60 ppm
Triethylamine	NMT 5000 ppm	< 500 ppm
Isooctane	NMT 5000 ppm	< 500 ppm
Residue on Ignition	NMT 0.2%	< 0.1%
Specific Optical Rotation	-140° to -122°	-131 °
D9-THC level on ppm basis	For information	<4 ppm, ND

ND: Not-Detected

Conclusion: All limits are met

NMT: Not More Than

Ref: CMH SCCS CBD dossier

3.1.5 Impurities / accompanying contaminants

The typical Organic Impurities for Cannabidiol are listed below (respondent 1)

Table 4. Typical organic impurities of cannabidiol.

Name/ IUPAC Name	CAS #	Chemical Structure	Process/ Degradation Impurity
Olivetol (5-Pentylbenzene-1,3-diol)	500-66-3	OH, HO, CH_3	Process Impurity
Delta-9-Tetrahydrocannabinol (D9-THC) 2-(6aR,10aR) 6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3- pentyl-6H-dibenzo[b,d]pyran-l-ol	1972-08-3	CH_3, OH, H_3C, H_3C, O, CH_3	Process and Degradation Impurity
4-Monobromo-cannabidiol 4-Bromo-2-[(1R,6R)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol	112639-10-8	CH_3, OH, Br, H_3C, HO	Process Impurity

Ref: CMH SCCS CBD dossier

From Respondent 2

Impurities/accompanying contaminants: Depending on the production process:
Natural CBD: CBDV, CBDa, CBDB, THC, Δ^9-THC, Δ^8-THC)
Synthetic CBD: THC, Δ^9-THC, Δ^8-THC

SCCS comments
The composition of synthetic CBD differs from that prepared from natural source, and contains fewer impurities/ accompanying contaminants.

Table 5. Certificate of Analysis of Natural CBD Hemp isolate (Batch CBDB1901791000)

Results of Analysis					
CBDV	0,84	%	CBD äquiv.	n.d.	%
CBDVa	n.d.	%	CBD äquiv. total	99,94	%
CBC	n.d.	%	**CBD+CBDA**	**99,94**	**%**
CBD	99,94	%	CBG äquiv.	n.d.	%
CBG	n.d.	%	CBG äquiv. total	n.d.	%
CBDa	n.d.	%	**CBG+CBGA**	**n.d.**	**%**
CBGa	n.d.	%			
CBN	n.d.	%			
9-THC	n.d.	%			
8-THC	n.d.	%			
THCV	n.d.	%			
THCa	n.d.	%			
n.d. = not detectable = < 0,01%					

Ref: Responder 2, Annex II

Another Responder (Respondent 3), who is a supplier of raw materials and not a producer of cosmetic products, submitted data for OPTIMA BROAD EXTRACT XB (CBD Purity > 83%) and OPTIMA CBD XB (CBD Purity > 99%).

SCCS comment

Specification limits for the impurities for synthetic CBD were submitted by the respondent 1, based on Certificates of Analysis of various batches (presented in Table 3), where a representative certificate of analysis for batch 19124053 is shown. Respondent 2 also reported Δ9-tetrahydrocannabinol both as a process and degradation impurity, with a maximum allowable limit of 0.10%.

The SCCS notes that the composition of synthetic CBD differs from that prepared from natural source, and contains fewer impurities/ accompanying contaminants.

3.1.6 Solubility

From Respondent 1

The solubility of the proposed CBD for use as an ingredient in cosmetic products has been determined in a variety of substances, as outlined in **Table 6** below. Synthetic cannabidiol (97%-102% purity) is hydrophobic in nature and insoluble in water.

Table 6: Solubility of Cannabidiol

Solvent	Solubility (mg/ml)
Water	<0.1
DMSO (Dimethyl sulfoxide)	>100
Corn Oil	>250
Caprylic/Capric triglyceride	>250
Sesame oil	211
Sunflower seed oil	>100

Ref: responder 1, CBD dossier

3.1.7 Partition coefficient (Log Pow)

ACD/LogP: 7.03

SCCS comment

The log P value of 7.03 for cannabidiol, as calculated using ACD Labs platform , indicates that the substance is highly lipophilic.

3.1.8 Additional physical and chemical specifications

General Appearance: White to off white / pale yellow powder
pKa (Strongest Acidic): 9.13
pKa (Strongest Basic): -5.7
Melting range: 66 °C (responder 2), 67.5±0.3 °C (Stinchcomb *et al.*, 2004)
Topological polar: > 40.5 Å²
From CLH dossier
Boiling point: 428.51°C predicted (Adapted Stein & Brown method)
Relative density: 1.0±0.1 g/cm^3 predicted
Flash point: 206.3±23.3 °C , predicted
Vapor pressure: (25 deg °C): 2.75E-8 mm Hg estimated (Modified Grain method)

The UV-Vis analysis of cannabidiol is shown in Figure 2. The spectrum was obtained using a 0.3 mg/mL solution in 70:30 acetonitrile: water on a Waters H-Class equipped with a UPLC-PDA detector

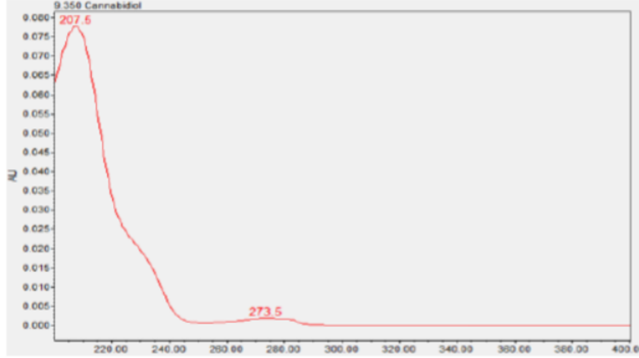

Figure 2. UV-Vis Spectrum of Cannabidiol
λmax = 208 nm, 273.5 nm

Ref: responder 1, CBD dossier

SCCS comment

A UPLC-PDA system was used to acquire the UV-Vis spectrum of cannabidiol.

3.1.9 Homogeneity and Stability

According to respondent 1, the cannabidiol proposed for assessment and use as a cosmetic ingredient, representative of synthetic cannabidiol of at least 97%-102% purity, is highly stable and has completed accelerated and long-term stability studies utilizing batches of cannabidiol manufactured at full scale. These batches have been stored per ICH Q1A (R2) guideline. All

results obtained have met specification and show no trends towards increased impurity levels or decrease in purity.

A 36-month shelf life/ retest date is proposed for the cannabidiol cosmetic ingredient determined after an evaluation of the data at accelerated conditions (40°C/75%RH) and intermediate conditions (30°C / 65%RH). Tables 7-9 below outlines a summary of some batch results obtained from these stability studies.

Table 7: Cannabidiol raw material Long Term Stability Table, Storage condition: 40° C/ 75% RH (Batch 20454051)

			Sampling time points (months) and test results			
Studied Characteristics	Method	Specification	0	1	3	6
Appearance	Visual	White to slightly beige powder	Conforms	Conforms	Conforms	Conforms
Chromatographic Purity (HPLC)	3.2.S.4.2.1	Individual specified impurity Olivetol ≤ 0.15% 4-Monobromo-Cannabidiol ≤ 0.15% Delta-9-Tetrahydrocannabinol ≤ 0.10% Each unspecified impurity ≤ 0.10% Total impurities ≤ 1.0%	< 0.05% < 0.05% ND < 0.05% < 0.1%	< 0.05% < 0.05% ND < 0.05% < 0.1%	< 0.05% < 0.05% ND < 0.05% < 0.1%	< 0.05% < 0.05% ND < 0.05% 0.1%
Assay By HPLC	3.2.S.4.2.1	98.0 - 102.0%	100.0%	99.6%	99.4%	99.3%
Water Content	USP <921>	≤ 0.5%	< 0.1%	< 0.1%	< 0.1%	< 0.1%

ND = None-Detected

Table 8: Cannabidiol raw material Long Term Stability Table, Storage condition: 30° C/ 65% RH (Batch 19MW849-4)

				Sampling time points (months) and test results					
Studied Characteristics	Method	Specification		0	1	2	3	6	9
Appearance	Visual Inspection	White to slightly beige powder		Pass	Pass	Pass	Pass	Pass	Pass
Identity by HPLC	3.2.S.4.2.1	Corresponds to Reference		Pass	Pass	Pass	Pass	Pass	Pass
Water Content by KF	USP <921>	NMT 0.5%		0.2%	0.0%	0.2%	0.2%	0.0%	0.1%
Assay by HPLC in % w/w	3.2.S.4.2.1	98.0% - 102.0%		100.7%	100.6%	100.0%	100.0%	100.4%	100.8%
Related Substances by HPLC in % w/w	3.2.S.4.2.1	Related Substances:							
		Olivetol	NMT 0.15% w/w	ND	ND	ND	ND	ND	ND
		4-Monobromo-cannabidiol	NMT 0.15% w/w	ND	ND	ND	ND	ND	ND
		Delta-9-Tetrahydrocannabinol	NMT 0.10% w/w	ND	ND	ND	ND	ND	ND
		Individual Unspecified Impurities	NMT 0.10% w/w	< 0.05%	ND	< 0.05%	< 0.05%	< 0.05%	< 0.05%
		Total Impurities	NMT 1.0% w/w	< 0.05%	ND	< 0.05%	< 0.05%	< 0.05%	< 0.05%

ND = Not Detected
NMT = Not More Than

Table 9: Cannabidiol raw material Long Term Stability Table, Storage condition: 30° C/ 65% RH (Batch 19MW849-4)

				Sampling time points (months) and test results		
Studied Characteristics	Method	Specification		12	18	24
Appearance	Visual Inspection	White to slightly beige powder		Pass	Pass	Pass
Identity by HPLC	3.2.S.4.2.1	Corresponds to Reference		Pass	Pass	Pass
Water Content by KF	USP <921>	NMT 0.5%		0.1%	0.3%	0.2%
Assay by HPLC in % w/w	3.2.S.4.2.1	98.0% - 102.0%		100.1%	100.0%	100.7%
Related Substances by HPLC in % w/w	3.2.S.4.2.1	Related Substances:				
		Olivetol	NMT 0.15% w/w	ND	ND	ND
		4-Monobromo-cannabidiol	NMT 0.15% w/w	ND	ND	ND
		Delta-9-Tetrahydrocannabinol	NMT 0.10% w/w	ND	ND	ND
		Individual Unspecified Impurities	NMT 0.10% w/w	< 0.05%	< 0.05%	0.07%
		Total Impurities	NMT 1.0% w/w	< 0.05%	< 0.05%	0.1%

ND = Not Detected
NMT = Not More Than

In addition, according to respondent 1, a forced degradation study conducted on CBD following ICH guidelines for stress testing. The study tested various stress conditions such as acid, base, oxidative, thermal (solution and solid), and light degradation. The results showed that CBD is most stable when subjected to light degradation and thermal degradation (in solid form), and least stable under acid, base, oxidation, and thermal (in solution) conditions.
Additionally, the Respondent commissioned a stability study of the proposed synthetic CBD ingredient combined with Medium Chain Triglyceride (MCT) coconut oil only. The concentrations analysed were 2.8% w/w and 5.6% w/w CBD in 10 mL MCT oil. These formulations where these samples were also stored according to ICH Q1A (R2) climatic zone II at both accelerated conditions (40°C/75%RH) for a period of 6 months and ambient (25°C/60%RH) conditions for a period of 36 months. All results obtained have met specifications and has shown that within an oil-based form synthetic CBD remains stable under both accelerated and real time conditions.

Respondent 2
The integrity of the CBD exposed to 25 ◦C ± 2° C/60% RH ± 5% remained statistically unchanged for 270 days. After one year, samples in open vials showed a slight decrease of 10.37 ± 0.51% and samples in closed vials showed a smaller decrease of 8.01 ± 0.67% (Kosovic *et al.*, 2021)

SCCS comment
The SCCS has noted that CBD is stable in oil solutions under normal ambient conditions.

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

From Respondent 1
This applicant did not provide any studies on dermal absorption and therefore proposed a default value of 50% dermal absorption, in the absence of an experimental value, as indicated on SCCS Notes of Guidance (testing of cosmetic ingredients and their safety evaluation - 12th revision).

From Respondent 2
An *in vitro* human skin permeation model (Casiraghi *et al.*, 2020) under occlusive conditions at 37°C using modified Franz diffusion cells assessed 1% CBD combined with four different solvents; Liquid paraffin, virgin olive oil, 80% propylene glycol (PG) and 80% Polyethylene glycol (PEG) 400. Interestingly of these 4 tested formulations, the 2 in which CBD was least soluble showed the highest rates of permeation and retention; liquid paraffin (LP) and 80% PG. Semi solid solutions were prepared using both these vehicles, on lipophilic ointment (which contained 49% LP) and a hydrophilic gel (containing 79% PG) of these 2 solutions it was noted that the hydrophilic gel containing 79% PG displayed a retention and permeability rate approximately 2-3 times higher than the lipophilic gel. Additionally, a transdermal patch containing 1% CBD was applied to the skin, which performed worse both in terms of permeation and retention than the semisolid preparations, however the overall dose was 10 times less that contained in the semisolid solutions although the overall concentration remained the same. The study also reference the permeation ability of PG alone as being able to diffuse through the full thickness of the subcutaneous and viable epidermis in a short period (Hoelgaard *et al.*, 1985); it is essentially a vehicle which will potentially increase the permeation of any molecule readily solubilised within it, of which CBD does not appear to be one.

The results of this study suggest that CBD alone or contained in a simple oil- based vehicle does not exhibit a favourable permeation rate and potentially would need to rely on dermal permeation enhancer to do so. The use of enhancers that are not lipophilic in nature raises concerns when formulating CBD products. Since CBD is a highly lipophilic compound, it is most soluble and compatible within lipid-based vehicles. When non-lipophilic carriers or enhancers are used, they may not adequately solubilize CBD, leading to instability, and degradation of the CBD

molecule. This incompatibility can compromise the overall stability of the product, meaning it may not meet stability-indicating criteria over its intended shelf life.

A publication by Ashraf Junaid *et al.*, 2022 stated that the delivery of CBD into the deeper skin layers could be challenging due to its extreme lipophilic nature and the possibility of depot formation in the outermost barrier layer of skin, the stratum corneum. Thus, sufficient permeation for CBD to reach the deeper skin layers, is predicted to be achievable via application of permeation enhancement strategies. The study investigates the impact of CBD concentration, chemical enhancers (transcutol, oleic acid, isopropyl myristate), and essential oils (eucalyptus, peppermint, lavender) on the delivery of CBD into human skin. Key findings from the study include: No significant difference in the amount of CBD absorbed into the skin between 5% and 10% CBD solutions, with 1% solution delivering a significantly lower amount. Oleic acid was found to be a useful enhancer, delivering significantly higher amounts of CBD into the skin compared to other enhancers tested, however CBD did not permeate into the receptor for any of the groups, and more than 80% of CBD absorbed was in the epidermis for both oleic acid and control. Essential oils tested had lower total CBD delivery compared to the control.

A recently published paper by Lapteva *et al.*, 2024 looked at the cutaneous delivery and biodistribution of CBD in human skin after topical application of colloidal formulations. This study aimed to assess the dermal absorption of a novel proprietary colloidal formulation, designed to encapsulate CBD (ACS 1% and 2% concentrations) in a lipophilic core surrounded by a monolayer of amphiphilic molecules. Two basic formulations containing CBD: 5% in Propylene Glycol (PG) (known to increase dermal absorption) and 6.6% in an oil solution were developed and two marketed formulations containing 1% CBD in a face cream (referred to as RP1) and serum (referred to as RP2) were obtained as comparators.

Under infinite conditions cutaneous deposition of CBD was measured, both the oil solution and ACS 2% solution outperformed the marketed solutions however in terms of transdermal permeation, the CBD across human skin from the comparator formulations (PG 5%, RP1 1%, and RP2 1%) was found to be low and under the Limit of Detection of the analytical method after application for 48 hours. The highest CBD permeation was observed for the ACS 2% at (16.8±5.0 ng/cm2) and the flux was 0.30 ± 0.24 ng/cm2/h for the linear range of 18-48 h; however, the concentrations were still below the LOQ. The permeation of the oil is not referenced under infinite conditions. It was observed also that the best delivery efficiency was found to be for ACS 2% (colloidal solution) followed by RP1 and RP2 with the delivery efficiency of the oil solution 6.6% displaying the lowest due to the high CBD content and the fact that the lipophilic nature of the formulation compromised the partitioning of the CBD into the lipophilic intercellular lipid matrix of the stratum corneum. As previously mentioned however overall transdermal permeation of all formulations were extremely low.

Under finite conditions the same formulations were assessed along with a Colloidal Gel formulated with 1% and 2% CBD in the proprietary colloidal solution. Under these conditions CBD's biodistribution profile in the human skin after 24 hours was assessed. The authors initial observations indicate that the concentration of CBD decreases considerably in the first 200 µg of skin depth for all formulations, which they suggest indicates that CBD is potentially mostly deposited and localized in the epidermis. These findings align with finding from Casiraghi *et al.*, 2020 and Junaid *et al.*, 2022 showing that CBD primarily exerts localized effects and displays an affinity for the stratum corneum, outer most skin barrier rather than being absorbed systemically. This suggests that CBD is more likely to provide benefits directly at the site of application, making it a promising candidate for topical skincare products.

Finally, the study concludes by comparing a recent clinical trial conducted published by Varadi *et al.*, 2023 which assessed the pharmacokinetics of CBD and THC in humans following topical application of 100mg CBD and 100mg THC; with blood levels monitored for 12 hours; CBD max concentration in the bloodstream following this dose was approx. 0.5ng/ml. Compared to an oral pharmacokinetic study carried out by Knaub *et al.*, 2019 that assessed oral doses of 25mg CBD using a self-emulsifying delivery system; which achieved a maximum concentration range of 6-20 ng/ml. Despite the dose being decreased in oral delivery by four- fold it resulted in 12-40-fold greater concentration levels of CBD in the bloodstream. Therefore, what this study suggest

is that oral administration of CBD is significantly more effective than topical application for achieving systemic absorption. Studies have demonstrated that, despite a four-fold lower dose, oral ingestion results in substantially higher plasma concentrations of CBD compared to transdermal application. This highlights the superior bioavailability of CBD when taken orally, as opposed to the limited absorption observed through the skin. Ironically, despite the enhanced systemic absorption of CBD through oral administration compared to topical application, the oral bioavailability of CBD remains notably low, with studies reporting absorption rates as low as 6%. This underscores the inherent challenges associated with CBD's pharmacokinetics, where a significant portion of the compound is lost due to extensive first-pass metabolism and other physiological barriers, resulting in limited systemic availability.

From Respondent 3

Delivery of CBD into the deeper skin layers could be challenging due to its extreme lipophilic nature (log P: 5.79) and the possibility of depot formation in the outermost barrier layer of skin, the stratum corneum (Junaid *et al.*, 2022, see also respondent 2)). The effect of concentration on the permeation of CBD into and across skin was evaluated. Solutions of CBD (1, 5, and 10% w/w) were prepared by dissolving the drug in propylene glycol. Solutions prepared (100 µL) were pipetted into the donor chamber, and the receptor samples were withdrawn at 0, 2, 4, 8, 22, and 24 h and analysed. At the end of the study, drug was extracted from the skin and analysed. Average cumulative amount of drug in receptor was significantly higher ($p \leq 0.0001$) for both 5% (29.03 ± 0.82 µg/cm2) and 10% (33.41 ± 1.04 µg/cm2) CBD concentration as compared to 1% (9.92 ± 0.61 µg/cm2). Also, 5% (188.05 ± 16.65 µg/cm2) and 10% (170.95 ± 13.14 µg/cm2) had significantly higher ($p \leq 0.005$) amounts of drug absorbed in epidermis as compared to 1% (12.18 ± 3.83 µg/cm2). 5% (25.33 ± 6.79 µg/cm2) delivered significantly higher ($p \leq 0.05$) amount of drug into dermis when compared to 1% (0.92 ± 0.39 µg/cm2) was observed among the groups. In terms of average total drug absorbed (skin + receptor drug amounts), 5% (242.41 ± 12.17 µg/cm2) and 10% (232.79 ± 20.82 µg/cm2) delivered significantly higher ($p \leq 0.0001$ and $p \leq 0.005$, respectively) than 1% (23.02 ± 4.74 µg/cm2). There was no significant difference ($p \geq 0.05$) between 5% and 10% in terms of epidermis, dermis, and total drug absorbed, although 10% (33.41 ± 1.04 µg/cm2) delivered significantly higher ($p \leq 0.05$) amount of drug in the receptor when compared to 5% (29.03 ± 0.82 µg/cm2) (Junaid *et al.*, 2022). It can be said that CBD absorption through the dermis is highly dependent on the dose, the vehicle and co-formulant use in the application (e.g., penetration enhancers). Many published studies tried to exploit the lipophilic potential of CBD to penetrate dermally, in order to meet therapeutic needs. However, there is poor evidence of dermal permeability of CBD up to desired systemic circulation.

Ex vivo skin absorption assay

One respondent provided a study to determine the skin absorption after application of the product "PSEUDOMA®" sample on human skin explants. This assay is based on the OECD 428 standard for the test of chemicals "Skin absorption: in vitro method". From the values obtained in this test, it is possible to differentiate (i) permeation, the amount of active substance that has penetrated through the skin, (ii) the amount in the surface of the skin (stratum corneum), and (iii) the amount of active substance that remains retained in the skin ("deposition") .
The main results are reported below:

• No permeation of CBD was found after 1 and 2 hours of product application.
• No CBD was found in the stratum corneum after 2 hours of product application.
• CBD deposition was 2.38 ± 1.52 µg/cm2 (15.85% of the applied amount) after 2 hours of product application.

SCCS comments

The SCCS noted several limitations in the provided study:

- GLP status of the study was not specified
- There was not enough information on the human skin samples used (origin, skin location, gender, age)

- The composition of the Pseudoma formulation was not specified
- The skin area used was 0.38 cm^2; whereas the SCCS recommend a minimum skin area of 0.64cm^2
- The assay duration was 2 hours, whereas 24 hours have been recommended with regular samplings
- The CBD has a logP of 7 and hence is highly lipophilic; however, CBD would have not been taken up into the hydrophilic receptor fluid (PBS).
- The mass balance was not provided.

The SCCS therefore considers that this study does not comply with the SCCS basic criteria for dermal absorption studies and therefore results from this study will not be used in this evaluation.

SCCS overall comments on dermal absorption
According to the data submitted to the SCCS, it appears that CBD has a low dermal absorption. Results from human studies, comparing topical and oral application of CBD (Varadi *et al.*, 2023 and Knaub *et al.*, 2019) have shown that the dermal absorption is between 48 and 160-fold lower than the oral absorption of CBD, which is between 6 and 25% (see below).

The SCCS considers that the highly lipophilic nature of CBD is likely to limit the rate of transfer between stratum corneum and epidermis, as well as diffusion across the aqueous layer of the skin, thus limiting the overall skin absorption. Therefore, the SCCS considers it appropriate to use a value of 10% for dermal absorption for CBD in this assessment.

3.2.2 Other studies on toxicokinetics

Inhalation ABSORPTION

FROM CLP report

Because it allows to reduce first-pass metabolism, its average bioavailability is increased when CBD is administered by inhalation (by smoking or vaping) compared to the oral route. Indeed, a study using radiolabelled CBD estimated the average bioavailability of CBD by the smoked route at 31 ± 13 % (range: 11-45 %) (Ohlsson *et al.* 1986). Inhalation of CBD leads to a higher Cmax and lower Tmax compared to the oral route

Ref: CLH REPORT FOR CANNABIDIOL, February 2025

Oral ABSORPTION

From EMA report

The pharmacokinetics of Cannabidiol (CBD) has been studied in healthy volunteers, patients and in special populations (Cf EMA report on Epidyolex).
Epidyolex is proposed for adjunctive therapy of seizures associated with Lennox-Gastaut syndrome (LGS) or Dravet syndrome (DS) in patients from 2 years of age and older.
Bioavailability of CBD was approximately 6.5 % following oral administration in fasting conditions. Due to significant food effect observed the bioavailability following administration with food can be expected around 14-25% (EMA).

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

From Respondent 1

Oral CBD bioavailability is notably low, as demonstrated by multiple studies. (Mechoulam *et al.*, 2002) found minimal absorption after ingestion. Grotenhermen *et al.*, 2003 reported a bioavailability of 13-19%, while Hawksworth and McArdle *et al.*, 2004 found CBD to be only 6% absorbed.

DISTRIBUTION

From EMA

In human
Plasma concentrations appear to follow a biphasic pattern suggesting a distribution into peripheral compartments. Apparent volume of distribution ranged in healthy volunteers for single doses of between 200 and 6000 mg CBD from 2820 to 42849 L.

In experimental animal
CBD was highly protein bound in rat, dog, and human plasma (> 94%), but less so in mouse and rabbit plasma (83% and 65%, respectively). 6-OH-CBD, 7-OH-CBD, and 7-COOH-CBD compounds showed high to very high binding in all species, returning values in the 98.8% to > 99.0% bound range.

CBD is highly lipid soluble and distributes widely into tissues with brown fat being the tissue with highest concentration after the liver. After 6 and 12 hours, the ratio of 14C-CBD ng equivalents in brown fat to white fat is 3080/879 = 3.5, 4690/1610 = 2.9, respectively. At 24 hours the radioactivity in brown and white fat is similar. The concentration of CBD in skin of non-pigmented and pigmented rats appeared to be similar and was not accumulating between the first dose and after 3 daily doses of 100 mg/kg.

The pharmacokinetics (tmax and t½) in brain appeared to be similar to plasma for both mouse and rat, however brain to plasma ratio determined using AUC0-24h obtained after i.p. administration was higher in rat with ratios of 1868/3144 = 0.6 and 5406/1987 = 2.7 in mouse and rat, respectively.

There was no significantly higher concentration in uveal tract or skin of the pigmented rats, thus CBD does not bind to melanin after single dosing. At 168 hours CBD was still quantified in epididymis and liver. No concentration was quantified 14 days post-dose. Distribution to adrenal and thyroid gland as well as testis was detected.

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

From Respondent 2

The mean half-life of CBD was reported to be 1.44–10.86 h after oromucosal spray (5–20 mg), 1.09–1.97 h after single oral administration (10 or 20 mg), 2–5 days after chronic oral administration, 24 h after intravenous administration, and 31 h after inhalation. The area-under-the-curve (AUC) and maximum serum concentration achieved (cmax) increased in a dose-dependent manner and were higher following inhalation relative to oromucosal spray or oral administration. Administration of CBD with a meal or in a lipid formulation also increased the maximum plasma concentration (cmax). The time to cmax (tmax) was reached between 0 and 5 h and was not dose dependent. CBD was rapidly distributed into tissues and appears to distribute to adipose tissue due to its high lipophilicity. Mean apparent volume of distribution for CBD was estimated to be 2520 L following intravenous administration, and around 30000 L following oromucosal spray (Gingrich *et al.*, 2023).

From CLP report

CBD penetrates highly vascularized tissues (such as brain, heart, liver or lungs), with subsequent equilibration into less vascularized tissues, and can be sequestered in fatty tissues (table 10). In male rats, concentrations of radioactivity in tissues was determined following single oral administration of 15 mg/kg of radiolabelled CBD (EMA CHMP 2019; FDA, 2017). Radioactivity concentrations in plasma peaked between 4 and 8 hours after dosing. Radioactivity was detected in adrenal gland, brain, epididymis, eye, fat, Harderian gland, heart, kidney, liver, lung, myocardium, spinal cord, spleen, testis and thyroid gland. Brown fat was the tissue with highest concentration after the liver, showing strong ability of CBD to accumulate and be stored in fatty tissues, thus prolonging exposure. A significant amount of CBD was found in liver 24 hours after administration, indicating a high metabolism rate. At 168 hours, CBD was still quantified in epididymis, white fat and liver but no concentration was quantified 14 days post-dose.

Table 10: Tissue concentration of [14C]-CBD in male rats. Abbreviations: GI: gastro-intestinal, BLQ: below limit of accurate quantification, †: above limit of accurate quantification.

Tissue	Concentrations of radioactivity µg equivalents CBD/gram of tissue			
	6 hours	12 hours	24 hours	168 hours
Adrenal gland	3.27	2.38	0.198	BLQ
Brain	**0.229**	**0.308**	BLQ	BLQ
Epididymis	**0.795**	**1.52**	**0.190**	0.158
Eye: whole	0.217	0.215	BLQ	BLQ
Fat: brown	**3.08**	**4.69**	**0.973**	BLQ
Fat: white	0.879	1.61	1.20	0.272
Harderian gland	1.86	2.57	0.454	BLQ
Heart blood	2.08	2.76	0.279	BLQ
Kidney: whole	2.85	2.29	0.479	BLQ
Liver	**7.76**	**8.20**	**2.18**	**0.294**
Lung	1.83	2.58	0.235	BLQ
Myocardium	1.31	1.74	0.110	BLQ
Spinal cord	0.230	0.381	BLQ	BLQ
Spleen	0.891	0.805	0.082	BLQ
Testis	**0.559**	**0.889**	**0.100**	**BLQ**
Thyroid gland	1.15	1.93	0.565	BLQ
GI tract (range)	1.35 – 109	1.28 – 276†	0.125 – 17.4	BLQ – 0.196
Remaining tissues (range)	BLQ – 18.6	0.190 – 2.40	BLQ – 0.354	BLQ

Ref: CLH REPORT FOR CANNABIDIOL, February 2025

Materno-foetal transfer

From CLP report

In human

Kim *et al.* 2018 found CBD in 9 out 12 umbilical cords (concentrations ranging from 9.8 to 335.3 ng/g), which associated meconium tested positive for THC, and/or its metabolites, and/or CBD

(). When perfused into human placenta ex vivo, CBD accumulated in the placental tissue and one fifth of maternal side concentrations reached the foetal side (Berman *et al.* 2023). Results of this study suggest that placenta is capable of sequestering CBD, thus partially restricting its movement to foetal tissues. Coherently with its ability to be stored in fatty tissues, authors also hypothesized that CBD may be temporally sequestered in the placenta, then slowly released in both foetal and maternal circulation

In experimental animal

In pregnant mice, when 3 mg/kg of CBD were daily subcutaneously injected from GD 5 to GD 18, CBD was detected in foetal brains, confirming materno-foetal transfer in this species (Maciel *et al.* 2022).
Furthermore, a single intravenous injection of 10 mg/kg bw of CBD in the maternal tail vein showed rapid (15 minutes post-maternal injection) transfer of CBD in the foetal brain, liver and gastrointestinal tract (Ochiai *et al.* 2021). In this study, dose and administration route induced a CBD Cmax of 2615.3 ng/ml in the maternal plasma, and of 598.7 ng/g in whole body foetuses, resulting in a 22% transfer. In another study, when 50 mg/kg bw/day of CBD were orally administered to pregnant mice from GD 5 through birth, CBD and 4 of its metabolites (6-OH-CBD, 7-OH-CBD, 7-COOH-CBD and CBD glucuronide) were found in dams and pups plasma at GD 18.5, PND 0 and PND 4 (Swenson *et al.* 2023). On GD 18.5, up to 96% of maternal CBD plasma concentrations were found in combined littermate plasma, highlighting the increase of the materno-foetal transfer due to prolonged exposure (and possibly administration route). In this study, CBD and its metabolites were cleared by PND 8.

Ref: CLH REPORT FOR CANNABIDIOL, February 2025

SCCS comment
From the available information, the SCCS has noted that CBD can reach systemic organs such as brain and testis of the exposed humans and other organisms and also can cross the placental barrier to reach the foetus.

METABOLISM

From EMA

CBD is extensively metabolized in vivo, likely following first pass effect by gut and liver metabolism.
The main isoforms responsible for phase I metabolism of CBD are CYP2C19 and to a lesser extent CYP3A4. Phase II metabolism is mediated by uridine 5’ diphospho glucuronosyltransferase (UGT) subtype 2B7 (UGT2B7), UGT1A7, and UGT1A9. The major CBD metabolites identified in human hepatocytes were 7 carboxy cannabidiol (7-COOH-CBD) and 7-hydroxy cannabidiol (7-OH-CBD). CYP2C19 is likely to be the major enzyme in vitro responsible for the hydroxylation of CBD to 7-OH-CBD. CYP3A4 is likely to be the major enzyme responsible for the further oxidation of 7-OH-CBD to 7-COOH-CBD. 6-hydroxy cannabidiol (6-OH-CBD) was identified as a CBD metabolite in HLMs, and CYP3A4 is likely responsible for its production. The most abundant metabolite was 7-COOH-CBD which was identified as having little or no intrinsic anticonvulsant efficacy. The exposure was 29-46 times higher than the mother compound. 7-OH-CBD was identified as an active metabolite with similar activity to CBD was present in lower concentrations than CBD, at approximately 40-60% of parent drug exposure.
The metabolite to parent ratios for 7-OH-CBD and 6-OH-CBD in healthy subjects were comparable with values observed in both patient populations, for 7-COOH-CBD there was very high variability among trials however significant difference between healthy subjects and patient population was not observed.
Based on in vitro data and the literature, the major metabolic pathways of CBD in human tissue are shown in the figure below.

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

ELIMINATION

From CLP

In humans, about 80% of CBD and its metabolites are eliminated through the hepatobiliary system and in feces, while the remainder is excreted through urine. Following a single oral dose of radiolabelled CBD at 5 mg/kg bw in healthy volunteers, radioactivity was excreted predominantly via the fecal route (84%) and smaller proportions of administered radioactivity recovered in the urine (8%), indicating that renal excretion is a minor elimination pathway for CBD.

Ref: CLH REPORT FOR CANNABIDIOL, February 2025

From EMA

In healthy subjects, the terminal t½ was approximately 60 hours after multiple dosing, although using 2 compartmental modelling (population PK) in healthy volunteers and in patient data suggested there may be a longer terminal slope with t½ estimates of between 85 hours and 202 hours. The population PK estimate of CBD CL/F after oral administration was 35.5 L/h, assuming a typical body weight of 70 kg from the population PK analysis.

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

SCCS overall comment on toxicokinetics:
From the available information, the SCCS has concluded that the oral bioavailability of CBD is low (6.5%). Nevertheless, food matrix effects have been observed and associated with an increase in the oral bioavailability (14-25%). From the available data**, the SCCS considers an oral bioavailability of 25%.** The SCCS has also noted that CBD distributes to adipose tissue

and, due to high lipophilicity, it is able to cross the placental barrier and reach the foetus.
CBD is also extensively metabolised in the liver and the gut, primarily by CYP2C19, CYP3A4 and UGT1A7, UGT1A9, and UGT2B7 enzymes. Two major circulating metabolites have been identified: 7-carboxy-cannabidiol (7-COOH-CBD) and 7-hydroxy-cannabidiol (7-OH-CBD). CBD has been found to undergo limited glucuronidation. Whilst UGT enzymes, such as UGT1A9, 2B7, and 2B17 may be involved in the glucuronidation of CBD, a minimal amount of the glucuronidated CBD was formed. The primary excretion route of CBD is through faeces (84%), followed by urine (8%), indicating limited absorption/metabolism following the oral route.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

From Respondent 1
This Respondent is a supplier of raw materials and not a producer of cosmetic products, OPTIMA BROAD EXTRACT XB and OPTIMA CBD XB.

From Respondent 2

The proposed use of synthetic cannabidiol would be for use in a topical cosmetic product within the skin care category, for the worst case and potential maximum absorption the respondent proposes to assess the product as a leave on product.

From Respondent 3
The Sponsor proposes various type of cosmetic products with different concentrations of CBD, ranging from 0.2% to 2%.
Here is a non-exhaustive list of possible products:

- Face cream with 1% CBD
- Body lotion with 0.20% CBD
- Body lotion with 1% CBD
- Body ointment with 2% CBD
- Body gel with 1.50% CBD

3.4 TOXICOLOGICAL EVALUATION

3.4.1. Irritation and corrosivity

3.4.1.1 Skin irritation

From Respondent 1

The Respondent has provided a Skin irritation rest report: assessment of the irritant potential of a test item on the model of EPISKINTM reconstructed epidermis model (OECD 439). From the results of the study, it could be concluded that CBD is not irritant.

From Respondent 2

The Respondent has additionally conducted a range of clinical and dermatological studies, including a 96-Hour Patch Test on participants with sensitive skin, a dermatologically controlled Erythemal index Report, a Human Repeat Insult Patch Test again on participants with sensitive skin, and two user trials involving over 100 subjects. These studies were conducted with an

internationally accredited ISO 9001:2015 and ISO 17025:2017 organization, have supported the Respondent cosmetic CBD products' safety and suitability for topical use on all skin types

From Respondent 3
This respondent provided information on a test which was performed in compliance with OECD TG 439 – In Vitro Skin Irritation: Reconstructed Human Epidermis test Method. The test item has been considered as no irritant.

SCCS comment
The Respondents provided a list of human studies for which subjects with sensitive skin were recruited, and tests indicated that CBD did not cause skin irritation when applied topically in a leave-on product.

3.4.1.2 Mucous membrane irritation / eye irritation

From Respondent 1
This respondent provided an Eye irritation (HET-CAM) study, and concluded that CBD was not irritating to the chloro-allantoic membrane of embryonated hen eggs

From Respondent 2
Current research on CBD for ocular use primarily focuses on its potential anti-inflammatory properties for treating ocular conditions. Studies have shown that CBD exhibits ocular hypotensive and neuroprotective effects.
To assess the irritant potential of CBD-containing formulations, an *in vitro* study (Maghfour *et al.*, 2021) was conducted utilizing the hen's egg chorioallantoic membrane (CAM) model with three CBD, PEA and hemp seed oil formulations; evaluated against a non-irritant control. The CAM model, a well-established method for evaluating ocular irritancy, demonstrated no significant irritant effects for any of the CBD-containing formulations.
Complementarily, a human patch test involving 20 participants confirmed the absence of skin irritation for the CBD formulations and various CBD concentrations.

From Respondent 3
Eye irritation potential of (-)-trans-Cannabidiol, synthesized (purity: 98.0-102.0%) was tested in-vitro. The test was performed in compliance with OECD GUIDELINE FOR THE TESTING OF CHEMICALS 492: Reconstructed human Cornea-like Epithelium (RhCE).
The test chemical is identified as not requiring classification and labelling according to UN GHS (No Category) if the mean percent tissue viability after exposure and post-exposure incubation is more than (>) the established percentage tissue viability cut-off value, i.e. tissue viability > 60% in EpiOcular™ Eye Irritation test. In this case no further testing in other test methods is required.
The test material (-)-trans-Cannabidiol, synthetised cannot be classified on the EpiOcularTM Eye Irritation test in isolation. The mean tissue viability after 6 h exposure and 18 h post-incubation was determined as 55.6% compared to negative control.

SCCS comment
These data collectively suggest that CBD-based products exhibit a low irritation profile in both ocular and cutaneous tissues.

3.4.2 Skin sensitisation

From Respondent 1

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From Respondent 2

Labib *et al.*, 2023 evaluated the skin sensitization potential of CBD using new approach methodologies, including a quantitative risk assessment in relation to CBD (>99% purity) at 0.3% concentration in a face cream application. Direct Reactivity Peptide Assay (DPRA), Human Cell line Activation test (h-CLAT) and Derek Nexus were integrated (ITSv1 DA) in accordance with OECD guideline No. 497. The DPRA results showed low reactivity with some lysine and cysteine depletion, while KeratinoSens results were negative but with a borderline increase in gene induction. h-CLAT results were positive, leading to a conclusion of CBD being a potential weak sensitizer using the OECD 2 out of 3 approaches.

An estimated Consumer Exposure Level (CEL) for CBD was established at 8.2 mg/cm2/ day using the SCCS Notes of Guidance. With an acceptable exposure level (AEL) determined to be around 105 µg/cm2. Data generated from Human Cell Line Activation Test (h-CLAT) and Direct Peptide Reactivity Assay (DPRA), and the prediction from Derek Nexus was used to convert into a total battery score. Based on this CBD is predicted to be a weak sensitizer with a predicted EC3 of 42 % (effective concentration of a chemical required to cause a threefold increase in proliferating lymphocytes (the stimulation index).

Further to this; Labib *et al.*, 2023 conducted a confirmatory clinical safety study using a dermatologist supervised Human Repeated Insult Patch Test (HRIPT) in 54 participants with a face cream formulation containing 0.3 % CBD (>99% purity). No reactions were observed in any of the test subjects under conditions of this study.

The potential for skin irritation, sensitization and phototoxicity of several CBD products, were assessed via patch testing on healthy human skin by Maghfour *et al.*, 2021 The products assessed included two formulations containing CBD and Palmitoylethanolamide (PEA), one containing hemp seed oil and four concentrations of CBD alone and isolated CBD at four concentrations (0.1%, 1%, 5% and 10%) in a simple grapeseed oil vehicle. Ocular toxicity was tested using a traditional hen's egg chorioallantoic membrane model with three CBD, PEA and hemp seed oil formulations. No irritation or sensitization was evident via patch testing on healthy participants with CBD concentrations from 0.1%-10% however mild phototoxicity of a hemp seed oil product was found at 48h time point compared with the negative control, whilst the in vitro experiment demonstrated comparable effects of cannabidiol product with historically non irritating products. The Respondent would like to point out Hemp and pure CBD should not be considered equivalent, as hemp contains a complex mixture of compounds, while pure CBD refers to an isolated cannabinoid. Hemp contains not only CBD but also other cannabinoids, terpenes, flavonoids, and numerous other plant compounds, including THC (tetrahydrocannabinol), which may vary in concentration depending on the strain. Additionally, hemp extracts can contain impurities such as pesticides, heavy metals, or residual solvents from the extraction process. These impurities can affect the safety, consistency, and efficacy of the product.

On the other hand, pure CBD refers to a single, isolated compound that can be precisely measured and controlled, ensuring a consistent and predictable effect. The presence of other compounds and potential contaminants in hemp introduces variability, which could impact the product's safety profile and performance, making hemp extracts less reliable for therapeutic use compared to pure CBD. Thus, they should be treated as distinct substances, with differing regulatory and safety considerations.

This aligns with the Respondents own data whereby we have applied CBD up to 6% concentration in an oil-based vehicle (CBD and MCT oil only) in a HRIPT test on 50 users with sensitive skin and no incidents of skin reactions were reported.

From Respondent 3

Skin sensitization potential of (-)-trans-Cannabidiol, synthesized (purity: 98.0-102.0%) was tested in vitro. The test was performed according to OECD TG 422D – In Chemico Skin Sensitization Assay addressing the Adverse outcome Pathway key event on covalent binding to proteins.

The mean percentage cysteine and lysine depletion value is calculated for each test chemical. Negative depletion is considered as "0" when calculating the mean. By using the cysteine 1:10/lysine 1:50 prediction model, the threshold of 6.38% average peptide depletion should be used to support the discrimination between skin sensitizers and non-sensitizers.

The test material (-)-trans-Cannabidiol, synthetized is a sample with no sensitizing potential. The measured average value of cysteine- and lysine-containing peptides is 0.52%. Therefore, according to the prediction model, the substance falls into the category of no or minimal reactivity (depletion 0.00 – 6.38%), the Direct Peptide Reactivity Assay (DPRA) test is therefore negative and the substance has no potential for skin sensitization.

Skin sensitization potential of (-)-trans-Cannabidiol, synthesized (purity: 98.0-102.0%) was tested *in-vitro*. The test was performed according to OECD TG 422D – In Vitro Skin Sensitization Assay Addressing AOP Key Event On Keratinocyte Activation: The ARE-Nf2 Luciferase LuSens test Method.

A test substance is considered as sensitizer, if the induction of luciferase activity is ≥1.5 compared to the solvent control and at the same time the viability is ≥70%.

A test substance is considered as non-sensitizer, if the induction of luciferase activity is <1.5 compared to the solvent control and at the same time the cell viability is ≥70%.

The test material (-)-trans-Cannabidiol, synthesized is a sample with no sensitizing potential. The measured values of luciferase activity induction reached values <1.5% and the same time the cell viability was not reduced below 70%.

SCCS comment

- Respondent 2 provided data from human studies, most of which do not provide any information on skin sensitisation. The exception is the HRIPT study. The SCCS has previously expressed ethical concerns about conducting human skin sensitisation tests, including the HRIPT (SCCP, 2008; SCCS, 2015; SCCS, 2018).
- Labib *et al.* (2023) investigated the skin sensitisation potential and potency of CBD in different New Approach Methods (NAMs). The SCCS did not have access to the raw data of these studies. Overall, integrating the data in the ITS showed that CBD is a category 1B (weak to moderate) skin sensitiser. This was also shown in another NAM - the SENS-IS assay, which predicted CBD to be a weak skin sensitiser. Also, in a previous guinea pig maximisation test, CBD was shown to be a moderate skin sensitiser.
- Respondent 2 used the risk characterisation done by Labib *et al* (2024). Consumer Exposure Level (CEL) was based on the use of a single product. As CBD will be used in multiple products, aggregate exposure assessment is needed to predict the CEL.
- Based on the data provided by Respondent 2, CBD is a moderate to weak skin sensitiser.

Based on the collective view of the data provided, the SCCS is of the opinion that CBD is weak/moderate skin sensitiser.

3.4.3 Acute toxicity

3.4.3.1 Acute oral toxicity

From Respondent 2

Acute administration of CBD, even at high doses (up to 1500 mg/day), has not shown significant adverse effects in humans. In the context of cosmetic product formulation, achieving a dosage that necessitates a substantial absorption of CBD would entail incorporating a prohibitively large quantity of CBD. Such a formulation approach is not practical due to the challenges associated with integrating such high concentrations of CBD into a commercially viable product. This suggests a high safety margin for acute toxicity.[37] Acute doses of CBD do not appear to have significant effects on physiological parameters (e.g., heart rate, blood pressure) or neuropsychological functions. Studies indicate no impact on cognitive functions or increased anxiety at typical therapeutic doses.

Preclinical studies have been performed in accordance with a tiered approach for safety, to support their Novel Food Application. These studies were conducted according to the principles of Good Laboratory Practice (GLP) at Charles River Laboratories, East Lothian, UK.

The study outlined below was performed using the synthetic CBD of 97%:

3-Day GLP Acute Dose Toxicity Study in Wistar Rats
In an acute, 3-day, dose study in Wistar rats (3 male and 3 female), CBD (dissolved in corn oil) was administered and an **MTD was established at 750 mg/kg**. Animals exhibited lethargy and hunched posture (observed in all animals) and ataxia (observed in three animals) at this dose, with no substantial difference in toxicity between sexes. A dose of **750 mg/kg** is extremely high for rats, especially considering the typical tolerance levels for most chemical compounds and molecules in preclinical studies. For most substances, such a high dose would likely lead to significant toxicity or adverse effects, as it far exceeds the maximum tolerated doses commonly observed in toxicology studies. Most chemicals, when administered at this level, would overwhelm the metabolic and excretory systems of the rats, potentially leading to organ damage, behavioural changes, or mortality. The fact that this dose was tolerated is highly unusual and not representative of typical dose-response profiles for many compounds. This suggests that the substance in question, CBD in this case, has an exceptionally wide safety margin compared to other molecules, which often show toxicity at much lower doses. This tolerance underscores the unique properties of CBD, distinguishing it from standard chemical agents that would not be tolerated at such high concentrations

From Respondent 3

The median lethal dose (LD50) of THC has been determined in various animal species through oral administration and ranges from 800 to 9000 mg/kg. In humans, there have been no recorded instances of fatal overdoses resulting from acute THC use. However, estimates for a potentially lethal human dose range from 4 to 15 g (Ng *et al.*, 2024).

3.4.3.2 Acute dermal toxicity

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3.4.3.3 Acute inhalation toxicity

No data submitted and are not needed for this assessment since CBD would be applied as a topical product leave on balm/ oil and is not proposed to be used in an aerosol product.

SCCS comment on acute toxicity
Acute oral toxicity studies in animals have shown that LD50 values for CBD are high, which indicate low acute toxicity. Further assurance about the acute toxicity of CBD may also be drawn from the data and reports on the use of Epidyolex®, which is a licensed medicine that can be administered to epilepsy patients up to a maximum daily dose of 20 mg/kg body weight (equivalent to 1,200 mg/ person per day). According to Vidal, the maximum daily dose may be up to 25 mg/kg/day.

3.4.4 Repeated dose toxicity

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

From Respondent 1
14-Day GLP Dose Range Finding Study in Wistar Rats

The objective of this study was to determine the potential toxicity of CBD (dissolved in corn oil), when given by oral gavage to Wistar rats for 14 days. This experiment allowed selection of a suitable dose range for a subsequent 90-day toxicity study. Groups of 3 male and 3 female rats received CBD (dissolved in corn oil) at doses of 71, 107, 143 or 714 mg/kg/day by oral gavage at a dose volume of 4 mL/kg body weight once daily. An identically comprised vehicle control group received the vehicle only (corn oil) under the same conditions. Administration of CBD once daily by oral gavage for 14 days was well tolerated in rats at dose levels of up to 143 mg/kg/day, with no biologically or statistically significant increase in the frequency or severity of any adverse events comparted to vehicle control. There were no statistically significant differences in haematology or serum chemistry parameters when compared to the control group. Therefore, **143 mg/kg/day was established as the NOAEL**.

From Respondent 2
The aim of this study was to assess the possible health hazards which could arise from repeated exposure of Full Spectrum Extract (REGULAR) via oral administration to rats over a period of 28 days.
The Full Spectrum Extract (REGULAR) is the test item with an intend use for novel food, this Hemp Fullspectrum Extract (Regular) contained 8.84% of CBD.
The test item Full Spectrum Extract (REGULAR) was administered daily via oral route in graduated doses to 5 groups of test animals, one dose level per group for a treatment period of 28 days. Animals of an additional control group were handled identically as the dose groups but received the vehicle instead of test item formulation. The groups comprised 3 male and 3 female Wistar rats each.
The following doses were evaluated:
Group no. 1: 0 mg/kg body weight
Group no. 2: 100 mg/kg body weight
Group no. 3: 250 mg/kg body weight
Group no. 4: 500 mg/kg body weight
Group no. 5: 1000 mg/kg body weight
Group no. 6: 2000 mg/kg body weight
The test item formulation was prepared freshly on each day of administration. The test item was suspended in hemp oil and administered daily during a 28 days treatment period to male and female animals. Dose volumes were adjusted individually based on weekly body weight measurement.
During the period of administration, the animals were observed precisely each day for signs of toxicity. Body weight and food consumption were measured weekly.
At the conclusion of the test, all animals were sacrificed and observed macroscopically.
There were no mortalities during the treatment phase.
There were no test item-related clinical signs of systemic toxicity observed during the treatment period in any of the animals.

In males and females, there was no test item-related effect on body weight or food consumption during the treatment period.
No toxicologically relevant effects on parameters of haematology and clinical biochemistry were observed in test item-treated animals.
Macroscopic examination revealed no toxicologically relevant findings at the end of the treatment period. There were no gross lesions that could be attributed to the treatment with the test item.
On the basis of this 28 days dose range finding study with Full Spectrum Extract (REGULAR) in male and female Wistar rats with dose levels of 100, 250, 500, 1000 and 2000 mg/kg body weight day the following conclusions can be made:
There was no test item-related effect observed on mortality, clinical signs, body weight development, food consumption, haematology, clinical biochemistry and gross pathological findings, in males and females sacrificed at the end of treatment period.

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

From EMA

Mouse study GWTX1503, 13week oral toxicity

The key findings in this study were indicative of changes in the liver. Mean alanine amino transaminase/alanine aminotransferase (ALT) levels were higher than controls during Week 7 and 13 in males given ≥ 150 mg/kg/day (by approximately 65% and 40%, respectively) and during Week 7 for females given 150 or 300 mg/kg/day (by 259% or 83%, respectively). Microscopic centrilobular hepatocyte hypertrophy in all animals given 300 mg/kg/day and in some animals given 100 or 150 mg/kg/day was associated with increased liver weight in all groups and macroscopic enlargement at ≥ 150 mg/kg/day. In conclusion, the no observed adverse effect level (NOAEL) was 300 mg/kg/day CBD-OS, corresponding to the respective Week 13 maximum measured plasma concentration (Cmax) and area under the concentration-time curve calculated to the last observable concentration at time t (AUC(0-t)) values of 9810 ng/mL and 44300 ng h/mL in males and 5770 ng/mL and 46400 ng·h/mL in females.

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

From Respondent 1
13-Week Dose Range Finding Study in Mouse
In a 13-week mouse study (0, 400, 550, or 700/625 mg/kg/day), there were 7 early deaths at the high dose that were attributed to drug-induced nephropathy. Clinical signs at 700 mg/kg/day included convulsions and tremor. This dose was subsequently lowered to 625 mg/kg/day beginning at week 6. Clinical chemistry changes included increased ALT (up to 2.5×), creatinine (up to 50%), cholesterol, total protein, and globulin. At necropsy, liver weights were increased in both sexes at all doses and large liver was noted macroscopically in some animals from all drug treated groups. A dose-related increase in the incidence and/or severity of nephropathy was observed microscopically in drug groups and was considered adverse at 550 mg/kg/day and 700/625 mg/kg/day males and in 700/625 mg/kg/day females due to the severity and extent of the lesion. None of these adverse findings were observed at the 400 mg/kg/day dose level in males or females. Based on the clinical observations, early deaths, and nephropathy noted at 500 mg/kg/day and 700/625 mg/kg/day males and 700/625 mg/kg/day females, the **NOAEL was 400 mg/kg/day for males** (C_{max} and AUC values of 6420 ng/mL and 37800 ng.h/mL) and **550 mg/kg/day for females** (C_{max} and AUC values of 8440 ng/mL and 41200 ng.h/mL).

Respondent 1 provided a non-published study described below:
90-Day GLP Toxicity Study in Wistar Rats
The objective of this study was to determine the potential toxicity of CBD (dissolved in corn oil), when orally administered via gavage for 90 days to Wistar rats.

Administration of CBD (dissolved in corn oil) to rats for at least 90 days at doses of 15, 36 or 72 mg/kg/day at a dose volume of 4 mL/kg body weight once daily was generally well tolerated.

During this study there was no sign of adverse events. Various parameters were assessed during this study such as, but not limited to, clinical observations, mobility, reaction to sensation, body temperature, ophthalmology (conditions relating to the eye), haematology (blood analysis), clinical chemistry (analysis of body fluids), organ and tissue examination, histology (microscopic structure of tissues) and sperm evaluation.

There were no deaths and no test item-related adverse clinical signs, nor any differences (compared to vehicle control) in body weight, food consumption, functional observation battery assessments or clinical pathology.

The only toxicologically relevant findings were in the liver and thyroid. Increases in mean body weight relative liver weights for males and females **given 36 or 72 mg/kg body weight/day** and in mean absolute and brain weight relative liver weights for both sexes at 72 mg/kg body weight/day were associated with minimal centrilobular hepatocyte hypertrophy (primarily in males).

From Respondent 2
In a 90-day study, rats were dosed with 0, 200, 400 and 800 mg test article/kg bw/day. The test article is a proprietary blend of 9% hemp extract and 91% organic extra virgin olive oil, which is produced by an isopropanol extraction method under current Good Manufacturing Practices. Fatty acids comprise approximately 88.70% of this extract, while the phytocannabinoid content is 6.96% (of this, 6.27% is CBD); the remaining 4.34% consists of fatty alkanes, sterols, terpenes and tocopherols. In the 90-day study, rats in Groups 5 to 8 had a 28-day recovery period before being sacrificed. In the 90-day study, male rats in Groups 1-8 were administered the test article daily for 93 days and female rats in Groups 1-8 were administered test article daily for 94 days. The recovery period was 30 and 31 days for the female and male rats, respectively. The NOAEL in the 90-day study was concluded to be 800 mg/kg bw/day and 400 mg/kg bw/day for female and male Sprague Dawley rats, respectively (Dziwenka *et al.*, 2020).

A 90-day repeated dose oral toxicity study was conducted in Hsd.Han Wistar male and female rats in order to evaluate the possible health hazards likely to arise from repeated oral exposure to the test article during postweaning maturation and growth well into adulthood. The test article was a supercritical CO2 extract of the aerial parts of hemp (C. sativa). Edible fatty acids comprise 61% of this concentrated extract, while phytocannabinoids are present at 26% (of this, approximately 96% is CBD and less than 1% is THC); the remaining 13% include fatty alkanes, plant sterols, triterpenes, and tocopherols. The main study was followed by a 28-day recovery period in which two satellite groups (5 additional animals per sex per group in the control and high dose groups) were observed in order to assess reversibility, persistence, or delayed occurrence of potential toxic effects. This GLP study was conducted in compliance with OECD 408 (1998) and FDA Redbook IV.C.4.a (2003). The test article was formulated in the vehicle (sunflower oil) just prior to administration. The test article was administered via gavage at doses of 0, 100, 360, and 720 mg/kg bw/day at a dosing volume of 5 mL/kg bw. These doses were based on the 14-day study lowest observed adverse effect level (LOAEL) of 1000 mg/kg bw/day (the lowest dose group tested) with the aim of inducing moderately toxic effects in the middle- and high-dose groups (without causing mortality or suffering) and determining a no observed adverse effect level (NOAEL) in the low-dose group. Animals assigned to the satellite groups were treated identically up to day 90 and then observed without treatment for four weeks. No toxicologically relevant, test article related changes were observed in male or female animals given 100 mg/kg bw/day or in female animals given 360 mg/kg bw/day for 90 days. **The NOAEL for the test article in this 90-day study was considered to be 100 mg/kg bw/day for male and 360 mg/kg bw/day for female** Hsd.Han Wistar rats (Marx *et al.*, 2018).

Respondent 2 provided 2 non-published studies described below:
The test item Full Spectrum Extract (REGULAR) was administered daily via oral route daily in graduated doses to 4 groups of test animals, one dose level per group for a treatment period of 90 days. Animals of an additional control group were handled identically as the dose groups but

received the vehicle instead of test item formulation. The groups comprised 10 male and 10 female Wistar rats each.
The following doses were evaluated:
Group 1: 0 mg/kg body weight
Group 2: 50 mg/kg body weight
Group 3: 250 mg/kg body weight
Group 4: 750 mg/kg body weight
Group 5: 1500 mg/kg body weight
The test item formulation was prepared within the stability time frame of 10 days at room temperature. The test item was suspended in hemp oil and administered daily during a 90-day treatment period to male and female animals. Dose volumes were adjusted individually based on weekly body weight measurement.
During the period of administration, the animals were observed precisely each day for signs of toxicity.
Body weight and food consumption were measured weekly. To detect possible delayed occurrence or persistence of, or recovery from toxic effects, animals in the recovery groups were observed for a period of 28 days following the last administration. In addition, animals for toxicokinetics (3 animals per sex for control, 9 animals per sex for dose groups) were included in the study.

On the basis of this 90-day repeated dose oral toxicity study with Full Spectrum Extract (REGULAR) in male and female Wistar rats with dose levels of 50, 250, 750 and 1500 mg/kg body weight/ day the following conclusions can be made:
No test item-related mortalities and no toxicologically relevant findings concerning clinical symptoms, detailed clinical observations or the functional observation battery occurred during the treatment or recovery period of the study.
Overall, the mean body weight increased during the treatment and recovery periods of the study in control as well as in all dose groups. There is a tendency to lower body weight in males in groups 4 and 5 when compared to group 1, which was not observed in females.
No treatment-related or toxicologically relevant effects on food consumption, blood coagulation, parameters of haematology and clinical biochemistry and hormones were observed during the treatment and recovery period of the study.
No toxicologically relevant effects on fertility parameters were detected in this study.
Histopathologically, microscopic changes that could be attributed to treatment with the test item were observed at >250 mg/kg bw/day in the liver of both sexes, at >750 mg/kg bw/day in the thyroid glands of both sexes, urinary bladder of males, and adrenal gland and ovaries of females, and at 1500 mg/kg bw/day in the kidneys of males. In addition, microscopic findings likely due to a stress-related secondary effect associated with inhibited body weight gain indicated by lower group mean values of final body weights were observed in the thymus and adrenal glands of males at >750 mg/kg bw/day.
The histopathological examination also revealed that, except for above-mentioned stress-related findings in males, treatment-related microscopic findings in the liver, thyroid glands, kidneys, urinary bladder, ovaries and adrenal glands were reversible under the conditions of this study.
Based on histopathology evaluation, due to presence of interstitial cell vacuolation of the ovary and micro-vesicular vacuolation in the zona fasciculata of the adrenal glands in females at >750 mg/kg bw/day, the no-observed-adverse-effect-level (NOAEL) was established at **250 mg/kg bw/day for females**.

In a second 90-day repeated dose oral toxicity study with Natural CBD Isolate (purity = 99.94%), suspended in corn oil, in male and female Wistar rats with dose levels of 25, 50, 75 and 150 mg/kg body weight/ day the following conclusions can be made: no test item-related mortalities and no toxicologically relevant findings concerning clinical symptoms, detailed clinical observations or the functional observation battery occurred during the treatment or recovery period of the study.

No treatment-related or toxicologically relevant effects on body weight, food consumption, blood coagulation, parameters of haematology and hormones were observed during the treatment and recovery period of the study. Some treatment related changes in clinical biochemistry parameters regarding the liver were observed that were considered not to be adverse.
No toxicologically relevant effects on fertility parameters were detected in this study.
The histopathological examination revealed treatment-related histomorphological changes in the liver, thyroid glands, adrenal glands and ovaries were reversible under the conditions of this study.
Based on microscopic findings recorded and considering presence of enhanced vacuolation (fatty change) indicating inhibition of steroid synthesis in the adrenal glands and ovaries at or above 50 mg/kg bw/day, the no-observed-adverse-effect-level (NOAEL) could be established at 25 mg/kg bw/day for both sexes. A histopathological no-observed-effect-level (NOEL) was not determined due to presence of centrilobular hepatocellular hypertrophy, which is of adaptive character, **at 25 mg/kg bw/day in both sexes.**

From Respondent 3
The respondent provided two 90-Day Repeated Dose Oral (Gavage) Toxicity Study in Sprague Dawley Rats with 28-Day Recovery Period (OECD 408).

In the first one, rats were treated by gavage with 30, 60 and 238 mg/kg bw/day in corn oil as vehicle. Repeated administration to Sprague Dawley Rats for 90 consecutive days had no test-item related effects on the general health of the animals, functional observatory battery parameters, clinical pathology parameters and gross pathology in both sexes. However, the observed changes in body weight, net body weight gain, feed consumption, organ weight and histopathology of the liver in the mid and high-dose group were reversible during the 28- day recovery period and were not considered an adverse effect of test item treatment. Based on the observed results under the experimental conditions employed in the study, it is concluded that the No Observable Adverse **Effect Level (NOAEL) is equal to or greater than 238 mg/kg bw/da**y.

In the second one, rats were treated by gavage with 25, 50 and 200 mg/kg bw/day of pure CBD isolate (99%) in corn oil as vehicle. Treatment related findings were observed in clinical chemistry, liver weight and histopathology of the adrenal and liver. In the main group animals, after 90 days of treatment, a significant increase in liver weight was observed in G3 (50 mg/kg b.w./d dose group) and G4 (200 mg/kg b.w./d dose group) male and female animals accompanied by minimal to a mild degree of cytoplasmic vacuolation of hepatocytes (G3 and G4 male and female) and increase in levels of cholesterol (G3 & G4 female), total bilirubin (G4 female), and low-density lipoproteins (G4 female). However, there were no liver enzyme alterations or inflammatory changes in the liver. There was also an increased incidence of minimal to moderate degree of cortical vacuolation in adrenal in G3 and G4 male and female animals without any inflammatory changes. At the end of 28 days of the recovery period, liver weights of G6 (200 mg/kg b.w./d dose recovery group) male and female animals were comparable to G5 (vehicle control recovery group). Total cholesterol, total bilirubin and low-density lipoprotein levels of G6 females also showed recovery and values were comparable to G5 female. There was no incidence of cytoplasmic vacuolation in the liver and adrenal cells of G6 female animals. However, in G6 male animals, decreased incidence and severity of cytoplasmic vacuolation of hepatocytes (4/5) and cortical cells of adrenal (3/5) was observed without any inflammatory changes and biochemical alterations. Based on the above findings the No Observed Adverse Effect Level (**NOAE**L) of CBD Isolate powder 99% +/ THC Free was found to be **25 mg/kg b.w./day** for Males and 200 mg/kg b.w./day for Females when administered to Sprague Dawley rats once daily for 90 consecutive days through oral route followed by 28 days recovery period under the conditions tested.

3.4.4.3 Chronic (> 12 months) toxicity

From EMA

Rat GWTX1412, 26-week oral toxicity study with 4-week recovery

In a 26-week Wistar male and female (N=10) rat study (0, 15, 50, or 150 mg/kg/day) with Epidyolex, the centrilobular hypertrophy in the liver of animals given ≥ 50 mg/kg/day, the main finding in this study, was associated with increased liver weight, macroscopic enlargement. A **NOAEL (150 mg/kg/day)** was associated with an increase in ALP and ALT activities. Thyroid follicular hypertrophy in both sexes, correlated with increased thyroid weights and macroscopic enlargement in males, was considered an indirect effect of treatment due to its recognized relationship with liver hypertrophy. Microscopically these were specified by liver centrilobular hypertrophy and thyroid follicular cell hypertrophy in both sexes along with increased adrenocortical vacuolation in males and minor ovarian interstitial cell hyperplasia in females. In liver, an organ enlargement was also associated with increased mean plasma ALT and alkaline phosphatase (ALP) activities at the highest dose tested. Effects in liver and thyroid were considered by Expert as non-adverse and representative of adaptive changes due to microsomal hepatic induction.

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

From Respondent 1

39-Week Toxicity Study in Beagle Dogs
In a 39-week study in Beagle dogs (0, 10, 50, or 100 mg/kg/day), there were no deaths, but bodyweight was reduced over the study in 50 and 100 mg/kg/day males and in females at all doses. There were consistent decreases in heart rate in 100 mg/kg/day males but no drug-related cardiac rhythm disturbances. Drug-related liver changes characterised by hepatocyte hypertrophy associated with increased liver weight, macroscopic enlargement, and marked increases in ALP (up to 8-fold compared to controls) were seen at all doses. These were considered adaptive and demonstrated a tendency for reversal at the end of the recovery period. No other toxicological effects were observed. The **NOAEL (100 mg/kg/day)** was associated with CBD exposures (AUC) of 20500 ng.h/mL in males and 22,400 ng.h/mL in females

SCCS comment
Extensive toxicological data and information from literature were provided from studies in mice, rats, rabbits, dogs, monkeys (including juvenile rats and juvenile dogs) with oral CBD (EMA, Respondents 1 to 3). In this Advice, only a factual description of the study results with a brief discussion of toxicity findings has been provided. Most of the studies found that the liver was one of the target organs:

-In mice, the target organs of toxicity in the 13-week study were the liver and the kidneys.

-In rats, the target organs for toxicities were liver, thyroid, and adrenals, presented by change in organ weight.

-In dogs, the target organ for toxicity was liver, with hepatocyte hypertrophy, macroscopic enlargement and increased liver weight.

From the 90-day repeated oral rat toxicity studies, the SCCS **considered a NOAEL of 25 mg/kg bw/d** for CBD based on liver toxicity (cytoplasmic vacuolation of hepatocytes) in rat.

3.4.5 Reproductive toxicity

Pivotal fertility, embryo-foetal developmental, and prenatal/postnatal development toxicity studies were performed with Purified CBD that was formulated in sesame oil and given *p.o.* by gavage. Preliminary (DRF) embryo-foetal and prenatal/postnatal development toxicity studies in rats and rabbits were performed to enable the selection of suitable doses for the pivotal studies.

3.4.5.1 Fertility and reproduction toxicity

From EMA

In a fertility and early embryonic development toxicity study, Han Wistar rats (20/sex/group) were given 0, 75, 150, or 250 mg/kg/day for 2 weeks prior to pairing until the day prior to necropsy for males and up to gestation day (GD) 6 for females (GWTX1456). There were no treatment-related deaths and no adverse clinical or post-dosing observations. During the post-pairing phase, there was a treatment-related reduction in the overall body weight gain of males given ≥ 150 mg/kg/day. There were no treatment-related necropsy observations in either sex and no test article-related effects on male or female reproductive indices, male reproductive organ weights, female estrus cycling, or any caesarean-section parameters at doses up to 250 mg/kg/day Purified CBD, which was determined to be the NOAEL. Evaluation of CBD effects on male and female reproductive performance is considered adequate, and it is agreed that no significant negative effects were observed in rat. A Safety margin of 60-fold was calculated for inclusion in the SmPC section 5.3 based on exposure measurements from the rat embryofetal study (GWTX1454) at 250 mg/kg/day dose level on Day GD17. Adjusted human AUC(0-24h) 2790 ng.h/ml was used for calculation.

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

From Respondent 2
Henderson et al 2023 conducted a Reproductive and Developmental Toxicity Extended Screening study following OECD test guideline 421 modified to include extended postnatal dosing through PND 42 and hormone analysis (testosterone and thyroid hormones) in rats under GLP conditions. This study is highly significant as it adheres to the rigorous standards set by the Organisation for Economic Co-operation and Development (OECD) and Good Laboratory Practice (GLP) guidelines, ensuring the reliability and reproducibility of its findings. As the most recent and comprehensive investigation into the effects of CBD on reproductive health, this study provides critical insights that challenge previous research, including concerns raised by the European Food Safety Authority (EFSA) regarding potential reproductive risks associated with CBD. The robust methodology and adherence to international standards underscore the study's importance in shaping future regulatory and safety assessments. The study used a source of CBD 99-08-101, 46% purity at the following dose levels: 0, 30, 400 or 300mg/kg-bw/day. No CBD-related effects were observed on precoital interval, oestrous cycle length, mating, fertility or pregnancy indices at any dose level. Maternal toxicity was however noted in the 300mg/kg-bw/day group with 2 females being euthanized in extremis, otherwise mean gestation lengths in CBD-treated groups were similar to those in the control group, and there were no significant differences in the mean number of implantation sites or proportions of post implantation loss. The following findings were reported in a non-clinical study conducted as part of the Epidyolex® submission to the FDA (FDA, 2018). Oral administration of cannabidiol (0, 75, 150, or 250 mg/kg/day) to pregnant rats throughout the period of organogenesis resulted in embryofoetal mortality at the highest dose tested (250 mg/kg/day.) There were no other product-related maternal or developmental effects. The highest no-effect dose for embryofoetal toxicity in rats was associated with maternal plasma cannabidiol exposures (AUC) approximately 16 times that in humans at the recommended human dose of 20 mg/kg/day.

When CBD (75, 150, or 250 mg/kg/day) was orally administered to rats throughout pregnancy and lactation, decreased growth, delayed sexual maturation, neurobehavioral changes (decreased activity), and adverse effects on male reproductive organ development (small testes in adult offspring) were observed in the offspring at the mid and high doses. These effects occurred in the absence of maternal toxicity. The no-effect dose for pre- and postnatal developmental toxicity in rats was associated with maternal plasma cannabidiol exposures approximately 9 times than in humans at the recommended human dose.

In an oral (gavage) Wistar rat embryofoetal development (EFD) study of purified CBD (Epidyolex® at 0, 75, 150, or 250 mg/kg/day), the dose of 250 mg/kg/day was associated with decreased body weight gain and total litter loss in 2 of 20 dams. The NOAEL for maternal toxicity was amended to 150mg/kg/day due to 100% loss of pregnancy in 2 dams at the high dose of 250 mg/kg/day. The NOAEL of 150 mg/kg/day equates to an estimated human equivalent dose of 24.2 mg/kg/day. This value is 1.2 times higher than the maximum approved dose for Epidyolex® (20 mg/kg/day) in humans.

In an oral (gavage) New Zealand white rabbit EFD study of purified CBD (Epidyolex® at 0, 50, 80, or 125 mg/kg/day), foetal body weights were decreased (10%) and foetal variations (unossified metacarpal, bulging eyes, and nonerupted incisors) increased at the 125 mg/kg/day dose, which was also associated with evidence of maternal toxicity. The NOAEL for embryofoetal developmental toxicity (80 mg/kg/day) was associated with a maternal exposure of 2,030 ng·h/mL (i.e., much lower exposures than in rats). This exposure in rabbits is approximately 1.2 times the AUC expected for a human dose of 1500 mg/day using the exposure in humans observed for a dose of 1500 mg/day (AUC0-inf of 1,618 ng.h/mL). Using allometric scaling the NOAEL of 80 mg/kg/day equates to an estimated human equivalent dose of 25.8 mg/kg/dayThis value is 1.3 times higher than the maximum approved dose for Epidyolex® (20 mg/kg/day) in humans.

In an oral (gavage) Wistar rat prenatal and postnatal development (PPND) study of purified CBD (Epidyolex® at 0, 75, 150, or 250 mg/kg/day), pup body weight was reduced at birth and throughout lactation at the 150 and 250 mg/kg/day doses and was associated with delays in achieving developmental landmarks (pinna unfolding, eye opening, pupillary reflex) including landmarks of male and female sexual maturation. Neurobehavioral changes (decreased locomotor activity) were observed in offspring tested after weaning and there was a dose-related increased number of males with small testis at all doses when animals were necropsied as adults. The lowest observed adverse effect level (LOAEL) for developmental toxicity (**75 mg/kg)** was associated with maternal CBD exposures of approximately 25,900 and 86,300 ng.h/mL on gestation days 6 and 17, respectively (based on toxicokinetics in the rat EFD study, since plasma levels were not collected in the PPND study).

SCCS comment

Anses proposed classification for cannabidiol (CBD) as a category 1B reproductive toxicant (may damage fertility, may damage the unborn child) and as hazardous to the breastfed child (may cause harm to breast-fed children). Anses (CLP report 2025 on CBD) pointed out studies (non GLP) from the Carvalho group which showed that subchronic peripubertal exposure of mice to CBD up to 30 mg/kg bw/day resulted in significant modification of spermatogenesis. In these studies, male Swiss mice were administered CBD (99.9% pure) by gavage for 34 consecutive days at doses of 0, 15, or 30 mg/kg bw/day.

The SCCS identified a number of other studies:

A) a study in rats (Marx *et al.,* 2018) showed that sperm morphology, and percentage of motile and immotile sperm cells were similar in the control and 720 mg/kg bw/day groups (OCDE, duration 42 days). The SCCS noted that this study has some limitations, notably due to the presence of other compounds (only 26% of CBD), which could alter the toxicokinetic and toxicodynamic effect of CBD. Therefore, this study was not selected to establish a PoD. The NOAEL for the test article in this 90-day study was considered to be

100 mg/kg bw/day for male (equivalent to 25 mg/kg bw/day of pure CBD) and 360 mg/kg bw/day for female rats (equivalent to 90 mg/kg bw/day of pure CBD).

B) In an OECD Test Guideline 421 GLP-compliant study in rats, Henderson *et al.* (2023), with extended postnatal dosing and hormone analysis, hemp-derived CBD isolate (0, 30, 100, or 300 mg/kg bw/d) was administered orally. The authors did not observe changes in sperm motility, viability, morphology, or enumeration in rats dosed with up to 300 mg/kg bw/d for up to 42 days in the F0 generation.

C) Another 90-day repeated dose oral toxicity study was provided by the respondent, carried out with Natural CBD Isolate (purity = 99.94%) in male and female Wistar rats with dose levels of 25, 50, 75 and 150 mg/kg body weight/day (see respondent 2, sub chronic toxicity section). The analysis of sperm parameters was performed, including sperm count, sperm motility and morphology No toxicologically relevant effects on fertility parameters were detected in this study, while all effects on thyroid glands, adrenal glands and ovaries were reversible at all doses.

In mechanistic terms, rodents, unlike humans, lack of a well-defined hemato-testicular barrier; and their Sertoli cells are therefore more sensitive to toxicants. As an example, Li *et al.* (2025), showed that primary human Sertoli cells and mouse Sertoli cells may respond differently to CBD.

Biochemical and physiological parameters are known to different between Human and rodent species and can lead to a different response to toxicants. Sertoli cell blood-testis barriers (BTB) in rats and humans share a similar foundational structure—tight junctions forming a physical seal—but differ significantly in molecular composition and permeability. Rodents often express specific junction proteins like occludin not found in human testes, while humans exhibit unique markers like claudin-3. These, along with differences in transporter dynamics, make rat models not directly relevant for studying male fertility effects (Johnson *et al*. 1980, Habert *et al*. 2014, Klein *et al*. 2014, Hau *et al*. 2021, Hau *et al*. 2023).

In view of all these considerations, the SCCS is of the opinion that the reported adverse effects relating to modification of spermatogenesis are inconsistent across studies, and do not provide a basis for deriving a point of departure (POD) for human health risk assessment.

3.4.5.2 Developmental Toxicity

From EMA

Embryo-foetal development was evaluated in rat and rabbit. Rabbit seemed to be more sensitive to effects of CBD compared to rat. This was evident by the observed dose-dependent body weight loss compared to controls in rabbit. Embryo-foetal development in rat was insensitive to high CBD exposure (Cmax up to 12800 ng/ml). The NOAEL for maternal toxicity was amended to 150mg/kg/day due to 100% loss of pregnancy in 2 dams at the high dose of 250 mg/kg/day. NOAEL for effects on embryo-foetal development in rabbit was 80 mg/kg/day. Foetal variations observed at 125 mg/kg/day CBD (e.g., unossified metacarpal, bulging eyes, and nonerupted incisors) were considered to be secondary to the reduced foetal weights. Maternal exposure at 80 mg/kg/day Purified CBD corresponded to GD 19 Cmax and AUC(0-t) values of 220 ng/mL and 2030 ng·h/ml, respectively. Cmax of this dose was lower than pharmacological relevant exposure in children and adults (approximately 290 ng/ml and 320 ng/ml, respectively). However, protein binding is lower in rabbit compared to rats and humans with 65% bound in rabbit and 95% and 94% in rat and humans, respectively. The non-existing safety margins for the rabbit study are reflected in SmPC section 5.3. and the rat NOAEL of 150 mg/kg/day is reflected to result in a safety margin of 50-fold.

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

From Respondent 2

The following findings were reported in a non-clinical study conducted as part of the Epidyolex® submission to the FDA (FDA, 2018). Oral administration of cannabidiol (0, 75, 150, or 250 mg/kg/day) to pregnant rats throughout the period of organogenesis resulted in embryofoetal mortality at the highest dose tested (250 mg/kg/day.) There were no other product-related maternal or developmental effects. The highest no-effect dose for embryofoetal toxicity in rats was associated with maternal plasma cannabidiol exposures (AUC) approximately 16 times that in humans at the recommended human dose of 20 mg/kg/day.

In an oral (gavage) Wistar rat prenatal and postnatal development (PPND) study of purified CBD (Epidyolex® at 0, 75, 150, or 250 mg/kg/day), pup body weight was reduced at birth and throughout lactation at the 150 and 250 mg/kg/day doses and was associated with delays in achieving developmental landmarks (pinna unfolding, eye opening, pupillary reflex) including landmarks of male and female sexual maturation. Neurobehavioral changes (decreased locomotor activity) were observed in offspring tested after weaning and there was a dose-related increased number of males with small testis at all doses when animals were necropsied as adults. The lowest observed adverse effect level (LOAEL) for developmental toxicity (75 mg/kg) was associated with maternal CBD exposures of approximately 25,900 and 86,300 ng.h/mL on gestation days 6 and 17, respectively (based on toxicokinetics in the rat EFD study, since plasma levels were not collected in the PPND study).

SCCS comments

According to the EMA report, there were no effects on fertility, embryo-fetal developmental toxicity, and prenatal/postnatal development below the dose of 75 mg/kg/day (NOAEL).

SCCS overall comments

From the data provided, and the recent classification proposal from ANSES (CLP report 2025 on CBD), there are indications that CBD exerts developmental toxicity. The SCCS considers that this effect is manifested at a relatively a high-dose level (75 mg/kg or higher) and this is therefore covered by the Point of Departure of 25 mg/kg identified for liver toxicity.

3.4.6 Mutagenicity / genotoxicity

The European Medicines Agency (EMA, 2019) has evaluated the genotoxic potential of CBD in a standard test battery of in vitro and in vivo assays according to ICH S2(R1). All tests concluded CBD to be negative for genotoxic potential.

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

SCCS overall comments

Several studies have been conducted as part of the regulatory approval process for CBD-containing medications like Epidyolex®. An extensive list of genotoxicity assays, including the Ames test, chromosomal aberration assays, and micronucleus tests in mammalian cells has been assessed, as have *in vivo* studies performed in a regulatory context for medicinal uses. A summary of available data of relevance to the current assessment, including the assessment of studies from the respondents plus additional studies, is described in the Annex.

The SCCS assessed the available information obtained from the literature search regarding the genotoxicity of CBD as well as the studies provided by the respondents. The details on the studies and the SCCS comments are provided in annex.

Although two studies reported positive findings *in vitro* using the pure compound (Russo *et al.*, 2019; Kolar, 2024) and one old study *in vivo* with limited reliability also with pure CBD (Zimmerman and Raj, 1980), the overall weight of the evidence from all the available studies indicates that CBD does not have mutagenic or genotoxic properties.

Overall, considering all the available evidence from *in vitro* and *in vivo* studies, the SCCS considers that CBD does not raise a concern regarding mutagenicity or genotoxicity.

3.4.7 Carcinogenicity

From EMA. 2019

A 104- weeks carcinogenicity study was conducted in rats with CBD as CBD BDS by the oral dietary route of administration at doses 5, 15, or 50 mg/kg/day. Overall, no concerns of tumour findings were found. Interestingly, at 50 mg/kg/day CBD there was a reduced incidence of tumours generally associated with hormonally-mediated neoplasia in aging animals. The clinical relevance of this finding is uncertain.

Exposure was adequate to provide safety margin to clinical exposure at the high dose. However, was very low in comparison to clinically achievable exposures and standard safety margins for carcinogenicity studies.

Ref: EMA/458106/2019 Committee for Medicinal Products for Human Use (CHMP)

From Respondent 1

The carcinogenic potential of CBD BDS (Botanical Drug Substance) was evaluated in a 2-year carcinogenicity study in rats (GW Study No JJG003). No apparent effects on survival were noted. There was no increased incidence of any factor contributory to death when treated animals were compared with Controls. Clinical signs observed were those expected for rats of this age and strain and were considered to be unaffected by administration of CBD BDS. There was no evidence of an adverse effect of the drug on the incidence or time of onset of palpable masses.

A clear treatment and dose related reduction in overall bodyweight gain (Weeks 1 – 104) was seen for males and females given 15 or 50 mg/kg bw/day; at 50 mg/kg bw/day, males had a 26 % reduction and females had a 35 % reduction in bodyweight gain compared with Controls. A dosage related reduction in food consumption and food conversion efficiency was present for both sexes throughout the study.

There were no effects of treatment with CBD BDS on haematological parameters in males or females during Weeks 52 or 78. During Week 103 only, the white blood cell counts were statistically significantly lower than those of Controls for males given 15 or 50 mg/kg bw/day; however individual values were within the background ranges found in this laboratory and the differences from Controls were considered to be of no toxicological significance. There was no evidence of an increased incidence of leukaemia in the CBD BDS treated groups.

There was an apparent increase in the incidence of abnormal size of the thyroid glands in CBD BDS-treated males. There was a reduction in the number of skin masses recorded in both males and females of the 50 /kg bw/mg/kg bw/day group and in the number of findings recorded in the pituitary gland and mammary tissue in the females of this group. In association with the reduced number of findings in the pituitary gland there was a reduction in the number of ventral depressions in the brain that are generally caused by pituitary enlargement.

There was no indication of carcinogenic potential. Indeed, there was, in animals given 50 /kg bw/mg/kg bw/day, an apparent reduction in the incidence of tumours generally associated with hormonally-mediated neoplasia in ageing animals. Non-neoplastic findings considered to be associated with treatment included an increased incidence of centrilobular hypertrophy in the liver of males in the 15 /kg bw/mg/kg bw/day and the 50 /kg bw/mg/kg bw/day groups and

females in the 50 /kg bw/mg/kg bw/day group. There was an increase in focal follicular hyperplasia in the thyroid glands of males given 50 /kg bw/mg/kg bw/day.

It was concluded that administration of both 15 and 50 /kg bw/mg/kg bw/day of CBD BDS in the diet resulted in a greater than 10 % reduction in overall bodyweight gain in both sexes and there was good survival in all groups over 104 weeks of treatment. There was no evidence that administration of CBD BDS at dose levels of up to 50 /kg bw/mg/kg bw/day to the HsdBrlHan:WIST rat influenced tumour formation. There was no apparent increase in the incidence of neoplasia, alteration in the time of tumour onset or induction of rare tumours. There was some evidence of reduction in some of the commonly seen hormone mediated ageing changes, especially those seen in ageing females (Sativex monograph).

From Respondent 2

Current scientific evidence suggests that CBD does not exhibit carcinogenic properties. Several key studies, including those conducted as part of the regulatory approval process for CBD-containing medications like Epidyolex®, have specifically evaluated the compound for carcinogenic potential. The findings from these studies consistently show no evidence of CBD being carcinogenic. Here are some points based on the available data:

1. Long-Term Animal Studies: Long-term studies in rodents, often required for assessing the carcinogenic potential of pharmaceuticals, have shown no increase in tumour incidence associated with CBD administration. For example, preclinical toxicology studies on Epidiolex® demonstrated no evidence of carcinogenicity after chronic administration in rats and mice, even at doses much higher than the recommended human dose.

2. In Vitro and In Vivo Genotoxicity Testing: Genotoxicity tests a few mentioned above described, which evaluate whether a substance damages genetic material, are often predictive of carcinogenic risk. CBD has been subjected to a battery of genotoxicity assays, including the Ames test (a test for mutagenicity), chromosomal aberration assays, and micronucleus tests in mammalian cells. The results of these tests consistently indicate that CBD does not have genotoxic or mutagenic properties, further reducing the likelihood of carcinogenicity.

3. Epidemiological Data: Although human long-term epidemiological studies specifically evaluating CBD's carcinogenic potential are still limited, available clinical data from the use of purified CBD in therapeutic settings (such as in patients with epilepsy) has not raised any red flags regarding cancer risk.

4. Cannabis Studies and Clarification on Impurities: While some studies on cannabis products as a whole have raised concerns about carcinogenicity, these concerns are largely attributed to other compounds found in the plant, such as polycyclic aromatic hydrocarbons (PAHs) formed during smoking, or impurities such as pesticides, which may be present in non-pure CBD extracts. Studies that focus on purified or synthetic CBD, free from these contaminants, do not show evidence of a carcinogenic effect.

Recent studies suggest that CBD may have potential anti-cancer properties without being carcinogenic itself. A comprehensive genotoxicity evaluation found CBD unlikely to pose a genotoxic hazard (Henderson *et al.*, 2023).105 CBD has demonstrated anticancer effects through various mechanisms, including modulation of ceramide production, ER-stress, autophagy, apoptosis, angiogenesis, and matrix remodelling (Zhelyazkova *et al.*, 2020). In experimental models, a CBD-rich Cannabis sativa extract reduced colon carcinogenesis and inhibited colorectal cancer cell proliferation via CB1 and CB2 receptor activation (Romano *et al.*, 2014). CBD has shown anti-tumour effects on various cancer types in preclinical studies, exerting some of its effects through modulation of the endocannabinoid system (Massi *et al.*, 2013). While these findings are promising, further research is needed to fully understand CBD's specific molecular mechanisms and potential therapeutic applications in cancer treatment. Therefore, from the available data it appears CBD does not to exhibit clastogenicity and aneugenicity and displays a well-tolerated safety profile, in acute and repeated dose toxicity studies. Additionally,

there is some pre-clinical evidence to suggest that pure CBD isolate may reduce cancer cell viability. Overall, the available evidence suggests that CBD is generally well-tolerated and does not accumulate to a significant extent in most individuals. Considering also the poor dermal absorption rate and known poor bioavailability of CBD the carcinogenicity is not considered to be a major risk based on the proposed use of the product as a topically applied cosmetic. Further substantiation that a molecule is not classified as a CMR (carcinogenic, mutagenic, or toxic to reproduction) substance is supported if the ingredient is approved and permitted for oral consumption, as outlined in Appendix 5 of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation, 12th revision. According to this guidance, if a substance is approved for use in food, it is not considered a CMR substance, reinforcing its safety profile for cosmetic applications. As previously detailed in this dossier, synthetic CBD, specifically the Applicant's synthetic CBD, has received a positive safety opinion from the UK FSA, deeming it safe for oral consumption for general population. Notably, CBD is also authorized as a medicine for paediatric use, even at high concentrations, underscoring its established safety profile. The approval of CBD as a therapeutic agent in children highlights the rigorous safety assessments it has undergone, particularly given the sensitivity of this population due to their higher rates of cell division and growth. It would be scientifically and ethically unacceptable to authorize a medication with carcinogenic properties in paediatric patients, regardless of the severity of the condition being treated. This further supports the conclusion that CBD does not pose carcinogenic risks, especially in such vulnerable populations.

SCCS comment
In view of the available information, the SCCS is of the opinion that CBD does not raise concerns about carcinogenicity.

3.4.8 Photo-induced toxicity

3.4.8.1 Phototoxicity / photo-irritation and photosensitisation

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3.4.8.2 Photomutagenicity / photoclastogenicity

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3.4.9 Human data

EMA has approved the application for Epidyolex in the treatment of use as adjunctive therapy of seizures associated with Lennox-Gastaut syndrome (LGS) or Dravet syndrome (DS) in conjunction with clobazam only, for patients 2 years of age and older.
Monitoring of patients receiving EPIDYOLEX focuses primarily on liver function, due to the risk of hepatocellular damage associated with cannabidiol. Indeed, cannabidiol is associated with dose-dependent elevations of hepatic transaminases (alanine aminotransferase [ALAT] and/or aspartate aminotransferase [ASAT]), generally occurring within 2 months of initiating treatment. In a randomized clinical trial, Florian et al. (2025) found that 5.6% of healthy adults administered CBD 5 mg/kg/d for up to 28 days had liver enzyme level elevations greater than 3 times the clinical upper limit of normal range.

3.4.10 Special investigations

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3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

From Respondent 1

In order to give a holistic assessment of CBD >97% purity we have used NOAEL's across both Henderson et al (2023) and Tallon and Child et al (2023) OECD compliant 90- day repeated-dose oral toxicity studies referenced above along with both **36 mg/kg-bw/day and 72 mg/kg-bw/day** doses. Studies pertaining to crude hemp extracts containing lower levels of CBD were not considered representative and therefore discounted, it is our opinion that the NOAEL's obtained from the aforementioned studies represent new data pertaining to highly purified CBD ingredients.

MOS= NOAEL/SED

MOS assessment for Hand oil 6% CBD in MCT oil -limited body areas (% SED)

NOAEL (mg/kg bw)	Reference	MOS calculation
140	Henderson et al 2023	140/0.196= **714.29**
230	Tallon and Child 2023	230/0.196 = **1173.47**
72	Applicant study	72/0.196 = **367.35**
36	Applicant study	36/ 0.196 =**183.67**
120	*Averaged out*	*120/0.196=* ***612.25***

MOS assessment for 1.3% CBD in a balm- 5.0 g per day application (%SED)

NOAEL (mg/kg bw)	Reference	MOS calculation
140	Henderson et al 2023	140/0.108**= 1296.30**
230	Tallon and Child 2023	230/0.108**= 2129.63**
72	Applicant study	72/0.108= **666.67**
36	Applicant study	36/ 0.108= **333.33**
120	*Averaged out*	*120/0.108=* ***1111.11***

From Respondent 2

In a 90-day study commissioned by the sponsor in order to gain data for an EFSA submission, male and female rats were dosed orally with Natural CBD Isolate, vehicled in corn oil. The established NOAEL was 25 mg/kg bw/day for both sexes.

Therefore, the Point of Departure (PoD) for Margin of Safety (MoS) calculation is considered 25 mg/kg bw/day.
CBD oral absorption (OA) is low and has values between 6-14%
It has been decided to consider the maximum OA (14%) because the test item used in the study was vehicled in corn oil, which has been shown to promote oral absorption of CBD.
Therefore, the systemic PoD (PoD_{sys}) for CBD is:

$$\boldsymbol{PoD_{sys}} = PoD \times OA = 25 \times 0.14 = \mathbf{3.5\ mg/kg\ bw/day}$$

	PoD_{sys} (mg/kg bw/day)	**SED** (mg/kg bw/day)	**MoS** (-)
Body ointment 2%	3.5	0.2464	**14.20**
Body lotion 1%	3.5	0.1232	**28.41**
Face cream 1%	3.5	0.02414	**144.99**

Because MoS for body products is largely below 100, is calculated also the MoS for a body product with 0.2% CBD.

	$E_{product}$ (mg/kg bw/day)	**Concentration** (% w/w)	**E_{dermal}** (mg/kg bw/day)
Body lotion 0.2%	123.20	0.002	**0.2464**

	E_{dermal} (mg/kg bw/day)	**Dermal Absorption** (-)	**SED** (mg/kg bw/day)
Body lotion 0.2%	0.2464	0.1	**0.02464**

	PoD_{sys} (mg/ kg bw/day)	**SED** (mg/kg bw/day)	**MoS** (-)
Body lotion 0.2%	3.5	0.02464	**142**

From Responder 2, for THC evaluation

THC is not intended to be added intentionally in cosmetic products, but it could be present in CBD derived from extract as impurity.
The Sponsor provided a hemp extract for toxicological tests. Maximum concentration of THC, as impurity, in hemp extract is 0.20%. Considering 0.20% of THC as maximum impurity in CBD, in products mentioned before, THC concentration in final product is:

	CBD concentration (-)	THC concentration (-)
Body ointment 2%	0.02	0.00004
Body lotion 1%	0.01	0.00002
Face cream 1%	0.01	0.00002
Body lotion 0.2%	0.002	0.000004

For calculating the PoDsys is considered the same as CBD oral absorption of 14% due to similar physico-chemical properties.

$$\boldsymbol{PoD_{sys}} = PoD \times OA = 0.0014 \times 0.14 = \mathbf{0.000196}\ \boldsymbol{mg/kg\ bw/day}$$

Since this value is referred to humans and not to animals, and variations within the human population are already taken into account in the UF applied, the reference MoS is 1.

In conclusion, MoS for THC used in concentration and products afore mentioned, is:

	PoD$_{sys}$	**SED**	**MoS**
	(mg/kg bw/day)	(mg/kg bw/day)	(-)
Body ointment 2%	0.00196	0.00025	**7.84**
Body lotion 1%	0.00196	0.00012	**16.33**
Face cream 1%	0.00196	0.00002	**98**
Body lotion 0.2%	0.00196	0.000025	**78.4**

From Respondent 3

Taking into account the NOAEL of 24.75 mg/kg b.w./d for CBD from the 90-day repeated oral rat toxicity study conducted on raw material OPTIMA CBD XB, the maximum recommended concentrations are as follows:

Table 11: Maximum recommended concentrations for raw material OPTIMA CBD XB taking into account the NOAEL of 24.75 mg/kg b.w./d for CBD

Product	Relative daily exposure (mg/kg b.w. /d)	Oral NOAEL Value (mg/kg b.w./d)	Systemic PoD Value (mg/kg b.w./d)	Dermal absorption (%)	Maximum CBD concentration (%)	Corresponding maximum OPTIMA CBD XB concentration (%)
Shower gel	2.79	24.75	12.38	50	1*	1*
Shampoo	1.51				1*	1*
Body lotion	123.2				0.20	0.20
Face cream	24.14				1*	1*
Hand cream	32.7				0.76	0.76
Deodorant non-spray	22.08				1*	1*
Lipstick	0.9		24.75	100	1*	1*
Toothpaste	2.16				1*	1*
Mouthwash	32.54				0.76	0.77

Taking into account the NOAEL of 197.54 mg/kg b.w./d for CBD from the 90-day repeated oral rat toxicity study conducted on raw material OPTIMA BROAD EXTRACT XB, the maximum concentrations are as follows:

Table 12: Maximum recommended concentrations for raw material OPTIMA BROAD EXTRACT XB taking into account the NOAEL of 197.54 mg/kg b.w./d for CBD

Product	Relative daily exposure (mg/kg b.w./d)	Oral NOAEL Value (mg/kg b.w./d)	Systemic PoD Value (mg/kg b.w./d)	Dermal absorption (%)	Maximum CBD concentration (%)	Corresponding maximum OPTIMA BROAD EXTRACT XB concentration (%)
Shower gel	2.79	197.54	98.77	50	1*	1*
Shampoo	1.51				1*	1*
Body lotion	123.2				1*	1*
Face cream	24.14				1*	1*
Hand cream	32.7				1*	1*
Deodorant non-spray	22.08				1*	1*
Lipstick	0.9		197.54	100	1*	1*
Toothpaste	2.16				1*	1*
Mouthwash	32.54				1*	1*

SCCS comment

The SCCS has considered an Oral NOAEL of 25 mg/kg b.w./day from the 90-day repeated oral rat toxicity studies. This corresponds to a systemic PoD for CBD of 6.25 mg/kg b.w./day, considering an oral bioavailability of 25%.

For the calculation of aggregate exposure, a dermal absorption of 10% was considered for dermally applied products. For oral products, an absorption of 25% was used that is considered to integrate both oral and dermal availability.

The maximum CBD concentrations for dermal and oral products were determined as follows:

If the MoS is greater than 100 **for aggregate (dermal and oral)** with a concentration of 0.19 % of CBD in the cosmetic product, then the maximum concentration for each product taken individually is also considered safe.

Table 13: Calculation of aggregate exposure and MoS for CBD

Product families	Product categories	Dermal or oral absorption	Eproduct normalized	Dermal and oral SED	NOAEL syst	MOS	Max. conc
		(%)	mg/kg bw /d	mg/kg bw /d	mg/kg bw /d		%
Rinse-off skin & hair cleansing products (except hand wash)	Shower gel	10	2.79	0.0005301	6.25	11790	0.19
	Hair conditioner	10	0.67	0.0001273		49097	
	Shampoo	10	1.51	0.0002869		21785	
Hand wash soap	Hand wash soap	10	3.33	0.0006327		9878	
Leave on skin and hair products	Body lotion	10	123.2	0.023408		267	
	Face cream	10	24.14	0.0045866		1363	
	Hand cream	10	32.7	0.006213		1006	

	Deodorant non-spray	10	22.08	0.0041952		1490
	Hair styling	10	5.74	0.0010906		5731
Face make-up products	Liquid foundation	10	7.9	0.001501		4164
	Make-up remover	10	8.33	0.0015827		3949
Eye make up	Eye make-up	10	0.33	0.0000627		99681
	Mascara	10	0.42	0.0000798		78321
	Eyeliner	10	0.08	0.0000152		411184
oral care products	Toothpaste	25	2.16	0.001026		6092
	Mouthwash	25	32.54	0.0154565		404
Lip products	Lipstick, lip salve	25	0.9	0.0004275		14620
aggregate dermal and oral exposure				0.061		102

NOAEL syst = 25 mg/kg (NOAEL)x 25% (oral absorption) = 6.25 mg/kg/day

Based on the aggregate dermal and oral exposure, the SCCS considers the use of a CBD dose of up to 0.19% in both dermal and oral cosmetic products as safe.

Delta 9THC evaluation

THC is not intended to be added intentionally in cosmetic products, but it could be present in CBD derived from extract as impurity.

EFSA (CONTAM Panel2015) established an ARfD of 1 µg/kg bw/day via oral route, derived from a human LOAEL of 2.5 mg/day, adjusted for a person with a weight of 70 kg and divided for an UF of 30 (3 for extrapolation from the LOAEL to a NOAEL and 10 for interindividual differences).

For calculating the PoDsys the oral absorption of 6% is considered based on specific data on delta 9 THC (EFSA 2015). For dermal absorption, the SCCS used a value of 10% (similar to CBD based on similar physico-chemical properties).

Since this value refers to humans and not to animals, and variations within the human population are already taken into account in the UF applied, the reference MoS is 1.

The maximum delta 9 THC concentrations for dermal and oral product were determined as follows:

If the MoS is greater than 1 **for aggregate (dermal and oral)** with a concentration of 0.00025% of THC in the cosmetic product, then the maximum concentration for each product taken individually is also considered safe. The SCCS rounded to 1 the value of 0.9.

Table 14

Product families	**Product categories**	**Dermal or oral absorption**	**Eproduct normalized**	**Dermal and oral SED**	**Acute reference dose**	**MOS ***	**Maximum conc**
		(%)	mg/kg bw /d	mg/kg bw /d	mg/kg bw /d		%
Rinse-off skin & hair cleansing products (except hand wash)	Shower gel	10	2.79	6.975E-07	0.001	86	0.00025
	Hair conditioner	10	0.67	1.675E-07		358	
	Shampoo	10	1.51	3.775E-07		159	
Hand wash soap	Hand wash soap	10	3.33	8.325E-07		72	
Leave on skin and hair products	Body lotion	10	123.2	0.0000308		2	
	Face cream	10	24.14	0.000006035		10	
	Hand cream	10	32.7	0.000008175		7	
	Deodorant non-spray	10	22.08	0.00000552		11	
	Hair styling	10	5.74	0.000001435		42	
Face make-up products	Liquid foundation	10	7.9	0.000001975		30	
	Make-up remover	10	8.33	2.0825E-06		29	
Eye make up	Eye make-up	10	0.33	8.25E-08		727	
	Mascara	10	0.42	0.000000105		571	
	Eyeliner	10	0.08	0.00000002		3000	
oral care products	Toothpaste	6	2.16	0.000000324		185	
	Mouthwash	6	32.54	0.000004881		12	
Lip products	Lipstick, lip salve	6	0.9	0.000000135		444	
aggregate dermal and oral exposure			0.0001			1	

* for the MOS calculation, an oral absorption 6% was applied to the ARfD

3.6 DISCUSSION

Physicochemical properties

The characteristics of the synthetic production of CBD are largely consistent across different suppliers, as they follow similar routes of synthesis

Toxicokinetics

The SCCS considers CBD as an extremely lipophilic substances, and therefore the rate of transfer between the stratum corneum and the epidermis can become the limiting factor in overall skin absorption. Moreover, its hydrophobic nature limits diffusion across the aqueous layer of the skin.
Thus, the dermal penetration to be used for the calculation of the MoS will be 10%.

For oral absorption, the SCCS has considered a value of 25%

Exposure

The SCCS has applied a dermal absorption of 10% and oral absorption of 25 % to determine a safe concentration of CBD in cosmetic products, based on the MOS of aggregate dermal and oral exposure.

Toxicological Evaluation

Irritation and corrosivity
CBD was not considered to be irritating to the skin and not irritating for the eye.

Skin sensitisation

In view of the absence of reports on contact sensitisation in humans and the relatively low level of skin exposure, the SCCS considers the risk of sensitisation from exposure to CBD as negligible.

Acute toxicity

CBD is of low acute toxicity by oral or dermal route

Repeated dose toxicity

The SCCS has considered an Oral NOAEL of 25 mg/kg b.w./day from the 90-day repeated oral rat toxicity studies associated with liver toxicities. This corresponds to a systemic POD for CBD of 6.25 mg/kg b.w./day considering a default oral bioavailability of 25%.

Reproductive toxicity

The NOAEL derived from the chronic oral toxicity study 25 mg/kg/day(which is used in the current assessment) is below the NOAEL of 75 mg kg/day for reproductive and developmental effects.
Mutagenicity / genotoxicity

CBD does not have genotoxic or mutagenic properties

Carcinogenicity

The SCCS is of the opinion that CBD is not likely to be carcinogenic.

Photo-induced toxicity

CBD has no phototoxic effect

Human data

Data and report on Epidyolex®, which is a license medicine for epilepsy, provides adequate data to assess the efficacy and safety of CBD. Epidyolex® can be administered at a maximum daily dose of up to 20mg per kilogram of body weight. Epidyolex is used to treat severe medical conditions and that the decision to treat patients is based on a risk-benefit analysis performed by medical professionals.

Special investigation

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Safety assessment

This assessment has been carried out according to the principles of the SCCS Notes of Guidance. Uncertainties regarding this assessment can be addressed through provision of adequate data/ information by interested Applicants.

4. CONCLUSION

1. *Taking under consideration the information/data submitted via the respective call for data, the SCCS is requested:*

(a) to assess the maximum concentration of Cannabidiol that is considered safe when used in cosmetic products

Based on the limited available data, the SCCS considers CBD safe when used at concentrations up to 0.19% in dermal cosmetic products and oral cosmetic products – whether used separately or in combination.

(b) to identify the maximum safe level of Delta- 9-tetrahydrocannabinol (THC) present as a contaminant in Cannabidiol preparations

The SCCS considers the presence of THC impurities as safe at concentrations up to 0.00025% in dermal and oral cosmetic products – whether used separately or in combination.

The SCCS acknowledges that the current evaluation may have some limitations because of the paucity of data/information received from a few respondents to the Commission's Call for data. These limitations can be addressed as and when adequate data / information can be made available by interested Applicants.

2. *Does the SCCS have any further scientific concerns with regard to the use of CBD and the possible non-intended presence at trace levels of other cannabinoids, including THC, in cosmetic products?*

This assessment is based only on the safety of pure CBD.
This Scientific Advice does not consider the use of CBD in cosmetic products that may lead to exposure of the end-user's lungs by inhalation.

5. MINORITY OPINION

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6. REFERENCES

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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 – 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 – from page 158

9. ANNEX A

Mutagenicity / genotoxicity *in vitro*

From EMA 2019:

CBD, purified CBD and CBD as Botanical Drug Substance (BDS) were evaluated in a range of *in vitro* and *in vivo* standard genotoxicity assays. Only studies performed with purified CBD and Cannabidiol oral solution (CBS-OS) are summarised.

The genotoxic potential of CBD has been evaluated in a standard test battery of *in vitro* and *in vivo* assays according to ICH S2(R1). All tests concluded CBD to be negative for genotoxic potential.

A genotoxicity assessment of 7-carboxy-CBD (human metabolite; 7-COOH-CBD) using non-GLP test material in an Ames Test (GLP GWTX18016) was provided. Results from this study showed that 7-COOH-CBD did not induce mutation in 5 *Salmonella typhimurium* strains (TA98, TA100, TA102, TA1535 and TA1537) under the conditions selected for this study. However, test item output from the scaled-up manufacture will produce appropriately characterised material to conduct genotoxicity GLP studies planned with both 7-OH-CBD and 7-COOH-CBD. GLP genotoxicity studies are awaited via post-authorization measure commitment.

Ref.: Assessment report. Epidyolex. International non-proprietary name: cannabidiol. Procedure No. EMEA/H/C/004675/0000. 25 July 2019. EMA/458106/2019. Committee for Medicinal Products for Human Use (CHMP)

SCCS comment
The composition of the test substance used in the genotoxicity studies was not clearly provided. The SCCS does not evaluate formulations, only ingredients.

In this submission, the applicant provided the following new studies on genotoxicity:

- On OPTIMA BROAD EXTRACT XB
  - Reverse Mutation Assay using Bacteria - EUROFINS Munich STUGC20AA2201-3 01/10/2020
  - In Vitro Mammalian Cell Micronucleus Test-(OECD 487) - CHARLES RIVER 20/194-013C-08/01/2021

- On OPTIMA CBD XB
  - Reverse Mutation Assay using Bacteria (Salmonella typhimurium and Escherichia coli) (OECD 471) EUROFINS Munich STUGC20AA2201-2 13/10/2020
  - In Vitro Mammalian Cell Micronucleus Test (OECD 487) CHARLES RIVER 20/195-013C 03/02/2021

A summary of available data of relevance to current assessment, including the assessment of these studies plus additional studies in the literature, is described below.

In vitro study #1: Gene mutation test in bacteria (Ames test) on Natural Hemp Isolate CBD

Guidelines/Methods:	OECD TG 471 (26 Jun 2020)
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101
GLP:	Yes
Replicates:	3 test plates per dose or per control; 2 independent experiments
Test substance:	Natural Hemp Isolate CBD
Batch (Purity):	CBDB1901770001 (99.83%)
Vehicle:	Dimethyl sulfoxide (DMSO)
Concentrations:	Experiment I (plate incorporation test) 31.6, 100, 316, 1000, 2500 and 5000 µg/plate Experiment II (plate preincubation test): 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA98, TA100 and TA1535 (with metabolic activation); E. coli WP2 uvrA (pKM101)) 0.316, 1.00, 3.16, 10.0, 31.6 and 100 µg/plate (TA98, TA100 and TA1535 (without metabolic activation); TA1537)
Positive controls:	-S9-mix: Sodium azide (10 µg/plate for TA 1535, TA 100) 4-Nitro-o-phenylendiamine (10 µg/plate for TA 98; 40 µg/plate for TA1537) Methylmethanesulfonate (1 µg/plate) for E. coli WP2 uvrA, pKM101) +S9-mix: 2-aminoanthracene: 2.5 µg/plate for S. typhimurium and 10 µg/plate for E. coli
Negative control:	Vehicle (DMSO)
Date of report:	2021, May 10
Study period:	February-May 2021
Reference:	Eurofins Munich Study No.: STUGC21AA0231-2

The test item Natural Hemp Isolate CBD was investigated for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and tester strain E. coli WP2 uvrA (pKM101).

No precipitation of the test item was observed in any tester strain used in experiment I and II (with and without metabolic activation). In experiment I, toxic effects of the test item were noted in tester strain TA100 at concentrations of 2500 µg/plate and higher (without metabolic activation). No further toxic effects of the test item were noted in experiment I. In experiment II toxic effects of the test item were noted at concentrations of 10.0 µg/plate and higher (without metabolic activation) and at concentrations of 316 µg/plate (with metabolic activation), depending on the particular tester strain.
No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with Natural Hemp Isolate CBD at any concentration level, neither in the presence nor absence of metabolic activation in experiment I and II. All criteria of validity were met.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, Natural Hemp Isolate CBD did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used.

Therefore, Natural Hemp Isolate CBD is considered to be non-mutagenic in this bacterial reverse mutation assay.

Ref: Respondent 3

From the Respondent 2

During a toxicological evaluation for the use of CBD as a novel food, in addition to literature, it was considered necessary to perform a Genotoxicity basic test battery on the intended products CBD as substance as requested by the EFSA Guidance on Novel Food (2012).
The test item is natural CBD, not synthetic one. However, the purity of the substance (above 98.0 % as HPLC assay) allow the read across.
Studies performed have been:
- Bacterial Reverse Mutation assay (OECD 471)
- In vitro Mammalian Cell Micronucleus test (OECD 487)

In the Bacterial Reverse Mutation assay (OECD 471) CBD Natural Hemp Isolate, did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used under the experimental conditions reported.
Therefore, Natural CBD Isolate is considered to be non-mutagenic in this bacterial reverse mutation assay.

SCCS comment

The SCCS considers the study valid and the results negative. Natural Hemp Isolate CBD is considered to be non-mutagenic in bacterial reverse mutation assay.

In vitro study #2. Gene mutation test in bacteria (Ames test) on CBD Isolate Powder 99%+

Guidelines/Methods:	OECD TG 471 (26 Jun 2020)
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101
GLP:	Yes
Replicates:	3 test plates per dose or per control; 2 independent experiments
Test substance:	CBD ISOLATE POWDER 99%+
Batch (Purity):	SL-20-ISO-04, Purity: 99.46%
Vehicle:	Dimethyl sulfoxide (DMSO)
Concentrations:	Experiment I: 31.6, 100, 316, 1000, 2500 and 5000 µg/plate Experiment II: 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA98, TA100 (with metabolic activation), TA1535 (with metabolic activation), TA1537 and E. coli WP2 uvrA (pKM101)) 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA100, (without metabolic activation)) 0.1, 0.316, 1.0, 3.16, 10.0, 31.6 and 100 µg/plate (TA1535 (without metabolic activation))
Positive controls:	-S9-mix: Sodium azide (10 µg/plate for TA 1535, TA 100) 4-Nitro-o-phenylendiamine (10 µg/plate for TA 98; 40 µg/plate for TA1537) Methylmethanesulfonate (1 µg/plate) for E. coli WP2 uvrA, pKM101) +S9-mix: 2-aminoanthracene: 2.5 µg/plate for S. typhimurium and 10 µg/plate for E. coli
Negative control:	Distilled water

Solvent control:	DMSO
Date of report:	2020, October 13
Study period:	July-October 2020
Reference:	Eurofins Munich Study No.: STUGC20AA2201-2

The test item CBD ISOLATE POWDER 99%+ was investigated for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and tester strain E. coli WP2 uvrA (pKM101).
No precipitation of the test item was observed in any tester strain used in experiment I and II (with and without metabolic activation).
Toxic effects of the test item were noted in one tester strain used in experiment I and in three tester strains used in experiment II:
- In experiment I toxic effects of the test item were observed at a concentration of 5000 µg/plate (without metabolic activation) in one particular tester strain.
- In experiment II toxic effects of the test item were noted at concentrations of 3.16 µg/plate and higher (without metabolic activation) depending on the particular tester strain.
No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with CBD ISOLATE POWDER 99%+ at any concentration level, neither in the presence nor absence of metabolic activation in experiment I and II.
All criteria of validity were met.
In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, CBD ISOLATE POWDER 99%+ did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used.
Therefore, CBD ISOLATE POWDER 99%+ is considered to be non-mutagenic in this bacterial reverse mutation assay.

Ref.: Reverse Mutation Assay using Bacteria (Salmonella typhimurium and Escherichia coli) with CBD ISOLATE POWDER 99%+. Eurofins Munich Study No.: STUGC20AA2201-2

SCCS comment
The SCCS considers the study valid and the results negative. CBD ISOLATE POWDER 99%+ is considered to be non-mutagenic in bacterial reverse mutation assay.

***In vitro* study #3. Gene mutation test in bacteria (Ames test) on PCR BROAD SPECTRUMS HEMP EXTRACT 85%**

Guidelines/Methods:	OECD TG 471 (26 Jun 2020)
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101
GLP:	Yes
Replicates:	3 test plates per dose or per control; 2 independent experiments
Test substance:	PCR BROAD SPECTRUMS HEMP EXTRACT 85%
Batch (Purity):	SL-20-BSE-01, Purity: 85.92% CBD
Vehicle:	Dimethyl sulfoxide (DMSO)
Concentrations:	plate incorporation test (experiment I) and the pre-incubation test (experiment II): 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate
Positive controls:	-S9-mix: Sodium azide (10 µg/plate for TA 1535, TA 100) 4-Nitro-o-phenylendiamine (10 µg/plate for TA 98; 40 µg/plate for TA1537)

	Methylmethanesulfonate (1 µg/plate) for E. coli WP2 uvrA, pKM101) +S9-mix: 2-aminoanthracene: 2.5 µg/plate for S. typhimurium and 10 µg/plate for E. coli
Negative control:	Distilled water
Solvent control:	DMSO
Date of report:	2020, October 1
Study period:	July-October 2020
Reference:	Eurofins Munich Study No.: STUGC20AA2201-3

The test item PCR BROAD SPECTRUMS HEMP EXTRACT 85% was investigated for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and tester strain E. coli WP2 uvrA (pKM101).
No precipitation of the test item was observed in any tester strain used in experiment I and II (with
and without metabolic activation). Toxic effects of the test item were noted in two tester strains used in experiment I and in all tester strains used in experiment II:
- In experiment I toxic effects of the test item were observed at concentrations of 100 µg/plate and higher (without metabolic activation) and at concentrations of 2500 µg/plate and higher (with metabolic activation), depending on the particular tester strain.
- In experiment II toxic effects of the test item were noted at concentrations of 31.6 µg/plate and higher (without metabolic activation) and at concentrations of 2500 µg/plate and higher (with metabolic activation), depending on the particular tester strain.
No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with PCR BROAD SPECTRUMS HEMP EXTRACT 85% at any concentration level, neither in the presence nor absence of metabolic activation in experiment I and II. All criteria of validity were met.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, PCR BROAD SPECTRUMS HEMP EXTRACT 85% did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used. Therefore, PCR BROAD SPECTRUMS HEMP EXTRACT 85% is considered to be non-mutagenic in this bacterial reverse mutation assay.

Ref.: Reverse Mutation Assay using Bacteria (Salmonella typhimurium and Escherichia coli) with PCR BROAD SPECTRUMS HEMP EXTRACT 85%. Eurofins Munich Study No.: STUGC20AA2201-3

SCCS comment
The SCCS considers the study valid and the results negative. PCR BROAD SPECTRUMS HEMP EXTRACT 85% is considered to be non-mutagenic in bacterial reverse mutation assay.

***In vitro* study #4: Gene mutation test in bacteria (Ames test) on Full Spectrum Extract (REGULAR)**

Data in Table below is taken from the dossier by Chemsafe S.r.l., 2020 and a study plan from Eurofins Munich Study No.: STUGC21AA1085-2.

Guidelines/Methods:	OECD TG 471 (26 June 2020)
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101
GLP:	Yes

Replicates:	3 test plates per dose or per control; 2 independent experiments
Test substance:	Full Spectrum Extract (REGULAR)
Batch (Purity):	PN20/002405 (mixture, purity unknown)
Vehicle:	Distilled water
Concentrations:	Experiment I: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA98, TA100, TA1537, E. coli WP2 uvrA (pKM101)) 3.16, 10.0, 31.6, 100, 316 and 1000 µg/plate (TA1535) Experiment II: 0.5, 1.58, 5.0, 15.8, 50 and 158 µg/plate (TA98, TA100 and TA1535 (without metabolic activation)) 5.0, 15.8, 50, 158, 500, 1580 and 3000 µg/plate (TA98, TA100 and TA1535 (with metabolic activation)) 15.8, 50, 158, 500, 1580 and 3000 µg/plate (TA1537 (without metabolic activation)) 15.8, 50, 158, 500, 1580, 3000 and 5000 µg/plate (TA1537 (with metabolic activation), E. coli WP2 uvrA (pKM101) (with and without metabolic activation))
Positive controls:	-S9-mix: Sodium azide (10 µg/plate for TA 1535, TA 100) 4-Nitro-o-phenylendiamine (10 µg/plate for TA 98; 40 µg/plate for TA1537) Methylmethanesulfonate (1 µg/plate) for E. coli WP2 uvrA, pKM101) +S9-mix: 2-aminoanthracene: 2.5 µg/plate for S. typhimurium and 10 µg/plate for E. coli
Negative control:	Vehicle (distilled water)
Date of study plan:	2021, May 31
Study period:	May-June 2021
Reference:	Eurofins Munich Study No.: STUGC21AA1085-2 cit in TOXICOLOGICAL EXPERT REPORT Cannabidiol (CBD) for cosmetic uses Prepared by Chemsafe S.r.l. Consulting company for chemical, pharmaceutical and medical device industries)

The test item Full Spectrum Extract (REGULAR) (Chemical name: Hemp Extract) was investigated for its potential to induce gene mutations according to the plate incorporation test (experiment I and experiment II) using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and tester strain E. coli WP2 uvrA (pKM101) (OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Bacterial Reverse Mutation Test", adopted 21 July 1997, corrected 26 June 2020).
No precipitation of the test item was observed in any tester strain used in experiment I and II (with and without metabolic activation). In experiment I toxic effects of the test item were observed at concentrations of 31.6 µg/plate and higher (without metabolic activation) and at concentrations of 316 µg/plate and higher (with metabolic activation), depending on the particular tester strain. In experiment II toxic effects of the test item were noted at concentrations of 50 µg/plate and higher (without metabolic activation) and at concentrations of 1580 µg/plate (with metabolic activation), depending on the particular tester strain. No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with Full Spectrum Extract (REGULAR) at any concentration level, neither in the presence nor absence of metabolic activation in experiment I and II.
In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, Full Spectrum Extract (REGULAR) did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used. Therefore, Full Spectrum Extract (REGULAR) is considered to be non-mutagenic in this bacterial reverse mutation assay.

Ref.: Respondent 2.

SCCS comment
According to the Applicants, the Full Spectrum Extract (REGULAR) has no bacterial gene mutation potential. Study not evaluated by SCCS (data not available).

***In vitro* study #5: Gene mutation test in bacteria (Ames test) on Hemp-derived CBD isolate**

Guidelines/Methods:	OECD TG 471
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101
GLP:	Yes
Replicates:	triplicates
Test substance:	Hemp-derived CBD isolate (CAS No. 13956-29-1)
Batch (Purity):	99.62% CBD
Vehicle:	DMSO
Concentrations:	0.25, 0.5, 1.0, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, or 5000 µg/plate +/-S9
Positive controls:	-S9-mix: 2-nitrofluorene (2NF; Sigma-Aldrich) at 2.5 µg/plate with TA98, sodium azide (NAAZ; Sigma-Aldrich) at 1.0 µg/plate with TA100 and TA1535, ICR-191 acridine (Sigma-Aldrich) at 0.5 µg/plate with TA1537, and 4-nitroquinoline-N-oxide (Acros Organics) at 2.0 µg/plate with E. coli WP2 uvrA. +S9-mix: 2-aminoanthracene: (2.5 µg/plate), and 10 µg/plate for E. coli WP2 uvrA.
Negative control:	DMSO
Date of study plan:	Unknown
Study period:	unkown
Reference:	Henderson et al., 2023 https://doi.org/10.1016/j.yrtph.2023.105425

CBD up to 5000 µg/plate was negative in Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537, and Escherichia coli strain WP2 uvrA, with and without metabolic activation.

These assays, performed according OECD TG and GLP, indicate that CBD is unlikely to pose a genotoxic hazard.

Ref: Henderson *et al.*, 2023 https://doi.org/10.1016/j.yrtph.2023.105425

SCCS comment
The study was considered valid and did not indicate any concerns about the mutagenic effects of Hemp-derived CBD isolate (99.62%).

***In vitro* study #6: Gene mutation test in bacteria (Ames test) on CBD isolate with lipid carrier**

The proprietary CBD test substance comprised a high purity The CBD isolate was diluted to a concentration of 31–33% using MCT oil supplied by cbdMD Inc (Charlotte, NC, USA). The CBD isolate was a whole plant ethanol extract of *Cannabis sativa* L. (Cannabaceae) and CBD, accounts for >97% of the total cannabinoids present (Batch No. 103501B, cbdMD Inc (Charlotte, NC,

USA). Additional cannabinoids present in the isolate included cannabigerol (1.71%), cannabinol (0.47%), and cannabidivarin (0.22%).

The ability of the CBD test substance (30% CBD in MCT oil) to cause mutations was assessed in Ames tests using a plate incorporation and preincubation method.. All strains were studied in the presence and absence of a rat liver metabolic activation system.

Guidelines/Methods:	OECD TG 471
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101
GLP:	no
Replicates:	triplicates
Test substance:	CBD isolate with its lipid carrier [medium chain triglyceride (MCT)]
Batch (Purity):	30% CBD in MCT oil
Vehicle:	DMSO
Concentrations:	4.8, 15.2, 47.9, 1517.5, 4795, and 15175 µg/plate
Positive controls:	-S9-mix: Daunomycin for TA98 (6 µg/plate), Sodium azide for TA100 and TA1535 (1 µg/plate), ICR 191 acridine for TA1537 (1 µg/plate), and Methyl methanesulfonate for E. coli WP2 (mix) (2.5 µg/plate). +S9-mix: 2-aminoanthracene
Negative control:	DMSO
Date of study plan:	Unknown
Study period:	unkown
Reference:	Tallon MJ, Child RB, Blum JL. Genotoxic assessment of a Cannabis sativa L. extract. Pharm Biol. 2025 May 6;63(1):357–363. doi: 10.1080/13880209.2025.2499075

No substantial increases in revertant colony numbers were observed in any of the five tester strains following treatment with CBD test substance at any concentration, in the presence or absence of metabolic activation (S9), using either the plate incorporation or the pre-incubation method.

Precipitation was observed in all strains at >4795 µg/plate in both conditions, but this did not obscure the counts or mutagenic evaluation.

In conclusion, under test conditions, the CBD test substance was considered non-mutagenic at concentrations of up to 1517.5 µg/plate on bacterial tester strains *S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2 uvrA.

Ref: Tallon MJ, Child RB, Blum JL. Genotoxic assessment of a Cannabis sativa L. extract. Pharm Biol. 2025 May 6;63(1):357–363. doi: 10.1080/13880209.2025.2499075

SCCS comment
The study was considered valid and did not indicate any concerns about mutagenic effects of 30% CBD in MCT oil.

***In vitro* study #7: Gene mutation test in bacteria (Ames test) on blend of 9%hemp extract and 91% organic extra virgin olive oil**

Three Ames tests, one on the extract diluted in olive oil and two on undiluted extracts were completed.

Guidelines/Methods:	OECD TG 471
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA
GLP:	Yes
Replicates:	triplicates
Test substance:	blend of 9% hemp extract and 91% organic extra virgin olive oil An Ames test was conducted on this test article and two additional Ames tests were conducted on undiluted extract, one an isopropanol extract and the other a supercritical CO2 extract.
Batch (Purity):	Fatty acids comprise approximately 88.70% of this extract, while the phytocannabinoid content is 6.96% (of this, 6.27% is CBD); the remaining 4.34% consists of fatty alkanes, sterols, terpenes and tocopherols.
Vehicle:	DMSO
Concentrations:	extract diluted in olive oil: 0.24, 0.76, 2.41, 7.633, 24.12, 76.33, 241.22, 763.33, 2412.2, 7633.5, 24122 and 76355 µg/plate undiluted isopropanol extract (both the initial and confirmatory tests): 1.58, 5.0, 15.8, 50, 158, 500, 1580 and 5000 µg/plate. undiluted supercritical CO2 extract; 1.58, 5.0, 15.8, 50, 158, 500, 1580 and 5000 µg/plate for the initial test and 0.5, 2.5 and 25 µg/plate for the confirmatory test.
Positive controls:	-S9-mix: sodium azide, ICR 191, daunomycin and methyl methanesulfonate for S. typhimurium strains TA100 and TA1535, TA1537, TA98 and E. coli WP2 uvrA +S9-mix: 2-aminoanthracene
Negative control:	DMSO
Date of study plan:	Unknown
Study period:	unkown
Reference:	Dziwenka M, Coppock R, Alexander M, Palumbo E, Ramirez C, Lermer S. Safety Assessment of a Hemp Extract using Genotoxicity and Oral Repeat-Dose Toxicity Studies in Sprague-Dawley Rats. Toxicol Rep. 2020 Feb 20;7:376-385. doi: 10.1016/j.toxrep.2020.02.014.

The Bacterial Reverse Mutation Assay (Ames test) conducted by Dziwenka *et al.* (2020) was carried out three time, two of which using the CBD extract not diluted in oil. The tested item showed not to be mutagenic to bacteria in the Ames test.

Due to toxicity noted for strains TA100 and TA1537 with the supercritical CO2 extract, a supplemental test was conducted to ensure five concentrations could be assessed without toxicity. Both the plate incorporation and pre-incubation methods were used as previously described at final doses of 0.5, 2.5 and 25 µg/plate.

There was no concentration related or substantial test article related increases in the number of revertant colonies for each of the strains tested in the presence or absence of metabolic activation (S9 mix), in either the plate incorporation or the pre-incubation methods (data not shown).

The mutagenicity testing showed that the extract diluted with olive oil as well as the extracts produced with an isopropanol and supercritical CO2 extraction method were not mutagenic to bacteria in the Ames assay.

Ref: Dziwenka M, Coppock R, Alexander M, Palumbo E, Ramirez C, Lermer S. Safety Assessment of a Hemp Extract using Genotoxicity and Oral Repeat-Dose Toxicity Studies in Sprague-Dawley Rats. Toxicol Rep. 2020 Feb 20;7:376-385. doi: 10.1016/j.toxrep.2020.02.014.

From Respondent 2

Literature data
The test article is a proprietary blend of 9% hemp extract and 91% organic extra virgin olive oil, which is produced by an isopropanol extraction method under current Good Manufacturing Practices. Fatty acids comprise approximately 88.70% of this extract, while the phytocannabinoid content is 6.96% (of this, 6.27% is CBD); the remaining 4.34% consists of fatty alkanes, sterols, terpenes and tocopherols. The mutagenicity potential of the test article as well as undiluted extracts were evaluated in the Bacterial Reverse Mutation Assay in accordance with FDA GLP (21 CFR Part 58, 1987) and US FDA Redbook 2000 (IV.C.1.a, 2007) and ICH guidelines.

There were no concentration-related or substantial test article related increases in the number of revertant colonies for each of the strains tested in the presence or absence of metabolic activation (S9 mix), in either the plate incorporation or the pre-incubation methods. Precipitation which interfered with lawn evaluation was noted for all strains at doses ≥7633.5 µg/plate but did not obscure counts in the test with the diluted test article. Precipitation which obscured lawn evaluation was seen in all strains with the supercritical CO2 extract at doses ≥ 1580 µg/plate with and without S9 in both the plate incorporation and pre-incubation methods. Toxicity was evident for strains TA 98, TA 1535, TA 1537 and E. coli WP2 uvrA at ≥50 µg/plate, with and without S9, in the plate incorporation and/or pre-incubation tests. Precipitation which obscured lawn evaluation was seen in all strains with the isopropanol extract at doses ≥ 1580 µg/plate with and without S9 in both the plate incorporation and pre-incubation methods. Toxicity was noted for strains TA 1537 and TA 100 at 500 and/or 1580 µg/plate without S9 in the pre-incubation method. (…)

The mutagenicity testing showed that the extract diluted with olive oil as well as the extracts produced with an isopropanol and supercritical CO2 extraction method were not mutagenic to bacteria in the Ames assay (Dziwenka *et al.*, 2020).

SCCS comment
The data from this study was not available in any corresponding papers (Dziwenka *et al.*, 2020), so the reliability could not be evaluated.

***In vitro* study #8: Gene mutation test in bacteria (Ames test) on CBD extract in oil**

Guidelines/Methods:	OECD TG 471
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA
GLP:	Yes
Replicates:	triplicates
Test substance:	CBD extract (26% cannabinoids, of which 96% CBD) in oil (supercritical CO2 extract of the aerial parts of hemp (C. sativa))
Batch (Purity):	Edible fatty acids comprise 61% of this concentrated extract, while

	phytocannabinoids are present at 26% (of this, approximately 96% is CBD and less than 1% is THC); the remaining 13% include fatty alkanes, plant sterols, triterpenes, and tocopherols
Vehicle:	DMSO
Concentrations:	5, 16, 50, 160, 500,1600, and 5000 g/plate
Positive controls:	-S9: 4-Nitro-1,2-phenylenediamine (NPD), (4 g/plate) was used for TA98, sodium azide (SAZ) (2g/plate) for TA100 and TA1535, 9-aminoacridine (9-AA) (50 g/plate) for TA1537, and methyl methanesulfonate (MMS) (2 g/plate) for WP2 +S9 2-aminoanthracene (2-AA) (2 g/plate and 50 g/plate for all S. typhimurium strains and the E. coli WP2uvrA strain, resp.).
Negative control:	DMSO
Date of study plan:	-
Study period:	-
Reference:	Marx TK, Reddeman R, Clewell AE, Endres JR, Béres E, Vértesi A, Glávits R, Hirka G, Szakonyiné IP. An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp. J Toxicol. 2018 Jun 7;2018:8143582. doi: 10.1155/2018/8143582.

No substantial increases in revertant colony numbers were observed in any of the five tester strains following treatment with the test article in the presence or absence of metabolic activation (S9) at any concentration level (see Tables 1 and 2). Sporadic increases in revertant colony numbers compared to vehicle control were observed in both experiments, reflecting the biological variability of the applied test system; however, there was no tendency of dose related increases and mutation rates remained within the historical control data range.

Ref: Marx TK, Reddeman R, Clewell AE, Endres JR, Béres E, Vértesi A, Glávits R, Hirka G, Szakonyiné IP. An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp. J Toxicol. 2018 Jun 7;2018:8143582. doi: 10.1155/2018/8143582.

From the Repondent:

Literature data
The mutagenic potential of the test article was evaluated in a bacterial reverse mutation test using Salmonella typhimurium (TA98, TA100, TA1535, and TA1537) and Escherichia coli WP2uvrA in the presence and absence of activated rat liver S9. The test article was a supercritical CO2 extract of the aerial parts of hemp (C. sativa). Edible fatty acids comprise 61% of this concentrated extract, while phytocannabinoids are present at 26% (of this, approximately 96% is CBD and less than 1% is THC); the remaining 13% include fatty alkanes, plant sterols, triterpenes, and tocopherols. (…)

The study was performed according to OECD Guideline No. 471 (1997), Environmental Protection Agency (EPA) Guideline Ofce of Prevention, Pesticides and Toxic Substances (OPPTS) 870.5100 (1998), European Commission (EC) No. 440/2008, and International Conference on Harmonisation (ICH) Guidance S2(R1) (2012). (…)

No substantial increases in revertant numbers were observed in any of the five tester strains following treatment with the test article in the presence or absence of metabolic activation (S9) at any concentration level. Sporadic increases in revertant colony numbers compared to vehicle control were observed in both experiments, refecting the biological variability of the applied test system; however, there was no tendency of dose related increases and mutation rates remained within the historical control data range (Marx *et al.*, 2018).

SCCS comment
The study was considered valid and did not indicate any concerns about the mutagenic effects of the tested CBD supercritical Fluid Extract of the Aerial Parts of Hemp.

In vitro study #9: Gene mutation test in bacteria (Ames test) on CBD isolate

Guideline	OECD TG 471 (Ames Fluctuation Test, MOLTOX® FT™ 471)
Species/strain	Salmonella typhimurium TA98, TA100, TA102
Replicates	Triplicates; assay repeated twice
Test substance	CBD isolate (CBD), purified from hemp seeds (purity ≥ 98%), was purchased from Mile High Labs (Lot: IL2004R007B; Loveland, CO, USA) For the in vitro assays, CBD dissolved in ethanol (Sigma-Aldrich, Steinheim, Germany) at a concentration of 60% (v/v).
Solvent	ethanol at a concentration of 60% (v/v).
Batch	IL2004R007B
Purity	≥98%
Concentrations	25, 50, 75, 100, 125 μM with and without S9 metabolic activation
GLP	-
Study period	Not dated in paper

For the assays performed, cannabidiol (CBD) and cannabigerol (CBG) were used. A CBD isolate (CBD), purified from hemp seeds (purity ≥ 98%), was purchased from MileHigh Labs (Lot: IL2004R007B; Loveland, CO, USA), and a CBG isolate (purity ≥ 98%) was obtained via fermentation by Amyris (Lot: 9194; Emeryville, CA, USA). For the in vitro assays, CBD and CBG were dissolved in ethanol (Sigma-Aldrich, Steinheim, Germany) at a concentration of 60% (v/v).

The Ames MOLTOX® FT™ 471 test was performed in accordance with the manufacturer's instructions and with OECD guideline Test No. 471, Bacterial Reverse Mutation Test [54]. In brief, the ability of cannabinoids to induce reversion of histidine-requiring Salmonella typhimurium strains TA98, TA100 and TA102 was evaluated. There was not a significant increase in the number of revertants.

Ref: Luz-Veiga M, Mendes A, Tavares-Valente D, Amorim M, Conde A, Pintado ME, Moreira HR, Azevedo-Silva J, Fernandes J. Exploring Cannabidiol (CBD) and Cannabigerol (CBG) Safety Profile and Skincare Potential. Int J Mol Sci. 2024 Nov 14;25(22):12224. doi: 10.3390/ijms252212224.

SCCS comment
The study was negative with some limitations: only 3 of the 5 strains recommended in OECD TG were included and only results on fold-increase increase were presented.

In vitro study #10: Gene mutation test in bacteria (Ames test) on CBD isolate

Guideline	OECD TG 471 (plate incorporation method)
Species/strain	Salmonella typhimurium TA97a, TA98, TA100, TA102, TA1535
Replicates	3 plates per concentration, per strain
Test substance	CBD isolate (CBD CP, crystalline powder, 99.4% CBD, obtained by distillation/crystallization, PharmaHemp d.o.o., Slovenia)
Solvent	Ethanol (3.7% v/v as solvent control)
Batch	Not specified (from PharmaHemp d.o.o.)
Purity	99.4%
Concentrations	0.0005 – 5 mg/plate, with and without 10% rat S9
Positive controls	4-nitroquinoline-N-oxide (NQO; 0.25 µg per plate) for TA97a and TA98; mitomycin C (0.05 µg/plate) for TA102; and sodium azide (NaN3; 0.25 and 0.125 µg/plate, respectively) for TA100 and TA1535. Benzo[a] pyrene (BaP) served as the positive control for S9-dependant mutagenicity for TA97a at 5 µg/plate and 2.5 µg/plate for TA98, TA100, TA102 and TA1535
GLP	Yes
Study period	Not explicitly dated

CBD did not induce mutations in *S. Typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535, under the test conditions applied in this study.

Both CBD samples were toxic for the strain TA102 at concentrations ≥ 0.05 (CBD CP) and 0.0158 mg/plate (CBD EX) without external (S9) metabolic activation. CBD EX was toxic also in the test with S9, at concentrations ≥ 1.58 mg/plate for strain TA102 and strain 1535, both with and without S9, at the highest tested concentration (5 mg/plate).

Ref: Štern A, Novak M, Kološa K, Trontelj J, Žabkar S, Šentjurc T, Filipič M, Žegura B. Exploring the safety of cannabidiol (CBD): A comprehensive in vitro evaluation of the genotoxic and mutagenic potential of a CBD isolate and extract from Cannabis sativa L. Biomed Pharmacother. 2024 Aug;177:116969. doi: 10.1016/j.biopha.2024.116969.

SCCS comment
The study was negative and is valid.

In vitro study #11: Gene mutation test in bacteria (Ames test) on CBD extract

Guideline	OECD TG 471 (plate incorporation method)
Species/strain	Salmonella typhimurium TA97a, TA98, TA100, TA102, TA1535
Replicates	3 plates per concentration, per strain
Test substance	high-content CBD cannabis extract (CBD EX, prepared by extraction using supercritical CO2, cannabinoid concentration and removal of THC using preparative chromatography), with a CBD content of 63.6 %, respectively.
Solvent	Ethanol (3.7% v/v as solvent control)
Batch	Not specified (from PharmaHemp d.o.o.)
Purity	63.6% CBD content (contained also other cannabinoids including CBDV (12.78 %), THCV (1.677 %), CBG (1.077 %), and others in small amounts)
Concentrations	0.0005 – 5 mg/plate, with and without 10% rat S9
Positive controls	4-nitroquinoline-N-oxide (NQO; 0.25 µg per plate) for TA97a and TA98; mitomycin C (0.05 µg/plate) for TA102; and sodium azide (NaN3; 0.25 and 0.125 µg/plate, respectively) for TA100 and TA1535. Benzo[a] pyrene (BaP) served as the positive control for S9-dependant mutagenicity for TA97a at 5 µg/plate and 2.5 µg/plate for TA98, TA100, TA102 and TA1535

GLP	Yes
Study period	Not explicitly dated

CBD EX did not induce mutations in *S. Typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535, under the test conditions applied in this study

Both CBD samples were toxic for the strain TA102 at concentrations ≥ 0.05 (CBD CP) and 0.0158 mg/plate (CBD EX) without external (S9) metabolic activation. CBD EX was toxic also in the test with S9, at concentrations ≥ 1.58 mg/plate for strain TA102 and strain 1535, both with and without S9, at the highest tested concentration (5 mg/plate).

Ref: Štern A, Novak M, Kološa K, Trontelj J, Žabkar S, Šentjurc T, Filipič M, Žegura B. Exploring the safety of cannabidiol (CBD): A comprehensive in vitro evaluation of the genotoxic and mutagenic potential of a CBD isolate and extract from Cannabis sativa L. Biomed Pharmacother. 2024 Aug;177:116969. doi: 10.1016/j.biopha.2024.116969.

SCCS comments
The study was negative and is valid.

SCCS comments on gene mutation:
The summary of analysis of all reports and publications available to the SCCS on gene mutation testing in the Ames tests shows that CBD was tested in the form of different products in which its content ranged from 6.27% to 100% (97-102%). Among the test substances, CBD was used in a pure form. Although the SCCS does not assess formulations, in the case of CBD, all products were considered. The analysis of all the data consistently showed no mutagenic potential of the pure CBD or CBD-containing products. Hence, the SCCS is of the opinion that CBD does not induce gene mutations in bacteria.

In vitro study #12: *In vitro* micronucleus test on CBD Isolate Powder 99%+

Guideline:	OECD TG 487
GLP:	Yes
Test system:	Mouse lymphoma L5178Y TK+/- 3.7.2 C cells
Test substance:	CBD Isolate Powder 99%+, Cannabidiol, 2-(6-Isopropenyl-3-methyl-cyclohex-2-enyl)-5-pentyl-benzene-1,3-diol
Batch (Purity):	SL-20-ISO-04, Purity: 99%+
Vehicle:	Dimethyl sulfoxide
Assay medium:	RPMI-1640 medium
Concentrations:	2.5, 1.25 and 0.625 µg/mL (3h without metabolic activation) 1.25, 0.625 and 0.3125 µg/mL (3h with metabolic activation and 24h without metabolic activation)
Exposure duration/ S9 use:	3-hour treatment with metabolic activation (in the presence of S9-mix) 3-hour and 24-hour treatment without metabolic activation (in the absence of S9-mix)
Cytochalasin B	Not used
Positive controls:	Without metabolic activation: Mitomycin C 0.5 µg/mL and 1 µg/mL and Colchicine, 0.5 µg/mL With metabolic activation: Cyclophosphamide monohydrate 6.0 µg/mL
Negative control:	Dimethyl sulfoxide
Study period:	02 November 2020- 17 December 2020

Results:	Negative
Reference:	Charles-River 2021. A GLP In Vitro Mammalian Cell Micronucleus Test of CBD Isolate Powder 99%+ Test Facility Study Code: 20/195-013C

In Assay 1, Marked cytotoxicity was observed in in the 3-hour treatment without metabolic activation at 5 and 2.5 µg/mL concentrations. The same effect was observed in the 24-hour treatment without metabolic activation at 5, 2.5 and 1.25 µg/mL. In Assay 2, marked cytotoxicity was observed in the 3-hour treatment with metabolic activation at 5, 2.5 and 1.25 µg/mL concentrations (cytotoxicity value was excessive in the first case, and 23% and 46%, respectively).

None of the treatment concentrations caused a biologically or statistically significant increase in the number of micronucleated cells when compared to the appropriate negative (vehicle) control value in the experiments with and without metabolic activation.

Ref: Charles-River 2021. A GLP In Vitro Mammalian Cell Micronucleus Test of CBD Isolate Powder 99%+ Test Facility Study Code: 20/195-013C

SCCS conclusion
This study was considered valid and did not indicate any concerns about clastogenic/aneugenic effects resulting from the use of CBD Isolate Powder.

***In vitro* study 13: *In Vitro* Mammalian Cell Micronucleus Test of Broad Spectrum Hemp Extract 85%.**

Guideline:	OECD TG 487
GLP:	Yes
Test system:	mouse lymphoma L5178Y TK+/- 3.7.2 C cells
Test substance:	Broad Spectrum Hemp Extract 85% - Cannabidiol, 2-(6-Isopropenyl-3-methyl-cyclohex-2-enyl)-5-pentyl-benzene-1,3-diol
Batch (Purity):	SL-20-BSE-01, Purity: 85% (15% of other cannabinoids and terpenes, the supplied material does not contain vehicle or solvent)
Vehicle:	Dimethyl sulfoxide
Assay medium:	RPMI-1640 medium
Concentrations:	3-hour treatment: 30, 20, 15, 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 µg/mL (with and without metabolic activation) 24-hour treatment: 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.07813, 0.03906, 0.01953 and 0.00977 µg/mL (without metabolic activation)
Exposure duration/ S9 use:	3-hour treatment with metabolic activation (in the presence of S9-mix) 3-hour and 24-hour treatment without metabolic activation (in the absence of S9-mix)
Cytochalasin B	Not used
Positive controls:	Without metabolic activation: Mitomycin C 0.5 µg/mL and 1 µg/mL and Colchicine, 0.5 µg/mL With metabolic activation: Cyclophosphamide monohydrate 6.0 µg/mL
Negative control:	Dimethyl sulfoxide
Study period:	26 October 2020- 18 November 2020
Results:	Negative

Reference:	Charles-River 2021. A GLP In Vitro Mammalian Cell Micronucleus Test of Broad Spectrum Hemp Extract 85%. Test Facility Study Code: 20/194-013C. 08 January 2021

In order to investigate a possible potential of Full Spectrum Extract (REGULAR) (Chemical name: Hemp Extract) to induce micronuclei in human lymphocytes an *in vitro* micronucleus assay was carried out.

Marked cytotoxicity was observed in the 3-hour treatment with metabolic activation at 30, 20 and 15 µg/mL concentrations (cytotoxicity values were 8%, 50% and 55%, respectively, and in the 3-hour treatment without metabolic activation at 30, 20, 15, 10, 5, 2.5 and 1.25 µg/mL concentrations. The same effect was observed in the 24-hour treatment at 5 and 2.5 µg/mL concentrations (cytotoxicity values were 18% and 39%, respectively).

Therefore, concentrations of 20, 10 and 5 µg/mL (a total of three) were chosen for evaluation in case of the short treatment with metabolic activation, concentrations of 2.5, 1.25 and 0.625 µg/mL (a total of three) were chosen for evaluation in case of the short treatment without metabolic activation and concentrations of 2.5, 1.25 and 0.625 µg/mL (a total of three) were chosen for evaluation in case of the long treatment without metabolic activation.

None of the treatment concentrations caused a biologically or statistically significant increase in the number of micronucleated cells when compared to the appropriate negative (vehicle) control value in the experiments with and without metabolic activation.

In conclusion, it can be stated that during the study described and under the experimental conditions reported, the test item Full Spectrum Extract (REGULAR) did not induce structural and/or numerical chromosomal damage in human lymphocytes.

Ref: Charles-River 2021. A GLP In Vitro Mammalian Cell Micronucleus Test of Broad-Spectrum Hemp Extract 85%. Test Facility Study Code: 20/194-013C. 08 January 2021

SCCS comment
The study was considered valid and did not indicate any concerns about clastogenic/aneugenic effects resulting from the use of Broad-Spectrum Hemp Extract 85%.

***In vitro* study #14: *In Vitro* Mammalian Cell Micronucleus Test of Hemp-derived CBD isolate**

Guideline:	OECD TG 487
GLP:	Yes
Test system:	Human lymphoblast TK6 cells
Test substance:	Hemp-derived CBD isolate (CAS No. 13956-29-1)
Batch (Purity):	99.62% CBD
Vehicle:	Dimethyl sulfoxide
Assay medium:	-
Concentrations:	0.100, 0.250, 0.500, 1.00, 2.00, 4.00, 6.00. 8.00, 8.00. 9.00, 10.0, 11.0 µg/mL, due to cytotoxicity observed in the dose range-finding assay with 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500, 1000, or 2000 µg/mL.
Exposure duration/ S9 use:	4-hour treatment with metabolic activation (in the presence of S9-mix) 4-hour and 27-hour treatment without metabolic activation (in the absence of S9-mix)
Cytochalasin B	Not used Flow cytometry analysis

Positive controls:	Without metabolic activation: vinblastine sulfate (VIN; Sigma-Aldrich, target dose levels 0.003 and 0.0025 µg/mL) for the 27-h treatments and mitomycin C (MMC, Sigma-Aldrich, target dose levels 0.125 and 0.0625 µg/mL) for the 4-h treatments With metabolic activation: cyclophosphamide monohydrate (11.9 and 4.7 µg/mL) for the 4-h treatments
Negative control:	Dimethyl sulfoxide
Study period:	-
Results:	Negative
Reference:	Henderson et al., 2023 https://doi.org/10.1016/j.yrtph.2023.105425

Excessive cytotoxicity was observed at ≥ 8 µg/mL in the 27-h treatment without metabolic activation; at ≥ 12 µg/mL in the 4-h treatment without metabolic activation; and at ≥ 11 µg/mL in the 4-h treatment with metabolic activation. The vehicle and positive control data were comparable to the relevant historical control values.

The in vitro micronucleus assay was negative in human TK6 cells up to 10–11 µg/mL, with and without metabolic activation.

Ref: Henderson *et al.*, 2023 https://doi.org/10.1016/j.yrtph.2023.105425

From the Respondent 2
During a toxicological evaluation for the use of CBD as a novel food, in addition to literature, it was considered necessary to perform a Genotoxicity basic test battery on the intended products CBD as substance as requested by the EFSA Guidance on Novel Food (2012).
The test item is natural CBD, not synthetic one. However, the purity of the substance (above 98.0 % as HPLC assay) allow the read across.
(…)

On the *in vitro* Mammalian Cell Micronucleus test (OECD 487) it can be stated that during the study described and under the experimental conditions reported, the test item CBD Natural Hemp Isolate did not induce structural and/or numerical chromosomal damage in human lymphocytes.

SCCS comment
The study was considered valid and did not indicate any concerns about the clastogenicity/aneugenicity of Hemp-derived CBD isolate.

In vitro study #15: *In Vitro* Mammalian Chromosomal Aberration Test of CBD extract in oil

Guideline:	OECD TG 473
GLP:	Yes
Test system:	V79 Chinese hamster lung cells
Test substance:	CBD extract (26% cannabinoids, of which 96% CBD) in oil (supercritical CO2 extract of the aerial parts of hemp (C. sativa).)
Batch (Purity):	Edible fatty acids comprise 61% of this concentrated extract, while phytocannabinoids are present at 26% (of this, approximately 96% is CBD and less than 1% is THC); the remaining 13% include fatty alkanes, plant sterols, triterpenes, and tocopherols
Vehicle:	DMSO
Assay medium:	-

Concentrations:	3-hour exposure + S9: 50, 70, and 90 g/mL -S9: 10, 20, and 30 g/mL 20h exposure -S9: 1.25, 2.5, and 5 g/mL
Exposure duration/ S9 use:	Yes
Positive controls:	ethyl methanesulfonate and cyclophosphamide
Negative control:	DMSO
Study period:	-
Results:	Non clastogenic
Reference:	Marx TK, Reddeman R, Clewell AE, Endres JR, Béres E, Vértesi A, Glávits R, Hirka G, Szakonyiné IP. An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp. J Toxicol. 2018 Jun 7;2018:8143582. doi: 10.1155/2018/8143582.

At least 400 metaphase cells from each experimental group, containing 22 ± 2 centromeres, were evaluated for structural aberrations (slides were coded and scored blind).

In the negative control group, the percentage of cells with structural aberrations was equal to or less than 5%, confirming the suitability of the V79 cell line used. The concurrent positive controls caused the expected biologically relevant increases of cells with structural chromosome aberrations as compared to current solvent and historical controls.

The test article did not induce an increase in the number of cells with aberrations or rates of polyploidy or endoreduplicated metaphases at concentrations ranging from 10 to 90 g/mL. There were no statistically significant differences between treatment and the solvent control groups, and no dose-response relationships were noted.

Ref: Marx TK, Reddeman R, Clewell AE, Endres JR, Béres E, Vértesi A, Glávits R, Hirka G, Szakonyiné IP. An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp. J Toxicol. 2018 Jun 7;2018:8143582. doi: 10.1155/2018/8143582.

From the Respondent 2

An *in vitro* mammalian Chromosomal Aberration test was conducted by Marx *et al.* (2018) to determine whether the test article could induce structural chromosomal aberration in V79 Chinese hamster lung cell, following OECD 473. *"The test article did not induce an increase in the number of cells with aberrations or rates of polyploidy or endoreduplicated metaphases at concentrations ranging from 10 to 90 µg/mL. There were no statistically significant differences between treatment and the solvent control groups, and no dose-response relationships were noted."*

SCCS comment

The study was considered valid and did not show evidence of induction of chromosomal aberrations by CBD extract in oil.

***In vitro* study #16: *In Vitro* Mammalian Cell Micronucleus Test of CBD isolate with lipid carrier**

The proprietary CBD test substance comprised a high purity. The CBD isolate was diluted to a concentration of 31–33% using MCT oil supplied by cbdMD Inc (Charlotte, NC, USA). The CBD isolate was a whole plant ethanol extract of *Cannabis sativa* L. (Cannabaceae) and CBD, accounts

for >97% of the total cannabinoids present (Batch No. 103501B, cbdMD Inc (Charlotte, NC, USA). Additional cannabinoids present in the isolate included cannabigerol (1.71%), cannabinol (0.47%), and cannabidivarin (0.22%).

Guideline:	OECD TG 487
GLP:	-
Test system:	human lymphoblastoid cells (TK6 Cells
Test substance:	CBD isolate with its lipid carrier [medium chain triglyceride (MCT)]
Batch (Purity):	30% CBD in MCT oil
Vehicle:	Dimethyl sulfoxide
Assay medium:	RPMI-1640 medium
Concentrations:	60, 40, 20, 15, 10, 8, 6, 4, 3, 2, 1, and 0.5 μg/mL for 4 h with metabolic activation (+S9) and 30, 25, 20, 15, 10, 8, 6, 4, 3, 2, 1, and 0.5 μg/mL for 4 h and 24 h without metabolic activation (−S9).
Exposure duration/ S9 use:	4-hour treatment with metabolic activation (in the presence of S9-mix) 4-hour and 24-hour treatment without metabolic activation (in the absence of S9-mix)
Cytochalasin B	Not used flow cytometry analysis
Positive controls:	Vinblastine sulfate (3.75 and 0.75 ng/mL) for the 4- and 24-h treatment without metabolic activation, and Cyclophosphamide monohydrate (3ug/mL) for 4 h with metabolic activation
Negative control:	Dimethyl sulfoxide
Study period:	-
Results:	Negative
Reference:	Tallon MJ, Child RB, Blum JL. Genotoxic assessment of a Cannabis sativa L. extract. Pharm Biol. 2025 May 6;63(1):357–363. Doi: 10.1080/13880209.2025.2499075

Excessive cytotoxicity was observed at 25 and 30 μg/mL in the 4- and 9-h treatment without metabolic activation, therefore 15 μg/mL was considered the highest analyzable concentration for this condition. The lowest test concentration to induce the OECD recommended range was 15 μg/ml and this was considered as the highest analyzable concentration. In the 4 h with S9, CBD test substance exposures at 60 μg/mL exceeded OECD cytotoxicity guidance, so 40 μg/mL was considered to be the highest analyzable concentration for this condition.

Table 2. CBD test substance *in vitro* human lymphocyte micronucleus assay (OECD 487).

Treatment group	4-h Without metabolic activation (−S9 mix)		4-h With metabolic activation (+S9 mix)		24-h Without metabolic activation (−S9 mix)	
	Cytotoxicity (%)	Mean MN (%)	Cytotoxicity (%)	Mean MN (%)	Cytotoxicity (%)	Mean MN (%)
DMSO	7.1	1.29	3.8	1.04	5.2	1.06
CBD test substance (µg/mL)						
0.5	4.4	0.82†	3.9	0.91†	7.4	0.39†
1	5.6	0.97†	3.9	1.23†	7.4	0.77†
2	6.1	1.16†	3.9	1.17†	7.3	0.51†
3	5.4	1.10†	4.3	1.16†	7.7	0.67†
4	6.9	0.67†	4.2	0.84†	8.3	0.37†
6	6.4	1.09†	4.3	1.41†	8.8	0.50†
8	7.9	0.81†	4.8	0.85†	10.5	0.90†
10	9.1	0.70†	6.3	0.73†	13.1	0.73†
15	14.4	0.79†	7.3	0.85†	18.6	1.22†
20	14.9	0.69	9.4	0.69†	20.2	1.43
25	20.4	0.95	–	–	35.4	4.19
30	20.5	0.66	–	–	48.2	7.80
40	–	–	23.1	2.10‡	–	–
60	–	–	38.5	1.08	–	–
VIN (3.75 ng/mL)	24.8	14.91	–	–	–	–
VIN (0.75 ng/mL)	–	–	–	–	22.5	22.78*
CP (3.0 µg/mL)	–	–	11.7	5.75*	–	–

Cytotoxicity (%): apoptotic/necrotic cells; MN: micronucleated cells; DMSO: dimethyl sulfoxide; CP: cyclophosphamide monohydrate; VIN: vinblastine sulfate.
*Significant at $p < 0.05$ (Student's *t*-test).
†Not significant (ANOVA/Dunnett's for comparison against vehicle control and linear regression modelling for trend test, significance at $p < 0.025$).
‡Significant (ANOVA/Dunnett's for comparison against vehicle control and linear regression modelling for trend test, significance at $p < 0.025$.

Following treatment with CBD isolate, no statistically significant increases in the incidences of micronucleated cells were observed at any dose level compared to current negative control values. In addition, there was no dose–response relationship, and all incidences of micronucleated cells were within historical controls, with the exception of 40 µg/mL for 4 h + S9 (Table 2). The 40 µg/mL CBD exposure at (4 h with S9) was statistically significant compared to the concurrent control; however, this was below the OECD cytotoxicity limit.

Under the test conditions, there was no indication of clastogenicity or aneugenicity as measured by micronucleus induction following exposure to human lymphocytes for 4 h with or without S9 or 24 h in the absence of S9.

Ref: Tallon MJ, Child RB, Blum JL. Genotoxic assessment of a Cannabis sativa L. extract. Pharm Biol. 2025 May 6;63(1):357–363. doi: 10.1080/13880209.2025.2499075

SCCS comment
Although the authors claim excessive cytotoxicity at the highest concentrations, cytotoxicity exceeding OECD guidance of 55 ± 5% is not shown in the table of the results and therefore the results cannot be considered valid. In the 24-h study without metabolic activation (−S9 mix), the concentration of 30 µg/mL induced 48.2% cytotoxicity (acceptable) and a 7-fold MN increase, which is not regarded as statistically significant.

In vitro study #17: *In Vitro* Mammalian Cell Micronucleus Test of CBD isolate

Guideline	OECD TG 487
Test system	TK6 cells
Replicates	Three independent experiments; three replicates each condition
Test substance	CBD isolate (CAS 13956-29-1 Mile High Labs)
Batch	IL2004R007B, ≥98%,
Purity	≥98%
Vehicle	Unclear
Concentrations	50, 25 or 2.5 µM,
Exposure duration/ S9 use	24h/No S9 used
Cytochalasin B	No
Positive controls	MMC
GLP	not explicitly stated
Study period	-

To assess the impact of cannabinoids on chromosome structure, micronucleus analysis was performed using the MicroFlow® kit (BD Biosciences, Franklin Lakes, NJ, USA), according to the manufacturer's instructions and in accordance with OECD guideline Test No. 487: In Vitro Mammalian Cell Micronucleus Test.

The micronuclei assay results for both CBD and CBG indicate that neither of thesecannabinoids induced a significant development of micronuclei in the tested conditions. The use of Mitomycin C as a positive control, which led to a significant increase in micronuclei formation, confirms the sensitivity of the assay. Therefore, CBD and CBG appear to be safe in terms of mutagenicity potential under the conditions tested.

Ref: Luz-Veiga M, Mendes A, Tavares-Valente D, Amorim M, Conde A, Pintado ME, Moreira HR, Azevedo-Silva J, Fernandes J. Exploring Cannabidiol (CBD) and Cannabigerol (CBG) Safety Profile and Skincare Potential. Int J Mol Sci. 2024 Nov 14;25(22):12224. doi: 10.3390/ijms252212224.

SCCS comment
The study was negative with some limitations regarding OECD compliance: no use of S9, results shown only graphically, no short exposure, no data on number of cells scored.

In vitro study #18: In Vitro Mammalian Cell Micronucleus Test of CBD

Guideline	OECD TG 487
Test	Human lymphoblastoid TK6 cells
Replicates	≥3 independent experiments; 1000 cells scored per condition
Test substance	CBD (CAS 13956-29-1, 98.7% purity, LGC Standards)
Solvent	DMSO (control), culture medium (RPMI-1640 with supplements)
Batch	Not specified

Purity	98.3–99.6% (see above)
Concentrations	CBD: 5, 10, 15, 20 and 25 µM
Exposure/S9	4h exposure with and without 0.25% Mutazyme™ S9
Cytochalasin B	3 µg/mL
GLP	Not explicitly stated
Study period	-

The tested cannabinoids CBD yielded a significant induction of micronuclei in TK6 cells with at least one concentration after 4 h of treatment. The addition of the metabolic enzymes caused a significant reduction in the number of micronuclei for CBD.

Using anti-centromere antibody staining, micronuclei were analyzed for the presence of kinetochores. the percentage of kinetochore positive induced micronuclei was 63.7% for CBD.

CBD caused a mild but significant rise of micronuclei in cells after 4 h of treatment without metabolic activation. The addition of metabolic enzymes to the human lymphoblastoid TK6 cells with cannabinoid treatment led to a detoxification of the cannabinoids. Kinetochore staining revealed that the vast majority of the induced micronuclei were positive, meaning cannabinoids predominately are likely to induce the segregation of whole chromosomes into micronuclei, further supporting mitotic disturbance as a major driver in cannabinoid-induced micronucleus formation. The effects were seen at micromolar concentrations of cannabinoids, which are higher than concentrations found in consumers' blood.

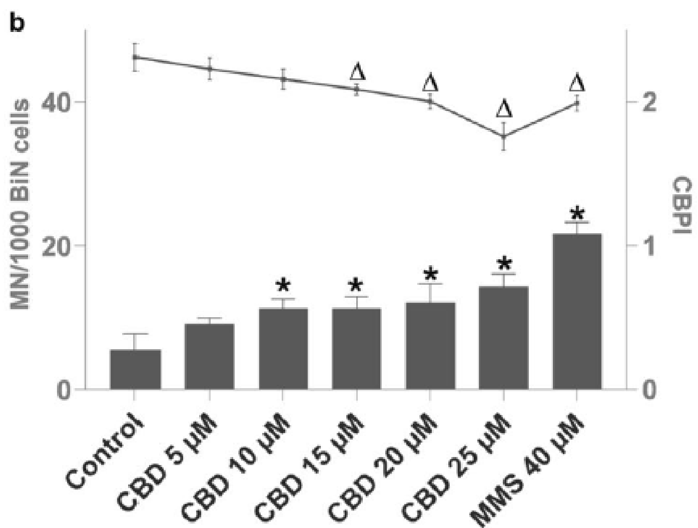

Fig. 4 Micronucleus induction (columns) and proliferation index (CBPI; line) in TK6 cells after 4 h of treatment with cannabidiol (CBD) (b)

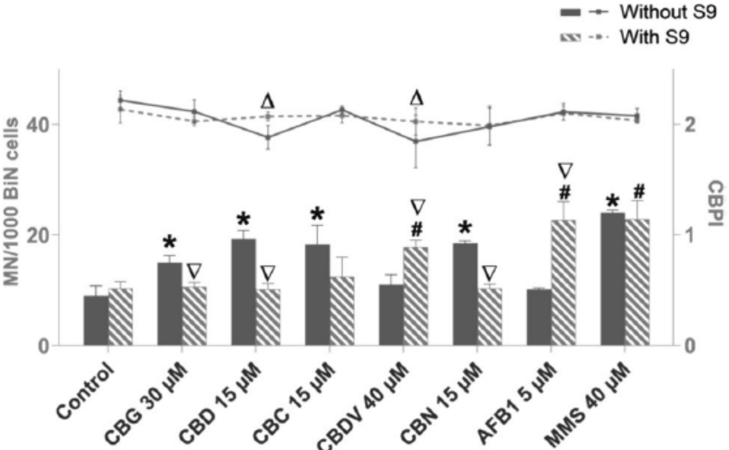

Fig. 5 Micronucleus induction (columns) and proliferation index (CBPI; line) in TK6 cells after 4 h of treatment with cannabigerol (CBG), cannabidiol (CBD), cannabichromene (CBC), cannabidivarin (CBDV), and cannabinol (CBN) and presence or absence of metabolic activation. The positive controls were methyl methane sulfonate (MMS) and aflatoxin B1 (AFB1); the control was solvent DMSO. *$p<0.05$ vs. corresponding control for MN/1000 BiN without metabolic activation, #$p<0.05$ vs. corresponding control for MN/1000 BiN with metabolic activation. Δ$p<0.05$ vs. corresponding control for CBPI without metabolic activation., ∇$p<0.05$ vs. respective dose without metabolic activation for MN/1000 BiN cells. *MN* micronucleus, *BiN* binucleated cells, *CBPI* cytokinesis-block proliferation index. Results are displayed as mean ± standard deviation (SD) from three independent experiments

Table 2 Kinetochore positive micronuclei (MNi) in human lymphoblastoid TK6 cells treated with cannabigerol (CBG) 30 µM, cannabidiol (CBD) 15 µM, cannabichromene (CBC) 15 µM, cannabidivarin (CBDV) 40 µM, and cannabinol (CBN) 15 µM for 4 h

	MNi	Kinetochore (+) MNi		Kinetochore (+) induced MNi[a]
	N	*N*	%	%
Control	200	62	31	0
CBG 30 µM	200	87	43.5	61.5
CBD 15 µM	200	94	47	63.7
CBC 15 µM	200	97	48.5	69.7
CBDV 40 µM	200	82	41	69.8
CBN 15 µM	200	92	46	63.3
MMS 40 µM	200	76	38	41.9
Vincristine 0.0121 µM	200	140	70	[b]

The control was solvent DMSO, and the positive controls were methyl methane sulfonate (MMS) and vincristine. The data are presented as number of counted micronuclei, number and percentage of kinetochore positive micronuclei and percentage of kinetochore positive induced micronuclei.

[a] Kinetochore (+) induced MNi= treatment – control

[b] Vincristine could not be evaluated for the percentage of induced micronuclei because the binucleated cells could not be identified clearly.

Ref: Kolar N, Bankoglu EE, Stopper H. Genotoxicity of selected cannabinoids in human lymphoblastoid TK6 cells. Arch Toxicol. 2024 Oct;98(10):3439-3451. doi: 10.1007/s00204-024-03826-y.

SCCS comment
The study was positive, however, the effects seem to be reduced in the presence of metabolic activation.

In vitro **study #19:** ***In Vitro*** **Mammalian Cell Micronucleus Test of CBD extract**

Guideline:	-
GLP:	Yes
Test system:	Human HepG2 cells
Test substance:	high-content CBD cannabis extract (CBD EX, prepared by extraction using supercritical CO_2, cannabinoid concentration and removal of THC using preparative chromatography), with a CBD content of 63.6 %, respectively.
Batch (Purity):	Not specified; supplier PharmaHemp d.o.o 63.6% CBD

Vehicle:	0.2 % EtOH
Assay medium:	MEM supplemented with 10 % FBS
Concentrations:	0.5, 1, and 5 µg/mL
Exposure duration/ S9 use:	24-hour exposures
Positive controls:	BaP, 30 µg/ml
Negative control:	cell culture medium and solvent (0.2 % EtOH)
Study period:	-
Notes	Comet assay and γH2AX and p-H3 assays also used

No significant increase in MNi, NBPs or NBUDs was observed after 24-h exposure to CBD CP and CBD EX at concentrations up to 5 µg/ml (or 15.9 µM of CBD in the CBD CP sample) (Fig. 7). Also, no impact on the nuclear division index (NDI) was observed, confirming that the tested concentrations were not cytotoxic and did not affect cell division.

In the comet assay, no significant increase in DNA damage, nor phosphorylation of the histone H2AX or H3 histones was observed in HepG2 cells after 4 and 24 h of exposure to CBD CP and CBD EX at concentrations of up to 5 µg/ml.

Ref: Štern A, Novak M, Kološa K, Trontelj J, Žabkar S, Šentjurc T, Filipič M, Žegura B. Exploring the safety of cannabidiol (CBD): A comprehensive in vitro evaluation of the genotoxic and mutagenic potential of a CBD isolate and extract from Cannabis sativa L. Biomed Pharmacother. 2024 Aug;177:116969. doi: 10.1016/j.biopha.2024.116969.

SCCS comment
The study is negative, with the following limitations: no S9 used and no short exposure. Additional endpoints (DNA damage, DSB, histone phosphorylation) were also negative.

In vitro **study #20:** ***In Vitro*** **Mammalian Cell Micronucleus Test of CBD isolate**

Guideline:	.
GLP:	Yes
Test system:	Human HepG2 cells
Test substance:	CBD isolate (CBD CP, crystalline powder, 99.4% CBD, obtained by distillation/crystallization, PharmaHemp d.o.o., Slovenia)
Batch (Purity):	Not specified; supplier PharmaHemp d.o.o 99.4%;
Vehicle:	0.2 % EtOH
Assay medium:	MEM supplemented with 10 % FBS
Concentrations:	0.5, 1, and 5 µg/mL
Exposure duration/ S9 use:	24-hour exposures
Positive controls:	BaP, 30 µg/ml
Negative control:	cell culture medium and solvent (0.2 % EtOH)
Study period:	-
Notes	Comet assay and γH2AX and p-H3 assays also used

No significant increase in MNi, NBPs or NBUDs was observed after 24-h exposure to CBD CP and CBD EX at concentrations up to 5 µg/ml (or 15.9 µM of CBD in the CBD CP sample) (Fig. 7).

Also, no impact on the nuclear division index (NDI) was observed, confirming that the tested concentrations were not cytotoxic and did not affect cell division.

Ref: Štern A, Novak M, Kološa K, Trontelj J, Žabkar S, Šentjurc T, Filipič M, Žegura B. Exploring the safety of cannabidiol (CBD): A comprehensive in vitro evaluation of the genotoxic and mutagenic potential of a CBD isolate and extract from Cannabis sativa L. Biomed Pharmacother. 2024 Aug;177:116969. doi: 10.1016/j.biopha.2024.116969.

SCCS comment
The study was negative, with the following limitations: no S9 used and no short exposure. Additional endpoints (DNA damage, DSB, histone phosphorylation) were also negative.

In vitro study #21: *In Vitro* Mammalian Cell Micronucleus Test of CBD

Guideline:	.
GLP:	-
Test system:	human hepatoma cell line (HepG2)
Test substance:	Cannabidiol (CBD, CAS 13956-29-1)
Batch (Purity):	purity 99.95% obtained from LGC Standards GmbH
Vehicle:	medium
Assay medium:	DMEM supplemented with 10 % FCS
Concentrations:	0.07–2 µM
Exposure duration/ S9 use:	3 h (serum-free)/ no S9
Positive controls:	Cyclophosphamide (final concentration 500 µg/mL)
Negative control:	cell culture medium and solvent (0.2 % EtOH)
Study period:	-
Notes	Comet assay also used

Both compounds caused induction of MNi in HepG2 cells at low concentrations (≥ 0.22 µM). Additionally, a significant increase of other nuclear anomalies (Nbuds and NPBs), as well as induction of cell death (necrosis and apoptosis) was observed after treatment with both drugs. *(Data from individual experiments can be found in supplementary tables SI 2A-B.)*
In addition, caused DNA damage (comet assay) in both cell types (HepG2 and human cell line TR146 is derived from buccal epithelial tissue). In the liver-derived cells, significant induction of damage was seen with both compounds at concentrations ≥ 6.0 µM after 3 h (Fig. 2a, b). When the cells were treated for 24 h, clear damage was observed with the lower concentrations (≥ 2.0 µM) (Fig. 2c, d).

Table 1 Impact of the two cannabinoids on MN formation and on the rates of various nuclear aberrations in HepG2 cells

Compounds	Concentrations (µM)	CPBI Mean ± SD	CT %	BN-MN[a] Mean (‰) ± SD	MNi[b] Mean (‰) ± SD	Nbuds Mean (‰) ± SD	NPBs Mean (‰) ± SD	Necrosis Mean (‰) ± SD	Apoptosis Mean (‰) ± SD
Neg. Ctrl	0	2.04 ± 0.03	–	5.25 ± 0.35	5.75 ± 0.35	4.75 ± 0.35	3.50 ± 0.71	6.25 ± 0.35	3.00 ± 0.71
CBD	0.07	2.00 ± 0.08	3.92	6.50 ± 1.41	6.50 ± 1.41	16.00 ± 2.12*	5.25 ± 0.35	16.25 ± 1.77*	13.50 ± 0.71*
	0.22	1.93 ± 0.04	10.60	21.00 ± 1.41*	31.00 ± 2.12*	25.50 ± 2.83*	8.50 ± 1.41*	21.00 ± 0.70*	25.25 ± 3.18*
	0.66	1.83 ± 0.04	20.22	31.25 ± 2.47*	46.25 ± 3.89*	37.25 ± 1.06*	10.00 ± 1.41*	30.75 ± 1.77*	29.00 ± 1.41*
	2.00	1.72 ± 0.01	30.76	39.25 ± 3.89*	53.25 ± 2.47*	43.00 ± 2.83*	14.00 ± 0.71*	33.50 ± 2.12*	37.25 ± 1.77*
SC[c]		1.80 ± 0.00	23.05	5.00 ± 1.41	6.25 ± 0.35	5.50 ± 1.41	3.25 ± 1.06	6.75 ± 1.06	3.00 ± 0.71
CBDV	0.07	1.95 ± 0.05	9.17	6.00 ± 0.71	6.00 ± 0.71	15.25 ± 1.77*	6.00 ± 2.12	15.25 ± 2.47*	13.75 ± 1.77*
	0.22	1.93 ± 0.04	10.60	26.00 ± 2.83*	29.75 ± 1.77*	36.25 ± 3.18*	10.00 ± 0.71*	18.50 ± 1.41*	21.75 ± 1.06*
	0.66	1.79 ± 0.01	24.03	32.00 ± 0.71*	45.50 ± 1.41*	40.00 ± 2.12*	13.25 ± 1.77*	24.5 ± 1.41*	28.75 ± 3.89*
	2.00	1.77 ± 0.03	25.97	41.25 ± 2.47*	51.25 ± 3.89*	45.75 ± 2.47*	16.00 ± 2.12*	34.75 ± 2.47*	30.00 ± 2.83*
SC[c]		1.81 ± 0.02	22.54	5.00 ± 00	5.75 ± 0.35	5.00 ± 0.71	3.25 ± 0.35	6.25 ± 1.06	3.00 ± 0.71
Pos. Ctrl	500 µg/mL	1.80 ± 0.01	23.54	42.25 ± 5.30*	56.75 ± 1.06*	35.50 ± 1.41*	11.75 ± 1.06*	16.25 ± 1.77*	9.25 ± 3.18

CBPI cytokinesis-block proliferation indices, *CT* cytostasis (%), HepG2 cells were treated with different concentrations of the test compounds for 3 h. Numbers represent results (means ± SD) obtained in two independent experiments, and in each experiment, two cultures were made per experimental point. Four slides were prepared and 2000 cells were evaluated. All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls.

BN–MNi binucleated cells with micronuclei, *MNi* micronuclei, *Nbuds* nuclear buds, *NPBs* nucleoplasmatic bridges, *Neg. Ctrl* cells cultivated in medium, *SC* solvent control, *Pos. Ctrl* cyclophosphamide (500 µg/ml)

*Significant differences from solvent control values (Dunnett test, $p \leq 0.05$)

[a]Number of binucleated cells with MN

[b]Total number of MN from binucleated cells

[c]Methanol was used as solvent control [0.06% (v/v) in experiments with CBD and 0.05% (v/v) in experiments with CBDV]

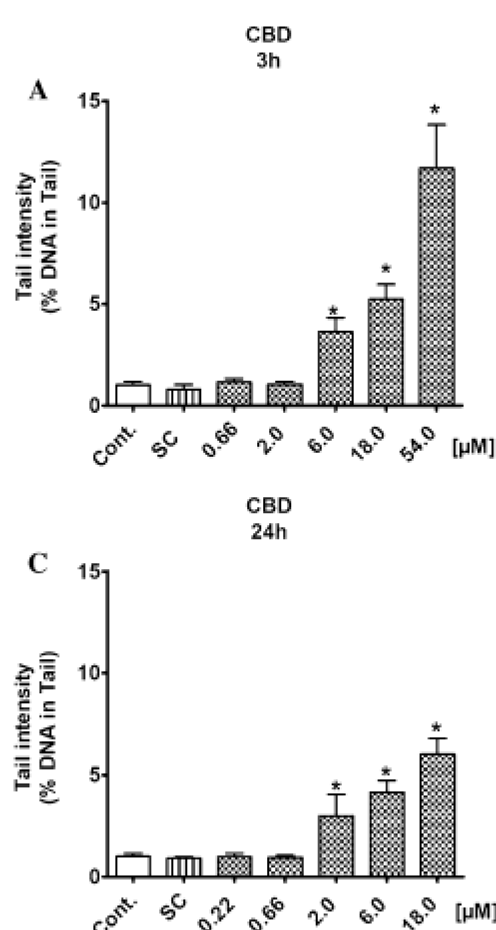

Fig. 2 **a, b** Induction of DNA damage by CBD and CBDV in a human-derived liver cell line (HepG2). The cells were treated with different concentrations of the test compounds for 3 and 24 h. Methanol was used as a solvent control [for 3 h CBD: 1.70% (v/v) and CBDV: 1.55% (v/v); for 24 h CBD: 0.56% (v/v) for CBDV: 0.52% (v/v)]. Hydrogen peroxide (50 µM) was used as a positive control (the cells were treated for 5 min on ice) and induced clear positive effects (26.57 ± 3.64% DNA in tail). Bars indicate means ± SD of results obtained with two parallel cultures per experiment (from each culture two slides were made and 50 cells were evaluated per slide). Stars indicate statistical significance ($p \leq 0.05$, ANOVA). All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls

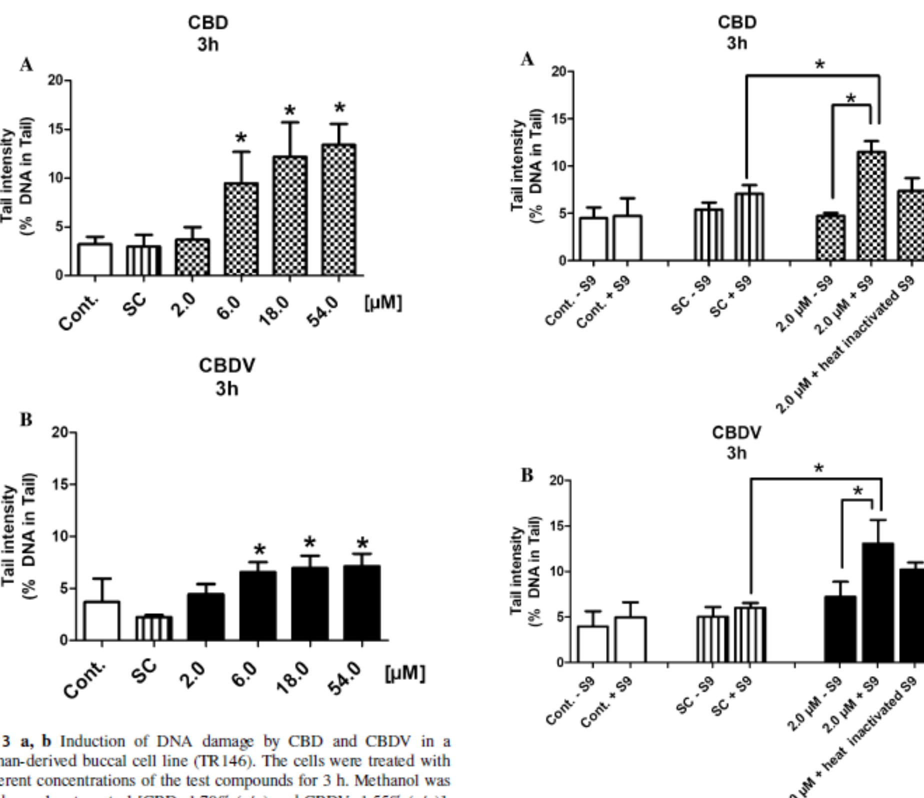

Fig. 3 a, b Induction of DNA damage by CBD and CBDV in a human-derived buccal cell line (TR146). The cells were treated with different concentrations of the test compounds for 3 h. Methanol was used as solvent control [CBD: 1.70% (v/v) and CBDV: 1.55% (v/v)]. Hydrogen peroxide (50 µM) was used as a positive control (the cells were treated for 5 min on ice). The peroxide induced clear positive effects (20.12 ± 1.84% DNA in tail). Bars indicate means ± SD of results obtained with two parallel cultures per experiment (from each culture two slides were made and 50 cells were evaluated per slide). Stars indicate statistical significance ($p \leq 0.05$, ANOVA). All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls

Fig. 4 a, b Impact of liver enzyme homogenate on the DNA-damaging activity of CBD and CBDV in TR146 cells. The cells were treated with 2.0 µM of the cannabinoids and in parallel with liver enzyme homogenate (for details see "Materials and methods"). Bars indicate means ± SD of results obtained with two parallel cultures per experiment (from each culture two slides were made and 50 cells were evaluated per slide). Stars indicate statistical significance ($p \leq 0.05$, Two-tailed paired t test). All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls

Ref: Russo C, Ferk F, Mišík M, Ropek N, Nersesyan A, Mejri D, Holzmann K, Lavorgna M, Isidori M, Knasmüller S. Low doses of widely consumed cannabinoids (cannabidiol and cannabidivarin) cause DNA damage and chromosomal aberrations in human-derived cells. Arch Toxicol. 2019 Jan;93(1):179-188. doi: 10.1007/s00204-018-2322-9.

SCCS comment
The study is valid and provides evidence of the genotoxic effects of CBD in liver cells, as well as DNA damage induction in buccal epithelial cells.

Mutagenicity / genotoxicity in vivo

From EMA, 2019

CBD, purified CBD and CBD as BDS were evaluated in *in vivo* standard genotoxicity assays (chromosomal aberrations and the Comet assay). Only studies performed with purified CBD and CBS-OS are summarised.

Table 3: Overview of genotoxicity studies performed with purified CBD or CBD-OS (EMA, 2019)

Type of test/ study ID/GLP	**Test system**	**Concentration range /metabolising system/dose**	**Results positive negative equivocal**
Chromosomal aberrations in vivo (GWOR0903/GLP)	Rat, micronuclei in bone marrow	125,250,500 mg/kg p.o. CBD-OS	Negative
DNA damage in vivo (GWTX1510/GLP)	Rat, Alkaline comet Assay	125,250,500 mg/kg p.o. purified CBD	Negative

The genotoxic potential of CBD has been evaluated in a standard test battery of in vivo assays according to ICH S2(R1). All tests concluded CBD to be negative for genotoxic potential.

Ref.: Assessment report. Epidyolex. International non-proprietary name: cannabidiol. Procedure No. EMEA/H/C/004675/0000. 25 July 2019. EMA/458106/2019. Committee for Medicinal Products for Human Use (CHMP)

SCCS comment
According to the official Epidiolex website, BD OS formulation includes 100 mg/mL of CBD concentration. Beside 100 mg of CBD, each ml of solution contains: 79 mg anhydrous ethanol, 736 mg refined sesame oil, 0.0003 mg benzyl alcohol, Anhydrous ethanol Sucralose (E955) and strawberry flavour (including benzyl alcohol).
It is not clear how the composition of the test substance used in the genotoxicity studies described in EMA report relates to the current submission. The SCCS does not consider formulations.

From the Respondent:

The table below summarise the available literature on CBD products (or hemp product) for supporting the safety profile of CBD.

	Test Material	Test	Comments/ results	GLP compliance	Ref.
1	hemp extract (9%) in olive oil (6,27% in CBD)	Bacterial Reverse Mutation Assay (Ames test)	Non mutagenic	Yes. (OECD 471)	(Dziwenka et al. 2020)
2	hemp extract – not diluted in oil	Bacterial Reverse Mutation Assay (Ames test)	Non mutagenic	Yes. (OECD 471)	(Dziwenka et al. 2020)
3	CBD extract (26% cannabinoids, of which 96% CBD) in oil	Bacterial Reverse Mutation Assay (Ames test) with and without metabolic activation (S9)	Non mutagenic	Yes. (OECD 471)	(Marx T. K., et al. 2018)
4	CBD extract (26% cannabinoids, of which 96% CBD) in oil	*In Vitro* Mammalian Chromosomal Aberration Test.	Non clastogenic	Yes. (OECD 473)	(Marx T. K., et al. 2018)
5	CBD extract (26% cannabinoids, of which 96% CBD) in oil	*In Vivo* Mouse Micronucleus Test	Negative	Yes. (OECD 474)	(Marx T. K., et al. 2018)

Test items of the genotoxicity tests hereto mentioned were different and, in some cases, lacking details (e.g. Study n°2 of the table) while other at very low level of CBD (eg. Study n°1 of the table on CBD (9%) in olive oil (91%) and CBD extract (26% cannabinoids, of which 96% CBD) in oil. However, all the tested items gave negative results.

REF: TOXICOLOGICAL EXPERT REPORT Cannabidiol (CBD) for cosmetic uses Prepared by respondent 2

SCCS comment
Looking at the medicinal use of supercritical CO2 extract of the aerial parts of the hemp (*C.sativa*) plant to investigate toxicological effects, Marx *et al.* (2018) tested the CBD extract (26% cannabinoids, of which 96% CBD) in oil using the *in Vivo* Mouse Micronucleus Test, with negative results.

Some other studies *in vivo* have been reported. Hemp-derived CBD isolate (99.08–101.46%; CAS No. 13956-29-1) was tested in the *in vivo* micronucleus test (Henderson *et al.*, 2023 https://doi.org/10.1016/j.yrtph.2023.105425). The study was considered valid and did not show concern for genotoxic effects.

Older studies are available, showing that CBD (99%) causes induction of micronucleus and chromosomal aberrations in bone marrow of mice (Zimmerman and Raj 1980). MN induction was reported after i.p. administration of CBD. The test was in partial agreement with general TG: several doses were tested, five animals were used per group, a sufficient number of cells was evaluated, and positive/negative controls were included. However, the impact of the drug on erythropoiesis, which may lead to false results, and OECD #474 were not taken into account, limiting the reliability of the study.

ANNEX B. Summary of results of the Ames tests on different CBD materials from different producers

Applicant#	Strains used	Test substance	Concentrations used	Result by Applicant	Result by the SCCS	Reference
Chemsafe S.r.l., 2020 on behalf of the EUROPEAN INDUSTRIAL HEMP ASSOCIATION (EIHA)	Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) and Escherichia coli (WP2 uvrA) -/+S9 Plate incorporation or the pre-incubation methods	From Charlotte's Web, Inc. Blend of 9% hemp extract and 91% organic extra virgin olive oil, which is produced by an isopropanol extraction method. Fatty acids comprise approximately 88.70% of this extract, while the phytocannabinoid content is 6.96% (of this, 6.27% is CBD); the remaining 4.34% consists of fatty alkanes, sterols, terpenes and tocopherols.	The final doses utilized for the extract diluted in olive oil were 0.24, 0.76, 2.41, 7.633, 24.12, 76.33, 241.22, 763.33, 2412.2, 7633.5, 24122 and 76355 µg/plate. For the undiluted isopropanol extract, the final doses utilized for both the initial and confirmatory tests were 1.58, 5.0, 15.8, 50, 158, 500, 1580 and 5000 µg/plate. For the undiluted supercritical CO_2 extract, the final doses utilized were 1.58, 5.0, 15.8, 50, 158, 500, 1580 and 5000 µg/plate for the initial test and	Negative -/+S9	Negative -/+S9	Dziwenka *et al.*, 2020 https://doi.org/10.1016/j.toxrep.2020.02.014

			0.5, 2.5 and 25 µg/plate for the confirmatory test.			
Chemsafe S.r.l., 2020 on behalf of the EUROPEAN INDUSTRIAL HEMP ASSOCIATION (EIHA)	Salmonella typhimurium (TA98, TA100, TA1535, and TA1537) and Escherichia coli WP2uvrA -/+S9 plate incorporation for initial test and 20-min preincubation for confirmatory test	From CV Sciences, Inc. (San Diego, CA) Supercritical CO2 extract of the aerial parts of hemp (C. sativa). Edible fatty acids comprise 61% of this concentrated extract, while phytocannabinoids are present at 26% (of this, approximately 96% is CBD and less than 1% is THC); the remaining 13% include fatty alkanes, plant sterols, triterpenes, and tocopherols. DMSO was used as the vehicle for the test article.	5, 16, 50, 160, 500, 1600, and 5000 µg/plate, were selected for the initial and confirmatory tests	Negative -/+S9	Negative -/+S9	Marx *et al.*, 2018 https://doi.org/10.1155/2018/8143582
Chemsafe S.r.l., 2020	Salmonella typhimurium strains TA98, TA100, TA1535,	Full Spectrum Extract (REGULAR) (Chemical name:	Experiment I: - 3.16, 10.0, 31.6, 100, 316,	Negative -/+S9	Negative -/+S9	Study sponsored by EIHA and Deep Nature

on behalf of the EUROPEAN INDUSTRIAL HEMP ASSOCIATION (EIHA)	TA1537 and tester strain E. coli WP2 uvrA (pKM101) -/+S9 plate incorporation	Hemp Extract) The hemp extract is a mixture of different compounds, primarily fats and polyphenols. CBD and CBDa were the main cannabinoids of interest along with THC (present up to 0.20%). Specification is the following: CBDV 0.07%, CBDVa 0.06%, CBC 0.47, CBD 8.84 %, CBG 0.22%, CBDa 1.61%, CBGa 0.02%, CBN 0.08%, Δ9-THC 0.19%, Δ8-THC 0.12%, THCV 0.05%, THCa n.d.	1000, 2500 and 5000 µg/plate (TA98, TA100, TA1537, E. coli WP2 uvrA (pKM101)) - 3.16, 10.0, 31.6, 100, 316 and 1000 µg/plate (TA1535) Experiment II: - 0.5, 1.58, 5.0, 15.8, 50 and 158 µg/plate (TA98, TA100 and TA1535 (without metabolic activation)) - 5.0, 15.8, 50, 158, 500, 1580 and 3000 µg/plate (TA98, TA100 and TA1535 (with metabolic activation)) - 15.8, 50, 158, 500, 1580 and 3000 µg/plate (TA1537 (without metabolic activation)) - 15.8, 50, 158, 500, 1580, 3000 and 5000 µg/plate (TA1537 (with			Project GmbH Dossier by Chemsafe S.r.l., 2020 Full report by Eurofins Munich Study No.: STUGC21AA 1085-2 was not available to the SCCS, only study plan.

			metabolic activation), E. coli WP2 uvrA (pKM101) (with and without metabolic activation))			
Chemsafe S.r.l., 2020 on behalf of the EUROPEAN INDUSTRIAL HEMP ASSOCIATION (EIHA)	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and tester strain E. coli WP2 uvrA (pKM101) - /+S9	Natural Hemp Isolate CBD (99.83% in Eurofins) Natural CBD isolate (99.4% pure) in oily vehicle. CBD was extracted by CO_2 from the raw dried hemp (whole plant) and then purified. THC (delta-9-THC or Δ9-THC) content is ≤ 0.015%.	Experiment I: - 31.6, 100, 316, 1000, 2500 and 5000 µg/plate Experiment II: - 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA98, TA100 and TA1535 (with metabolic activation); E. coli WP2 uvrA (pKM101)) - 0.316, 1.00, 3.16, 10.0, 31.6 and 100 µg/plate (TA98, TA100 and TA1535 (without metabolic activation); TA1537)	Negative - /+S9	Negative - /+S9	Study sponsored by Deep Nature Project GmbH and EIHA Eurofins Munich Study No. STUGC21AA 0231-2, 2021-05-10
Eurofins	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and tester strain E. coli WP2 uvrA	CBD ISOLATE POWDER 99%+ Batch No.: SL-20-ISO-02	Experiment I: 31.6, 100, 316, 1000, 2500 and 5000 µg/plate Experiment II:	Negative - /+S9	Negative - /+S9	EUROFINS Munich STUGC20AA 2201-2 13/10/2020

	(pKM101) -/+S9	Purity: 99.46% total CBD Vehicle: DMSO	- 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA98, TA100 (with metabolic activation), TA1535 (with metabolic activation), TA1537 and E. coli WP2 uvrA (pKM101)) - 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA100, (without metabolic activation)) - 0.1, 0.316, 1.0, 3.16, 10.0, 31.6 and 100 µg/plate (TA1535 (without metabolic activation))			
Eurofins	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and tester strain E. coli WP2 uvrA (pKM101) -/+S9	PCR BROAD SPECTRUMS HEMP EXTRACT 85% Batch No.: SL-20-BSE-01 Purity: 85.92% CBD Vehicle: DMSO	3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate	Negative -/+S9	Negative -/+S9	EUROFINS Munich STUGC20AA 2201-3 01/10/2020

Chanelle McCoy Health Dossier on Cannabidiol	S. typhimurium: TA98, TA100, TA1535, and TA1537, and at the tryptophan locus of Escherichia coli (E. coli) strain WP2uvrA -/+S9 A direct plate assay, followed by a pre-incubation assay.	CBD (97%-102% purity)	Epidiolex® was negative in the Ames test in vitro at up to 5,000 µg/plate, with or without metabolic activation (S-9).	Negative -/+S9	Detailed results not available.	Chanelle McCoy Health Dossier on Cannabidiol in accordance with the SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation 12th revision: Table 28 In Vitro Bacterial Reverse Mutation Test (Tier 1) [CONFIDENTIAL AND PROPRIETARY]
	S. typhimurium; TA98, TA100, TA1535, and TA1537), and at the tryptophan locus of Escherichia coli (E. coli) strain WP2uvrA -/+S9	Hemp-derived CBD isolate (99.08–101.46%; CAS No. 13956-29-1) from Canopy Growth USA (Evergreen, Colorado) produced by ethanol extraction method and subsequent crystallization. Purity: 99.62%	0.25, 0.5, 1.0, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, or 5000 µg/plate	Negative -/+S9	Negative -/+S9	Henderson, R. G., Welsh, B. T., Trexler, K. R., Bonn-Miller, M. O., & Lefever, T. W. (2023). Genotoxicity evaluation of cannabidiol. Regulatory Toxicology and Pharmacology, 142, 105425.

		Vehicle: DMSO				
	Salmonella typhimurium: TA97a, TA98, TA100, TA102, and TA1535	Two samples from PharmaHemp d.o.o. (Ljubljana, Slovenia) 1. CBD isolate (CBD crystalline powder; CBD CP, obtained by distillation and crystalization) with a CBD content: 99.4% 2. high-content CBD cannabis extract (CBD EX, prepared by extraction using supercritical CO_2, cannabinoid concentration and removal of THC using preparative chromatography), with a CBD content: 63.6%. Other cannabinoids including CBDV (12.78 %), THCV (1.677 %), CBG (1.077 %), and others in	0.5 – 5000 µg/plate	Negative -/+S9	Negative -/+S9	Štern, A., Novak, M., Kološa, K., Trontelj, J., Žabkar, S., Šentjurc, T., ... & Žegura, B. (2024). Exploring the safety of cannabidiol (CBD): A comprehensive in vitro evaluation of the genotoxic and mutagenic potential of a CBD isolate and extract from Cannabis sativa L. Biomedicine & Pharmacotherapy, 177, 116969.

		small amounts. Vehicle: ethanol				
	Salmonella typhimurium: TA98, TA100, TA1535, TA1537, and Escherichia coli WP2 uvrA plate incorporation test and pre-incubation test +S9	The CBD isolate was a whole plant ethanol extract of Cannabis sativa L. and CBD, accounts for >97% of the total cannabinoids present (Batch No. 103501B, cbdMD Inc (Charlotte, NC, USA). The CBD isolate was diluted to a concentration of 31–33% using MCT oil supplied by cbdMD Inc (Charlotte, NC, USA).	4.8, 15.2, 47.9, 1517.5, 4795, and 15175 µg/plate	Negative -/+S9	Negative -/+S9	Tallon MJ, Child RB, Blum JL. Genotoxic assessment of a Cannabis sativa L. extract. Pharm Biol. 2025 May 6;63(1):357–363. doi: 10.1080/13880209.2025.2499075
	Salmonella typhimurium: TA98, TA100, TA1535, TA1537, and Escherichia coli WP2 uvrA Plate-incorporation test and the main test with pre-incubation for 20 min. -/+S9	Imperial Oil®, a dark amber wax-like viscous liquid containing 86–89% total cannabinoids and 84–87% CBD. THC was non-detectable. The extract was manufactured using industrial hemp	5, 16, 50, 160, 500, 1600, and 5000 µg/plate	Negative -/+S9	Negative -/+S9	Clewell, A., Glávits, R., Endres, J. R., Murbach, T. S., Báldi, P. T., Renkecz, T., Hirka, G., Vertesi A., Beres, E., & Szakonyiné, I. P. (2023). An evaluation of the genotoxicity and 90-day repeated-dose toxicity of a CBD-rich

		biomass raw material, and denatured ethanol was utilized as the main solvent.				hemp oil. Journal of Applied Toxicology, 43(11), 1719-1747.
No report						Gijsbrechts JJA, 2020 [unpublished]. Prepared by Charles River Laboratories Den Bosch B.V., DD's Hertogenbosch, The Netherlands for Chanelle McCoy CBD Ltd., Loughrea, Galway, Ireland. Study Title: Cannabidiol: bacterial reverse mutation test. Confidential. (Test Facility Study No. 20203944) -Report not available.

10.ANNEX C

Table summarising all genotoxicity data available, as cited in the Advice

#	Substance	Purity	Endpoint	Test System	Result	SCCS comment	Ref
1	Natural Hemp Isolate CBD	99.83%	Bacterial Gene mutation	TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101	Negative	study valid and the results negative	Eurofins Munich Study No.: STUGC21AA 0231-2 and cited ad Annex VIII in "Cannabidiol (CBD) for cosmetic uses, Prepared by Chemsafe S.r.l."
2	CBD ISOLATE POWDER 99%+	99.46%	Bacterial Gene mutation	TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101	Negative	study valid and the results negative	Reverse Mutation Assay using Bacteria (Salmonella typhimurium and Escherichia coli) with CBD ISOLATE POWDER 99%+. Eurofins Munich Study No.: STUGC20AA 2201-2
3	PCR BROAD SPECTRUMS HEMP EXTRACT	85.92%	Bacterial Gene mutation	TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101	Negative	study valid and the results negative	Reverse Mutation Assay using Bacteria (Salmonella typhimurium and Escherichia coli) with PCR BROAD SPECTRUMS HEMP EXTRACT 85%. Eurofins Munich Study No.: STUGC20AA 2201-3

4	Full Spectrum Extract (REGULAR)	mixture, purity unknow	Bacterial Gene mutation	TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101	Negative	Study not evaluated by SCCS (data not available).	Dossier by Chemsafe S.r.l., 2020; Study plan by Eurofins Munich Study No.: STUGC21AA 1085-2.
5	Hemp-derived CBD isolate	99.62%	Bacterial Gene mutation	TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101	Negative	study valid and the results negative	Henderson *et al.*, 2023 https://doi.org/10.1016/j.yrtph.2023.105425
6	CBD isolate with its lipid carrier [medium chain triglyceride (MCT)]	30% CBD in MCT oil	Bacterial Gene mutation	TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM101	Negative	study valid and the results negative	Tallon MJ, Child RB, Blum JL. Genotoxic assessment of a Cannabis sativa L. extract. Pharm Biol. 2025 May 6;63(1):357–363. doi: 10.1080/13880209.2025.2499075
7	Hemp extract (9%) blend with 91% organic extra virgin olive oil	9% or extracts by isopropanol and supercritical CO_2 extraction	Bacterial Gene mutation	TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM102	Negative	Ames test was conducted on this test article and two additional Ames tests were conducted on undiluted extract, one an isopropanol extract and the other a supercritical CO_2 extract.; study data not shown - not valid	Dziwenka M, Coppock R, Alexander M, Palumbo E, Ramirez C, Lermer S. Safety Assessment of a Hemp Extract using Genotoxicity and Oral Repeat-Dose Toxicity Studies in Sprague-Dawley Rats. Toxicol Rep. 2020 Feb 20;7:376-385. doi: 10.1016/j.toxrep.2020.02.014

8	Hemp Extract - supercritical CO2 extract of the aerial parts of hemp (C. sativa).)	26% cannabinoids , of which 96% CBD	Bacterial Gene mutation	TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM103	Negative	study valid and the results negative	Marx TK, Reddeman R, Clewell AE, Endres JR, Béres E, Vértesi A, Glávits R, Hirka G, Szakonyiné IP. An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp. J Toxicol. 2018 Jun 7;2018:8143582. doi: 10.1155/2018/8143582.
9	CBD isolate (hemp-derived, supplier Mile High Labs)	98% purity, dissolved in tehanol at 60%	Bacterial Gene mutation	TA98, TA100, TA102	Negative	study was negative with some limitations: only 3 of the 5 strains recommended in OECD TG, only results on fold-increase increase were presented.	Luz-Veiga M, Mendes A, Tavares-Valente D, Amorim M, Conde A, Pintado ME, Moreira HR, Azevedo-Silva J, Fernandes J. Exploring Cannabidiol (CBD) and Cannabigerol (CBG) Safety Profile and Skincare Potential. Int J Mol Sci. 2024 Nov 14;25(22):12224. doi: 10.3390/ijms252212224
10	CBD isolate	99.4%	Bacterial Gene mutation	TA97a, TA98, TA100, TA102, TA1535	Negative	study valid and the results negative	Štern A, Novak M, Kološa K, Trontelj J, Žabkar S, Šentjurc T, Filipič M, Žegura B. Exploring the safety of cannabidiol

							(CBD): A comprehensive in vitro evaluation of the genotoxic and mutagenic potential of a CBD isolate and extract from Cannabis sativa L. Biomed Pharmacother. 2024 Aug;177:116969. doi: 10.1016/j.biopha.2024.116969
11	high-content CBD cannabis extract (prepared by extraction using supercritical CO_2)	63.6 %	Bacterial Gene mutation	TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA, pKM106	Negative	study valid and the results negative	Štern A, Novak M, Kološa K, Trontelj J, Žabkar S, Šentjurc T, Filipič M, Žegura B. Exploring the safety of cannabidiol (CBD): A comprehensive in vitro evaluation of the genotoxic and mutagenic potential of a CBD isolate and extract from Cannabis sativa L. Biomed Pharmacother. 2024 Aug;177:116969. doi: 10.1016/j.biopha.2024.116970
12	CBD Isolate Powder	99%+	Micronucleus test	Mouse lymphoma L5178Y TK+/- 3.7.2 C cells	Negative	study valid and the results negative	Charles-River 2021. A GLP In Vitro Mammalian Cell Micronucleus Test of CBD

							Isolate Powder 99%+ Test Facility Study Code: 20/195-013C
13	Broad Spectrum Hemp Extract	85%	Micronucleus test	Mouse lymphoma L5178Y TK+/- 3.7.2 C cells	Negative	study valid and the results negative	Charles-River 2021. A GLP In Vitro Mammalian Cell Micronucleus Test of Broad Spectrum Hemp Extract 85%. Test Facility Study Code: 20/194-013C. 08 January 2021
14	Hemp-derived CBD isolate	99.62%	Micronucleus test	Human lymphoblast TK6 cells	Negative	study valid and the results negative	Henderson *et al.*, 2023 https://doi.org/10.1016/j.yrtph.2023.105425
15	CBD extract in oil	26% cannabinoids, of which 96% CBD	Chromosomal aberration	V79 Chinese hamster lung cells	Negative	study valid and the results negative	Marx TK, Reddeman R, Clewell AE, Endres JR, Béres E, Vértesi A, Glávits R, Hirka G, Szakonyiné IP. An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp. J Toxicol. 2018 Jun 7;2018:8143582. doi: 10.1155/2018/8143582
16	CBD isolate with its lipid carrier [medium chain	30% CBD in MCT oil	Micronucleus test	human lymphoblastoid cells (TK6 Cells)	Negative	study not valid and the results negative	Tallon MJ, Child RB, Blum JL. Genotoxic assessment of a

	triglyceride (MCT)]						Cannabis sativa L. extract. Pharm Biol. 2025 May 6;63(1):357–363. Doi: 10.1080/13880209.2025.2499075
17	CBD isolate (Mile High Labs)	≥98%	Micronucleus test	human lymphoblastoid cells (TK6 Cells)	Negative	study was negative with some limitations regarding OECD compliance: no use of S9, results shown only graphically, no short exposure, no data on number of cells scored.	Luz-Veiga M, Mendes A, Tavares-Valente D, Amorim M, Conde A, Pintado ME, Moreira HR, Azevedo-Silva J, Fernandes J. Exploring Cannabidiol (CBD) and Cannabigerol (CBG) Safety Profile and Skincare Potential. Int J Mol Sci. 2024 Nov 14;25(22):12224. doi: 10.3390/ijms252212224.
18	CBD , LGC Standards	98.7%	Micronucleus test	Human lymphoblastoid TK6 cells	Positive -S9; Negative +S9	study valid and the results positive, however the effects seem to be reduced in the presence of metabolic activation.	Kolar N, Bankoglu EE, Stopper H. Genotoxicity of selected cannabinoids in human lymphoblastoid TK6 cells. Arch Toxicol. 2024 Oct;98(10):3439-3451. doi: 10.1007/s00204-024-03826-y
19	CBD cannabis extract (supercritical CO2, cannabinoid concentration and removal of THC u)	63.6 %	Micronucleus test	Human HepG2 cells	Negative	study is negative, with limitations: no S9 used, no short exposure. Additional endpoints (Dna damage,	Štern A, Novak M, Kološa K, Trontelj J, Žabkar S, Šentjurc T, Filipič M, Žegura B. Exploring the safety of cannabidiol

						DSB, histone phosphorilation) also negative.	(CBD): A comprehensive in vitro evaluation of the genotoxic and mutagenic potential of a CBD isolate and extract from Cannabis sativa L. Biomed Pharmacother. 2024 Aug;177:116969. doi: 10.1016/j.biopha.2024.116969.
20	CBD isolate	99,40%	Micronucleus test	Human HepG2 cells	Negative	study is negative, with limitations: no S9 used, no short exposure. Additional endpoints (Dna damage, DSB, histone phosphorilation) also negative.	Štern A, Novak M, Kološa K, Trontelj J, Žabkar S, Šentjurc T, Filipič M, Žegura B. Exploring the safety of cannabidiol (CBD): A comprehensive in vitro evaluation of the genotoxic and mutagenic potential of a CBD isolate and extract from Cannabis sativa L. Biomed Pharmacother. 2024 Aug;177:116969. doi: 10.1016/j.biopha.2024.116969.

21 CBD	99.95%	Micronucleus test	Human HepG2 cells	Positive	The study is valid and evidences genotoxic effects of CBD in liver cells, as well as DNA damage induction in buccal epithelial cells.	Russo C, Ferk F, Mišík M, Ropek N, Nersesyan A, Mejri D, Holzmann K, Lavorgna M, Isidori M, Knasmüller S. Low doses of widely consumed cannabinoids (cannabidiol and cannabidivarin) cause DNA damage and chromosomal aberrations in human-derived cells. Arch Toxicol. 2019 Jan;93(1):179-188. doi: 10.1007/s00204-018-2322-9.
22 CBD-OS	formulation	Chromosomal aberrations in vivo	Rat, micronuclei in bone marrow	Negative		Cit in Assessment report. Epidyolex. International non-proprietary name: cannabidiol. Procedure No. EMEA/H/C/004675/0000. 25 July 2019. EMA/458106/2019. Committee for Medicinal Products for Human Use (CHMP)
23 CBD	unkown	DNA damage in vivo	Rat, Alkaline comet Assay	Negative	Not assessed by SCCS	Cit in Assessment report. Epidyolex. International non-proprietary name: cannabidiol. Procedure No.

							EMEA/H/C/004675/0000. 25 July 2019. EMA/458106/2019. Committee for Medicinal Products for Human Use (CHMP)
24	CBD extract in oil	26% cannabinoids, of which 96% CBD	Micronucleus test	Mouse, bone marrow	Negative	Not assessed by SCCS	Marx TK, Reddeman R, Clewell AE, Endres JR, Béres E, Vértesi A, Glávits R, Hirka G, Szakonyiné IP. An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp. J Toxicol. 2018 Jun 7;2018:8143582. doi: 10.1155/2018/8143582
25	Hemp-derived CBD isolate	99.08–101.46%;	Micronucleus test	Mouse, bone marrow	Negative	Not assessed by SCCS	Henderson *et al.*, 2023 https://doi.org/10.1016/j.yrtph.2023.105425
26	CBD	99%	Micronucleus test and Chromosomal Aberrations	Mouse, bone marrow	Positive	Not assessed by SCCS: results of older studies are available (when no CBD-containing preparations were sold on the market). They show that CBD causes induction of MN and CA in bone marrow of mice	Zimmerman AM, Raj AY. Influence of cannabinoids on somatic cells in vivo. Pharmacology. 1980;21(4):277-87. doi: 10.1159/000137442. cit by: Russo *et al.*, 2019

(Zimmerman and Raj 1980). MN induction was found in three independent experimental series after i.p. administration of CBD; the test was in partial agreement with the U.S. EPA guidelines (Mavournin *et al.* 1990; OECD 2016), i.e., several doses were tested, five animals were used per group, a sufficient number of cells was evaluated and positive/negative controls were included. However, the impact of the drug on erythropoiesis, which may lead to false results and OECD #474 (Tweats *et al.* 2007) was not taken into account.