Scientific Committee on Consumer Safety

SCCS

OPINION ON

fragrance ingredient
Acetylated Vetiver Oil - AVO
(Vetiveria zizanioides root extract acetylated)

Submission III

The SCCS adopted this Opinion
at its the plenary meeting on 26 February 2019

Corrigendum of 20-21 June 2019

ACKNOWLEDGMENTS

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All Declarations of Working Group members are available on the following webpage:
http://ec.europa.eu/health/scientific_committees/experts/declarations/sccs_en.htm

This Opinion has been subject to a commenting period of a minimum eight weeks after its initial publication (from 02 July 2018 until 10 September 2018). Comments received during this period of time were considered by the SCCS.
For this Opinion, comments received resulted in the following main changes: *sections 3.1.5 impurities, 3.1.7 LogP values, 3.5 MoS calculation and Table 6, and inhalation toxicity (discussion and conclusion sections).*
Corrigendum made in the discussion part and in conclusion number 2, only for clarity of SCCS position regarding sensitisation.

1. ABSTRACT

The SCCS concludes the following:

1. On the basis of currently available information, does the SCCS consider Acetylated Vetiver Oil (AVO) safe for use as fragrance ingredient in cosmetic leave-on and rinse-off type products in a concentration limit(s) according to the once set up by IFRA as reported above?

On the basis of the safety assessment carried out using a conservative approach, the SCCS considers the use of Acetylated Vetiver Oil (AVO) with 1% alpha-tocopherol as a fragrance ingredient in cosmetic leave-on and rinse-off type products safe at the concentrations proposed by IFRA.

2. Does the SCCS have any further scientific concerns with regard to the use of Acetylated Vetiver Oil (AVO) as fragrance ingredient in cosmetic leave-on and rinse-off type products?

Acetylated Vetiver Oil (AVO) contains some constituents that belong to the chemical group of aldehydes and ketones that are known to be reactive towards biological entities, such as DNA and proteins. However, the overall health risk of such components is likely to be negligible at the concentrations intended to be used in cosmetics products.

The SCCS has noted that Acetylated Vetiver Oil (AVO) is a moderate skin sensitiser in test animals. Considering the results of the HRIPT study and the fact that AVO has been used for years in cosmetics without evidence of sensitising potential, it is unlikely that AVO would be causing contact allergy in humans.

Inhalation toxicity of Acetylated Vetiver Oil (AVO) was not assessed in this Opinion because no data were provided. Assessment of the inhalation risk would be needed if AVO was intended to be used in sprayable products.

Keywords: SCCS, scientific opinion, Acetylated Vetiver Oil (AVO), Regulation 1223/2009, Vetiverol acetate CAS 62563-80-8; Vetiveria zizanioides ext. acetylated, CAS 84082-84-8, EC 282-031-1, SCCS/1599/18

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SCCS

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

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

According to the Applicant Vetiver oil is produced for the fragrance industry by distillation of fresh or dried roots of Vetiveria (Chrysopogon) zizanioides originating from different geographical areas. The Vetiver oil is then subject to further processing to obtain Acetylated Vetiver Oil (AVO) (CAS No 84082-84-8, EINECS No 282-031-1).

Submission I on Vetiveryl acetate (AVO) was transmitted in 2005 by The European Flavour & Fragrance Association.

The Scientific Committee on Consumer Products (SCCP) adopted at its 7th plenary meeting held on the 28 of March 2006 the opinion (SCCP/0984/06)[1] on Vetiveryl acetate (sensitisation only) with the following conclusion:

"The SCCP is of the opinion that the information submitted is inadequate to assess the safe use of the substance.

Before any further consideration, the following information is required:
• *Characterisation of the test substance; clarification on purity and impurities;*
• *Data on sensitisation conforming to modern standards and guidelines;*
• *Appropriate information on all relevant toxicological endpoints as required to assess the safe use of the substance when used in cosmetic products."*

Submission II on Vetiveryl acetate was received in June 2013 from the International Fragrance Association (IFRA) .

In December 2014, the Scientific Committee on Consumer Safety (SCCS) adopted an opinion on Vetiveryl acetate (SCCS/1541/14)[2]. During the commenting period IFRA sent an updated dossier in which it was raised the necessity to modify the initial request on this substance, such as the identification/name of the substance and its use concentration in different cosmetic product types. The SCCS considered the request appropriate in order to finalize the opinion focusing on the substance Acetylated Vetiver Oil (AVO).

IFRA recommends a safe concentration limit for Acetylated Vetiver Oil (AVO) when it is used in the specific categories of cosmetic products as reported in the Table below.

[1] http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_054.pdf
[2] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_167.pdf

Table with concentration limits for Acetylated Vetiver Oil (AVO)

Product type	**% Acetylated Vetiver Oil** (AVO) **in consumer product**
Hydroalcoholic-based fragrances (e.g. Eau de Toilette, Perfume, Aftershave, Cologne)	0.90
Deodorants	0.05
Make up products (e.g. eye make-up, make-up remover, liquid foundation, mascara, eyeliner, lipstick)	0.05
Face cream	0.10
Hand cream	0.10
Body lotion	0.10
Hair styling	0.10
Bath cleansing products (e.g. soaps, shower gel, rinse-off conditioner, shampoo)	0.20

Terms of reference

1. On the basis of the currently available information, does the SCCS consider Acetylated Vetiver Oil (AVO) *safe for use as fragrance ingredient in cosmetic leave-on and rinse-off type products in a concentration limit(s) according the ones set up by IFRA as reported above?*

2. Does the SCCS have any further scientific concerns with regard to the use of Acetylated Vetiver Oil (AVO) *as fragrance ingredient in cosmetic leave-on and rinse-off type products?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

Vetiveryl acetate or Acetylated Vetiver Oil (AVO) is the commonly used name to refer to a natural complex substance. The starting material, Vetiver oil, is a UVCB substance (Unknown or Variable composition, Complex reaction products or Biological materials). The oil is then subjected to further processing.

A) repeated distillation (rectification) to yield 'Vetiverol' (Vetiver oil fraction rich in sesquiterpene alcohols), which is then followed by acetylation, purification and rectification,
B) acetylation (the generally applied method requiring acetic anhydride and phosphoric acid as process materials plus a temperature of 100–120 °C) to yield raw Acetylated Vetiver oil, which is then purified by neutralisation, washing steps and rectification(s)

Previously, a third manufacturing process was also used:

C) extraction of Vetiver alcohols using boric acid or phthalic anhydride to yield Vetiverol alcohols, followed by acetylation and rectification.

IFRA Standard (44th Amendment) describes the principles of three methods for the acetylation of Vetiver Oil.

3.1.1.1 Primary name and/or INCI name

Acetylated Vetiver Oil (AVO)
INCI name: Not applicable (mixture of many constituents, see 3.1.4)

3.1.1.2 Chemical names

SCCS comment - Submission II
The chemical names given relate to the main constituent of Vetiveryl acetate (about 15%). Vetiveryl acetate is a complex mixture of many constituents, and it cannot be identified as a single chemical substance (see 3.1.4.).

Submission III

According to the Applicant, 'Vetiveryl acetate' would be better described as AVO. A description of the production method used by fragrance industry was provided, according to which Vetiver oil is produced by distillation of fresh or dried roots of Vetiveria (Chrysopogen) zizanoides originating from various geographical areas as a UVCB substance (Unknown or Variable composition, Complex reaction products or Biological materials). The oil is then subjected to further processing (see 3.1.1 above).

According to the Applicant, the final product from both processes is Acetylated Vetiver Oil (AVO), which is described by the fragrance industry using the following identifiers:

- Vetiveria zizanioides, ext, acetylated CAS number 84082-84-8, EINECS number 282-031-1
- Oils, vetiver, acetylated CAS number 68917-34-0

3.1.1.3 Trade names and abbreviations

Acetylated Vetiver Oil (AVO)

As for Submission II, the Applicant has agreed to use CAS 84082-84-8 to represent the product in Europe that is associated with the name Acetylated Vetiver Oil (AVO).

Vetiver acetate
Vetivert acetate
Vetyvenyl acetate
Vetiverol acetate, dist, CAS number 73246-97-6
Vetiveryl acetate CAS number 117-98-6
Vetiveria zizanioides, ext., acetylated, CAS number 84082-84-8, EINECS number 282-031-1
Acetyver
Vetiveryl acetate 112 Extra Aetivenol
Oils, vetiver, acetylated, CAS number 68917-34-0

In the text of the Opinion, Acetylated Vetiver Oil (AVO) associated with CAS 84082-84-8 registered under REACH has always been used. Other related CAS numbers, e.g. 62563-80-8, 68917-34-0, and 73246-97-6, were used to describe the exact same material in other regions of the world.

Ref. 95 in Submission II

3.1.1.4 CAS / EC number

Acetylated Vetiver Oil - AVO
Vetiveria zizanioides root extract acetylated
CAS 84082-84-8
EINECS: 282-031-1

CAS: 62563-80-8
EINECS: 263-597-9

CAS: 68917-34-0

CAS: 73246-97-6

SCCS comment - Submission II
IFRA Standard (44th Amendment) describes following CAS No. for vetiveryl acetate:
117-98-6
62563-80-8
68917-34-0
73246-97-6
84082-84-8
According to description (see 3.1.4), vetiveryl acetate is a mixture of ca. 100 substances.
The rational for reporting up to five CAS and/or EC No. of vetiveryl acetate is not given.
According to the Reference AR1, vetiveryl acetate has CAS No. 68917-34-0
According to the Reference AR2, vetiveryl acetate has CAS No. 62563-80-8

SCCS comment - Submission III
The Applicant agreed that the available CAS numbers for substances derived from natural sources such as Acetylated Vetiver Oil (AVO) is highly confusing, and that registrations within the Chemical Abstract Survey register relate to global differences in requirements for

assigning specificity around UVCB regarding plant sections in certain regions of the world such as the USA.

According to the Applicant, in the EU, at least two CAS numbers for Acetylated Vetiver Oil (AVO) exist:

CAS number 84082-84-8, Vetiveria zizanioides, ext. acetylated, EINECS nr 282-031-1.

CAS number 62563-80-8 Vetiverol acetate, EINECS nr 263-597-9

According to the Applicant, the SCCS remark on the IFRA Standard would be taken into consideration updating the upcoming 48th Amendment, but stated that the global scope of IFRA regulations for the fragrance industry necessitated the inclusion of CAS numbers for Acetylated Vetiver Oil (AVO) from other regions of the world besides the EU. For the sake of relevance to this particular EU situation, however, the Applicant would only refer to the EU CAS number 84082-84-8 Vetiveria zizanioides ext. acetylated for this dossier. The Applicant also agreed that the CAS number 117-98-6 refers to a specific chemical (2,6-Dimethyl-9-isopropylidenbicyclo(5.3.0)dec-2-en-4-yl-acetate) and not to Acetylated Vetiver Oil (AVO) (as supported by the fragrance industry for this dossier) and would agree to remove this CAS number from the dossier. According to the Applicant, Reference 13 in Submission II referred to database information that the Applicant can no longer access but it is superseded by the information presented in the response above.

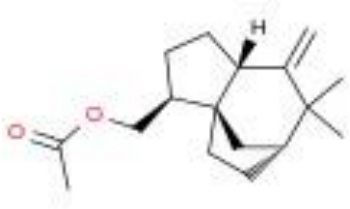
It was also noted by the SCCS that the test substances used in different toxicological studies had been described in terms of more than one CAS number. These included CAS 84082-84-8, 68917-34-0, 62563-80-8 and 117-98-6. Two of the CAS numbers (62563-80-8 and 117-98-6) have been listed in CosIng as Vetiveryl acetate/vetiverol acetate, with the IUPAC name of a specific substance (1,2,3,3a,4,5,6,8a-octahydro-2-isopropylidene-4,8-dimethylazulen-6-yl acetate). SCCS noted that only CAS number 62563-80-8 is correctly associated to 1,2,3,3a,4,5,6,8a-octahydro-2-isopropylidene-4,8-dimethylazulen-6-yl acetate) whereas CAS number 117-98-6 identifies 2,6-Dimethyl-9-isopropylidenbicyclo(5.3.0)dec-2-en-4-yl-acetate.

The Applicant explained that different CAS numbers had been incorrectly used in the past to describe the same commercial fragrance material, i.e. Acetylated Vetiver Oil (AVO), for which a single CAS 84082-84-8 is now proposed and used by the industry. The Applicant also confirmed that all the tests presented in Submission II Dossier of 11 June 2013 (Ref 1) had been conducted on Acetylated Vetiver Oil (AVO), and although some reports stated Vetiveryl acetate (CAS 117-98-6), the test article used in the studies was in fact what is now known as Acetylated Vetiver Oil (AVO) (CAS 84082-84-8).

Based on the Applicant's explanation, the SCCS is willing to accept that the studies referring to CAS 117-98-6 can be regarded as applicable to the Acetylated Vetiver Oil (AVO) (acetylated extract of Vetiveria zizanoides, CAS 84082-84-8) for the purpose of this assessment. However, the SCCS is also aware of the limitations placed by the GLP system on making any corrections/additions to a final report in the form of amendments which also need to be signed and dated by the Study Director. The SCCS considers it to be the sole responsibility of the Applicant to clarify/amend the CAS number in the study reports through relevant institutions/authorities. The SCCS also advises the Applicant to get the relevant CosIng entries amended so that the material is question is correctly defined in terms of a single identifiable CAS number.

3.1.1.5 Structural formula

SCCS comment - Submission II
According to the Reference AR2 in Submission II, the main component of Acetylated Vetiver Oil (AVO) is khushimyl acetate (CAS No. 61474-33-7) with following chemical structure:



SCCS comment - Submission III
According to the Applicant, supply of structural formulas for AVO, being a complex natural substance, is not appropriate. However, structural information is supplied where available for the 129 constituents of AVO recorded during an analysis in 2015 (Ref. 2 and 3.1.4 below).

3.1.1.6 Empirical formula

SCCS comment - Submission II
According to IFRA standard (44th Amendment), Vetiveryl acetate, CAS 117-98-6, has the empirical formula $C_{17}H_{26}O_2$.
Empirical formula of a mixture of many constituents, (see 3.1.4) is not possible.

SCCS comment - Submission III
According to the Applicant, this will be addressed in the next Amendment to the IFRA Standard. It is not possible to provide an empirical formula for a complex natural substance like Acetylated Vetiver Oil (AVO). In this respect, reference is made to the Industry dossier (mixture of many constituents, see 3.1.4).

3.1.2 Physical form

Almost colourless or pale-straw coloured, sometimes pale-olive green, slightly viscous liquid. Sweet and dry, fresh-woody and exceptionally tenacious odour. Poorer grades display conspicuous notes of vetiver oil (green earthy, rooty notes etc.)

Ref. 1 in Submission II

3.1.3 Molecular weight

Not applicable (mixture of many constituents, see 3.1.4)

3.1.4 Purity, composition and substance codes

SCCS comment - Submission II
AVO of a different origin (India, Indonesia, Haiti, Brazil etc.) may have a different composition. The quality of commercial AVO may differ considerably since several varieties of the grass vetiveria zizaniodes exist and since fresh as well as air dried roots of the grass are distilled and may vary according to the producer. Therefore the AVO prepared from different vetiver oils may have a different composition. The concentration differs in various constituents of AVO.

- Ca. 100 constituents with a concentration of > 0.01% are present in AVO, but identification of only 12 constituents (corresponding to 60% of the mixture) is described, leaving ca. 88 constituents unknown. Thus, more than 40% of the AVO is composed of the unknown ca. 88 constituents.
- No documentation was provided for the characterisation and quantification of the substances present in AVO.
- By polar/apolar GC, the GC peaks cannot be characterised as acetate, ketone or sesqiterpene. The method of determining acetate, ketone or sesqiterpene in vetiveryl acetate is not described.
- Composition of AVO prepared by acetylation of alcohols of vetiver oil (vetiverol) will be different from that prepared by acetylation of whole vetiver oil.
- No information is provided on the composition of various batches of AVO used in the submitted studies except that the ester content (varying from 46% to 99%) has been provided for some batches.
- It will not be possible to assess the toxicity profile of the constituents reported without chemical structure and CAS No. of the constituents of AVO.

Submission III

The Applicant provided an overview of constituents from analysis of Acetylated Vetiver Oil (AVO) during 2015 (Table 1). In addition, full details of constituents identified during analysis of AVO in 2007 and 2015 were provided separately.

Ref: 2

Table 1: Constituents of Acetylated Vetiver Oil (AVO)

Percentage of constituents				
		Average %	Max %	Min %
Acetate (AC)	AC	65.41	89.75	42.06
	AC identified	49.20	71.46	31.34
Sesquiterpene (SQ)	SQ	13.94	38.51	0.00
	SQ identified	12.05	32.21	0.00
Ketone (KT)	KT	16.80	24.89	7.85
	KT identified	12.63	19.85	5.03
Aldehyde (RCHO)	RCHO	1.39	2.87	0.00
	RCHO identified	1.05	2.87	0.00
Alcohol (ROH)	ROH	0.01	0.13	0.00
	Constituents identified	74.93		
	Chemical class identified	97.55		

Eighteen representative samples of AVO were analysed in 2015. The samples were manufactured by processing of AVO from Haiti, Java, Madagascar, Indonesia and Brazil and represented Process A (2 samples) and Process B (16 samples). Sample analysis was performed via GC-MS.

A multi-constituent substance has, as a general rule in accordance with Regulation EC 1907/2006 (REACH), a composition in which several main constituents are present at a concentration ≥ 10 % (w/w) and < 80 % (w/w). It is considered normal by the Applicant for constituents present at ≥ 1% to be specified, together with any known impurities present at lower concentration, that contribute to the Classification and Labelling according to Regulation EC 1272/2008 (CLP) of the material.
Each of the 129 listed constituents has a determined concentration range, 97.5 % of AVO composition is known in terms of chemical class, and 74.9 % of AVO constituents have been identified.
According to the Applicant, consideration of minimum, maximum and percentage range values relating to the 18 samples analysed in 2015, plus ECHA guidance on REACH registration, leads to the conclusion that it is correct to consider the AVO submitted for analysis as one multi-constituent substance, i.e. geographical origin of the AVO and use of production processes A or B do not affect the range of constituents present. A total of 22 constituents were listed as present at an average concentration ≥ 1 % during the 2015 analytical procedure (Table 2).

Table 2: Constituents of Acetylated Vetiver Oil (AVO) present at ≥ 1 % in 2015

ID	Constituent	Class	Av %	Min %	Max %
97	Khusimyl acetate	Acetate	13.99	9.57	24.01
105	(E)-Isovalencenyl acetate	Acetate	13.84	1.81	24.29
94	Vetiselinenyl acetate	Acetate	6.99	2.89	11.98
89	beta-Vetivone	Ketone	4.78	3.20	6.58
37	beta-Vetivenene	Sesquiterpene	2.99	0.00	8.52
83	Khusian-2-yl acetate	Acetate	2.90	2.10	4.29
82	Cyclocopacamphanyl acetate B	Acetate	2.69	1.75	3.98
95	alpha-Vetivone	Ketone	2.42	0.00	4.87
86	Ziza-6(13)-en-3a-yl acetate	Acetate	2.29	1.78	3.32
78	Ester SQ m/z 159(100), 91(40), 105(40), 131(35), 187(35), 202(30), 262(5)	Acetate	2.09	1.10	7.97
79	Cyclocopacamphanyl acetate A	Acetate	1.99	1.31	3.26
98	Unknown structure MW 262 & 264	Acetate	1.89	1.34	2.91
52	Unknown mixture MW 200, 202	Ketone	1.66	0.00	4.08
93	Isokhusimyl acetate	Acetate	1.58	0.00	5.20
58	13-nor-7,8-Epoxyeremophil-1(10)en- 11-one	Ketone	1.55	0.00	4.25
92	Unknown structure m/z 159(100), 218(20), 202(20)	Ketone	1.30	0.00	2.52
103	Unknown structure MW 262 m/z 187(100), 202(90)	Acetate	1.29	0.00	4.03
81	Ester SQ m/z 187(100), 159(70), 105(30), 174(30), 202(30)	Acetate	1.11	0.00	4.77
108	Unknown structure 218(100), 203(60),	Acetate	1.10	0.00	5.17
60	Unknown / Mixture	Unidentified	1.03	0.09	1.78
25	beta-Vetispirene	Sesquiterpene	1.00	0.00	2.79
28	delta-Amorphene	Sesquiterpene	1.00	0.00	4.11

The Applicant has concluded that the processed materials referred to collectively by the fragrance industry as AVO can be considered equivalent and should be treated as one multi-constituent substance during the discussion of the toxicological profile.
Results of the 2015 analytical procedure were compared with data from seventeen representative samples of AVO analysed during 2007. Chemical constituents were considered to be characteristic of AVO, notably the main constituents Khusimyl acetate and (E)-Isovalencenyl acetate. Although the groups of companies submitting samples of AVO for analysis were different in 2007 and 2015, three of the samples refer to the same commercial qualities (Sample 1 and 12 used for testing of sensitisation, and 18 used for several endpoints). Expansion of the data review to include all samples from 2007 and 2015 showed twelve constituents present at an average concentration of ≥ 1% in 17 samples analysed during 2007 (Ref. 2). The same twelve constituents were present in 18 samples characterised during 2015 (Table 3).

Table 3: Comparison of Acetylated Vetiver Oil (AVO) constituents present at ≥ 1% in 2007 and 2015

ID	Constituent	Average from all	Average from all 2015
97	Khusimyl acetate	15.37	13.99
105	(E)-Isovalencenyl acetate	14.80	13.84
94	Vetiselinenyl acetate	4.44	6.99
89	beta-Vetivone	4.24	4.78
82	Cyclocopacamphanyl acetate B	4.06	2.69
79	Cyclocopacamphanyl acetate A	3.08	1.99
83	Khusian-2-yl acetate	2.29	2.90
93	Isokhusimyl acetate	2.23	1.58
37	beta-Vetivenene	1.87	2.99
101	Isonootkatyl acetate	1.71	0.40
59	Ziza-6(13)-en-3-one	1.69	0.72
95	alpha-Vetivone	1.48	2.42

In summary, following detailed analysis of the compositional data, the Applicant found no relationship between either the geographical origin of the Vetiver Oil or the order in which the acetylation and distillation process were performed and the composition of the final AVO. In common with many other substances derived from natural sources, such variations in composition are to be expected as factors such as time of harvest, soil composition in the fields and variations in weather conditions from growing season to growing season will affect the composition of the Vetiver oil used as the starting material.
Three additional qualities of AVO (no longer produced by Givaudan) have been analysed in 2007 (origins: Java, Haiti and combined origins) and compared with Givaudan's quality of AVO (Vetiveryl acetate 112 Extra) (Table 4). These qualities were all produced following "Process B", acetylation of vetiver oil and subsequent purification.

Table 4: Analysis of 17 samples of Acetylated Vetiver Oil (AVO) in 2007 compared to 2015				
Substances	Vetiveryl acetate Haïti pure	Vetiveryl acetate Bourbon	Vetiveryl acetate Java DM	Vetiveryl acetate 112 Extra
Year of analysis	2007	2007	2007	Current quality
Sesquiterpenes	16%	10%	12%	16.04% (13.94%)
Ketones	24%	15%	21%	14.74% (16.80%)
Acetates	54%	65%	57%	65.45% (65.41%)
Unknowns	6%	10%	10%	3.77%

Ref: 2

SCCS comment
AVO is the acetylated form of a natural fragrance (vetiver oil), which is composed of around 129 constituents. Data presented by Industry (13 May 2015) (Ref 2) concerned the analysis of 18 samples of different AVO batches produced by 10 manufacturers comparing analytical data from 2007 and 2015 shows that the range of variability of the constituents of Acetylated Vetiver, considered during an extended period of time, can be accepted for samples of natural origin. The SCCS has considered this variation acceptable for a plant-derived material of natural origin and on the basis of this presumption SCCS considered AVO as a single entity on which to assess the toxicity.

3.1.5 Impurities / accompanying contaminants

Presence of residual process chemicals was investigated during analysis of 18 samples in 2015.

According to the Applicant, Acetic anhydride, acetic acid or any other residual solvents were not detected. The post process, likely fractionation, is the main parameter which contributes to the elimination of such potential residual traces. Water content was not measured but no evidence of cyclohexane, hexane or citric acid was detected in the samples. As such, it can be concluded that residual process chemicals are absent from Acetylated Vetiver Oil (AVO) supplied to the fragrance industry.

Analytical investigations performed on 18 commercial samples were free of these impurities. Acetic anhydride, acetic acid or any other residual solvents were not detected. The post process, likely fractionation, is the main parameter which contributes to the elimination of such potential residual traces.

3.1.6 Solubility

Not applicable. (Mixture of many substances, see 3.1.4)

3.1.7 Partition coefficient (Log P_{ow})

Partition coefficients n-octanol/water of Vetiveryl Acetate 112 Extra, for the 17 compounds that had relative areas of >1%, were: logPow in the range of 2.6 to 7.1.

SCCS comment – Submission III
Providing a measure of logK_{ow} for a complex multi-constituent substance such as Acetylated Vetiver Oil (AVO) is not meaningful, given the wide range of different structures and moieties. This could only result in a log Kow spanning several digits.

LogP values have been provided. However, the SCCS notes that chemical characterisation of the compounds that correspond to these seventeen logP values has not been provided.

3.1.8 Additional physical and chemical specifications

Boiling point: 285 °C
Specific gravity: 1

Ref. 1 in Submission II

3.1.9 Homogeneity and Stability

The stability and homogeneity of Acetylated Vetiver Oil (AVO) (batch VE00085543) in corn oil was assessed as part of the seven day repeated dose oral (gavage) range-finding study performed prior to the full 28-day study. Homogeneity was assessed by visual inspection of the test item formulations. Stability was determined by GC analysis of the test item formulations initially and then after storage at approximately 4 °C in the dark for 23 days. The test item formulations were deemed to be homogenous by visual inspection. Results of the GC analysis are presented in Table 5 below and show the formulations to be stable for at least 23 days. It should be noted that the same batch of AVO was used in the 28-day study, where formulations were prepared twice during the treatment period and stored at approximately 4 °C in the dark.

Table 5 Results of GC analysis from seven day repeated dose oral (gavage) range- finding study

Nominal concentration (mg/mL)	Concentration found initially (mg/mL)	Concentration found after storage for 23 days	
		(mg/mL)	(expressed as % of initial)
3.75	4.098	4.812	117
250	284	288	101

Stability of the test solutions was not assessed in any of the other studies where a solvent was used. However, based on the functional groups identified in AVO, the nature of the solvents used and the short time period between preparation and use of the solutions it is expected that they would be stable.
The shelf life of AVO claimed by manufacturers varies between one and two years when stored in full, sealed containers.

Typically, product shelf-life is determined after a series of analytical investigations over the time period claimed. Samples are checked regularly following the same initial control plan used for reception/manufacture.
The main investigations concern the physico-chemical and organoleptic measurements (specific gravity, refractive index, colour, odour) and GC comparison.
As an example, GC profiles from the same batch of AVO (Sample 1; not stabilised with antioxidant) measured at 0 and 14 months (a 12 month shelf-life is claimed) showed no significant change over this time period.

Ref. 2 in S
Submission II

SCCS comment
Stability data provided by the Applicant contain only raw data without any interpretation of the results. Based on the SCCS Notes of Guidance (SCCS/1602/18), more details on stability should have been provided.

3.2 FUNCTION AND USES

Acetylated Vetiver Oil (AVO), as used, is a mixture of many constituents, resulting from acetylation of crude vetiver oil. AVO is used as a fragrance in perfumes and in cosmetics.
Maximum use concentration of AVO in various types of cosmetic products is described in the following table below (provided by the Applicant).
According to the Applicant, these are the maximum concentrations they would like to defend in different cosmetic product categories. They have incorporated the product category of hydroalcoholic based fragrances/perfumes, which is of critical importance for them but not yet part of the systemic exposure calculation table as contained in the SCCS Notes of Guidance (2016) to derive the Margin of Safety.

Ref: Acetylated Vetiver Oil – Updated use levels for review by the SCCS, letter from IFRA to DG GROW – EU Commission, November 2016

Hydroalcoholic-based fragrances (e.g. Eau de Toilette, Perfume, Aftershave, Cologne)	0,90%
Deodorants	0,05%
Make up products (e.g. eye make-up, make-up remover, liquid foundation, mascara, eyeliner, lipstick)	0,05%
Face cream	0,10%
Hand cream	0,10%
Body lotion	0,10%
Hair styling	0,10%
Bath cleansing products (e.g. soaps, shower gel, rinse-off conditioner, shampoo)	0,20%

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

SCCS overall comment on acute oral toxicity - Submission II
Although LD50 in rats has been reported to be > 2000 mg/kg bw, the acute toxicity of Acetylated Vetiver Oil (AVO) cannot be evaluated as the composition of the test substances used in the submitted acute toxicity studies is not provided.

Ref. 16, 48 and 70 in Submission II

SCCS comment - Submission III
The SCCS has noted the analyses of the different samples of AVO, and has considered that the range of this variability can be accepted for samples of natural origin. Therefore the SCCS accepts the outcome of the acute oral toxicity studies. In view of the data provided AVO can be regarded as acutely orally nontoxic.

3.3.1.2 Acute dermal toxicity

SCCS overall comment on acute dermal toxicity - Submission II
The study could not be evaluated by the SCCS as the submitted original report only consisted of two pages in addition to the front page. The composition of the test substance is not known to the SCCS.

Ref. 16 in Submission II

3.3.1.3 Acute inhalation toxicity

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3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

SCCS overall comment on skin irritation - Submission II
Under the conditions of the OECD TG 404 study, the test substance is mildly irritating to rabbit skin. The SCCS noted that signs of skin irritation (slight to moderate erythema and oedema during the observation period) were also observed in the acute dermal toxicity study performed with a test substance labelled RIFM # 71-90' (Ref 16 in Submission II) (described as a brown liquid, no information on the ester content).
Based on the submitted studies the skin irritation potential of AVO cannot be evaluated as only partial and insufficient information on the composition of AVO on the market is reported and as the composition of the test substances used in the submitted skin irritation studies is not known to the SCCS.

SCCS comment - Submission III
The SCCS has noted the analyses of the different samples of AVO, and has considered that the range of this variability is acceptable for samples of natural origin. Therefore the SCCS has accepted the outcome of the irritation studies. In view of the data provided, AVO can be regarded as mildly irritating to rabbit skin. The SCCS agrees that the concentrations to be used in consumer products are not expected to carry a risk of skin irritation to the consumer.

3.3.2.2 Mucous membrane irritation / eye irritation

SCCS overall comment on eye irritation - Submission II
Under the conditions of the two OECD TG 405 studies, the test substances were either mildly irritating or irritating to the rabbit eye. Based on the submitted studies, the eye irritation

potential of AVO cannot be evaluated as only partial and insufficient information on the composition of AVO on the market is reported and as the composition of the test substances used in the submitted eye irritation studies is unknown to the SCCS.

Ref. 58, 59, 72 and 74 in Submission II

SCCS comment - Submission III
The SCCS has noted the analyses of the different samples and has considered that the range of this variability can be accepted for samples of natural origin. Therefore the SCCS has accepted the outcome of the irritation studies. In view of the data provided, AVO can be regarded as mildly irritating to the eye. The SCCS agrees that the concentrations to be used in consumer products are not expected to carry a risk of eye irritation to the consumer.

3.3.3 Skin sensitisation

SCCS overall comment on sensitisation - Submission II
Applicant has submitted Local Lymph Node Assays (LLNA) in which four different qualities of AVO have been tested for skin sensitising potential. Only these four
studies have been evaluated in this Opinion.
All four qualities of AVO tested in the LLNA have been shown to be moderate skin sensitisers. Based on the submitted studies, the skin sensitisation potential of AVO cannot be evaluated as only partial and insufficient information on the composition of AVO on the market is reported and as the composition of the test substances used in the submitted LLNA studies is unknown to the SCCS.

Ref. 79, 80, 81 and 86 in Submission II

SCCS comment - Submission III
The SCCS has noted the analyses of the different samples, and has considered that the range of this variability is acceptable for samples of natural origin. Therefore, the SCCS has accepted the outcome of the different LLNA's that show that the EC3 value of AVO is in the range of 9.3%-13.3%. In view of the data provided, AVO can be regarded as a moderate skin sensitiser.

3.3.4 Toxicokinetics

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3.3.5 Repeated dose toxicity

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

SCCS overall comment on 28 day oral toxicity study - Submission II
Kidney weights were increased in all treated male groups and were accompanied by histopathological changes including hyaline droplets. According to the study report authors, the alpha-2-microglobulin nature of the findings was confirmed by Mallory's Heidenhain staining. The SCCS considers that the exact mechanism by which the test substance used in this 28-day study causes kidney damage in male rats has not been elucidated. The SCCS agrees that the finding of hyaline droplets suggests that the mechanism behind the kidney

effects could be related to the accumulation of alpha-2-microglobulin in the male rat kidney. This mechanism is specific for the male rat and therefore unlikely to occur in humans who do not synthetise a protein equivalent to alpha-2-microglobulin. Kidney damage induced in male rats via alpha-2- microglobulin accumulation has been observed with a variety of hydrocarbons derived from petroleum but also from natural sources such as limonene, a monoterpene, which shares properties with some of the numerous sesquiterpenes in AVO.
Cholesterol, total protein and alanine aminotransferase were significantly increased in females at 1000 mg/kg bw/day with the effect in cholesterol also being observed in the recovery females. Cholesterol and alanine aminotransferase also increased in males at 1000 mg/kg bw/day, although this was not significantly different than in the control group. Relative liver weights increased in animals of either sex in all treated non-recovery groups with an increase of 50-55% in the high-dose group. SCCS considers increased cholesterol and increased relative liver weights of a magnitude above 50% at the highest dose level as adverse effects, although only in the absence of any associated microscopic changes in the liver, as histopathological changes in the liver cannot be expected to be observed in this study because its short duration (28 days).
Based on the findings in this 28-day study, the mid-dose level of 300 mg/kg bw/day is considered as the NOAEL for the test substance used in this study.
However, for AVO, based on the submitted study, a NOAEL for repeated dose toxicity cannot be evaluated as only partial and insufficient information on the composition of AVO on the market is reported and as the composition of the test substance used in the submitted 28-day study is unknown to the SCCS.

Ref. 84 in Submission II

SCCS comment - Submission III
The SCCS has noted the analyses of the different samples and has considered that the range of this variability can be accepted for samples of natural origin. Therefore the SCCS has accepted the outcome of the 28-day oral toxicity study. In view of the data provided, the SCCS confirms the evaluation performed in Submission II, which considers as adverse effects the variations of cholesterol, total protein and alanine transferase concentrations in females treated with 1000 mg/kg bw and the increase of absolute and relative liver weights identifying a NOAEL of 350 mg / kg bw for AVO.
The SCCS noted that the NOAEL value was incorrectly reported as 300 mg/kg bw in Submission II instead of 350 mg/kg bw.

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

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3.3.5.3 Chronic (> 12 months) toxicity

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3.3.6 Reproductive toxicity

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3.3.7 Mutagenicity / genotoxicity

3.3.7.1 Mutagenicity / genotoxicity *in vitro*

Submission II

First Ames study (Ref: 36)

SCCS comment - Submission II
Number of revertants decreased significantly in all 5 strains with S9-mix and in several Salmonella typhimurium strains, also without S9-mix. Because AVO showed bacteriotoxicity, results have limited value. The Applicant did not explain what vetiveryl acetate stab means. SCCS has considered that 'stab' means stabilised with alpha-tocopherol. Consequently this test has no value in the evaluation of AVO mutagenicity.

Ref. 36 in Submission II

Submission III

The obtained results reflect normal biological variations and normal differences in susceptibility. Cytotoxicity is quite often observed in the absence and presence of S9-mix at a varying degree and is known to be different for frame-shift vs. base-pair substitution strains. However, there were sufficiently high concentrations tested without cytotoxicity being apparent. These non-cytotoxic concentrations were in the range between 33 – 333 µg/plate and sometimes even higher in assays with metabolic activation. Without metabolic activation, cytotoxicity, if any, was predominantly observed at 5000 µg/plate. Thus, AVO was tested up to the maximum required concentration of 5000 µg/plate and induced at the higher concentrations, especially with metabolic activation, different degrees of cytotoxicity in this *in vitro* system. However, this should not be considered as a general anti-bacterial property. Toxicity to bacteria in the Ames is a typical observation and is used as a standard criterion to set the dose levels and may be used to demonstrate that adequate exposure has been achieved (SCCS1501/12). The OECD 471 test guideline indicates that the assay may not be suitable for testing some highly bactericidal chemicals (e.g. some antibiotics), but the level of toxicity should be much greater than was seen in this study. European regulators have indicated that the toxicity should be observed at levels below 10µg/plate before the test may be considered not relevant [Reference 12].

Second Ames study (Ref: 77)

SCCS comment - Submission II
Raw data on batch 9000429043 (stab) shows that there was decrease in number of revertants in samples with S9-mix in several Salmonella typhimurium strains showing bacteriotoxicity. No purity data were provided. The Applicant did not explain what vetiveryl acetate stab means. SCCS has considered that 'stab' means stabilised with alpha-tocopherol. Consequently this test has no value in the evaluation of AVO mutagenicity.

Ref. 77 in Submission II

Submission III

The obtained results reflect normal biological variations and normal differences in susceptibility. The arguments with regards to cytotoxicity (decrease in the number of revertants) are the same as above.

Third Ames study (Ref: 78)

SCCS comment - Submission II
Raw data on batch 9000428765 (extra stab) showed that there was a decrease in the number of revertants in samples with S9-mix in several Salmonella typhimurium strains indicating bacteriotoxicity. No purity data were provided. The Applicant did not explain what vetiveryl

acetate extra stab means. SCCS has considered that 'stab' means stabilised with alpha-tocopherol. Consequently this test has no value in the evaluation of AVO mutagenicity.

Ref. 78 in Submission II

Submission III

The obtained results reflect normal biological variations and normal differences in susceptibility. The arguments with regards to cytotoxicity (decrease in the number of revertants) are the same as above.

Fourth Ames study (Ref: 75)

SCCS comment - Submission II
Summary report says that no bacteriotoxicity was found. However, there was a reduced number of revertants reported in raw data as sign of bacteriotoxicity. The Applicant did not explain what vetiveryl acetate extra means. SCCS has considered that 'extra' means stabilised with alpha-tocopherol. Consequently this test has no value in the evaluation of AVO mutagenicity.

Ref. 75 in Submission II

Submission III

Toxicity was observed and was described in the study report, both in terms of effects on the background lawn and in reductions in revertant colony numbers. The summary in the Applicant's submission document did not state that 'no bacteriotoxicity was found', it simply did not mention that toxicity was observed. The SCCS comment seems to be incorrect on this point. In this context we would like to point out that the concentration ranges have been misquoted in the SCCS Opinion. The SCCS summary indicates a maximum dose level of 5 µg/plate, when in fact the maximum dose level used was 5 mg/plate. The maximum dose level used varied between strains and whether S9 was present or not, but only within the range of 0.5 to 5 mg/plate. Consequently, the levels of toxicity observed in this study on batch 20070028 are comparable to those seen in the Ames tests on the other samples.
Overall, this Ames test can be considered as key information within the evaluation of AVO's mutagenic potential. Furthermore, it clearly demonstrates that AVO is not mutagenic in the Ames test even when α-tocopherol has not been added to the preparation.

Fifth Ames study (Ref: 73)

SCCS comment - Submission II
Vetiveryl acetate (batch: 9000360016, ester: 65.0%) was tested with 1% alpha-tocopherol, which is a known antioxidant and can scavenge free radicals and prevent against induction of mutation. Consequently this test has no value in the evaluation of AVO mutagenicity.

Ref. 73 in Submission II

Submission III

The presence of α-tocopherol is considered to have no influence on the outcome of the study, as discussed below.
Overall, this Ames test can be considered as key information within the evaluation of AVO's mutagenic potential.

In vitro mammalian cell gene mutation test (Ref: 91)

SCCS comment - Submission II
The purity of 99% of AVO was assumed by the study report authors because the sample of the test substance contained 1% alpha-tocopherol. The composition of AVO is not known.
Only a short-term treatment experiment (3h with and without metabolic activation) was performed. This treatment may have been too short to discriminate mutagenicity, as for some compounds, for example compounds active in certain stage of cell cycle, the short treatment is not sufficiently long and longer, e.g. 24h treatment is needed.
AVO was tested with 1% alpha-tocopherol alpha and there is no justification for that. SCCS objects to testing AVO with alpha-tocopherol. Consequently, this test has no value in the evaluation of AVO mutagenicity.

Ref 91 in Submission II

Submission III

There was only a minor deviation to guideline requirements as in experiment 1 without metabolic activation only three instead of at least required 4 concentrations were in the range of acceptable cytotoxicity for evaluation.
The short treatment of 3 hours with and without metabolic activation is in line with OECD 476 guideline requirements. There is no specific requirement to test longer exposure periods. It is not correct to suggest that the mammalian cell assay was deficient because it did not include a group with a long exposure period. Long exposure periods (those that cover more than the time required for 1 cell cycle) are relevant for clastogenicity and aneuploidy but are not known to be relevant for gene mutations. The OECD 476 guideline does not require a long exposure period, although it is recommended for the L5178Y TK assay because it may detect both mutation and clastogenicity. In this case, the study used was the L5178Y HPRT assay, which does not detect clastogenic events. Indeed the latest draft of the revised OECD 476 guideline, which specifically excludes the TK assay, makes no mention of an extended exposure period (Paragraph 25 states 'Proliferating cells are treated with the test substance in the presence and absence of a metabolic activation system. Exposure should be for a suitable period of time (usually 3 to 6 hours is adequate)'.

In vitro mammalian cell chromosomal aberration test (Ref. 83)

SCCS comment - Submission II
Precipitation already occurred in relatively low concentrations in both experiments, both with and without S9-mix. Additionally, in one experiment, a statistically significant concentration-dependent increase in the number of cells with aberrations was observed. AVO was tested with 1% alpha-tocopherol and there is no justification for that. However, SCCS considers this test as positive.

Ref. 83 in Submission II

Submission III

Precipitation occurred in experiment 1 at ≥ 30 µg/mL (without S9 mix) and at ≥ 60 µg/mL (with S9 mix), while in experiment 2 precipitation was noted at 50 µg/mL (with S9 mix) but not without S9 mix up to 25 µg/mL. In each experiment a sufficient number of concentrations were available for evaluation. It is noteworthy to mention that precipitation was not observed in the test mentioned below in Human peripheral lymphocytes even at higher concentrations, using the same solvent (DMSO) and same metabolic activation system. Only the culture media were different, however, the presence of erythrocytes in the human lymphocyte cultures will have made the observation of precipitate very difficult (see next section). When the

concentration-response relationship is considered, only in experiment 1 was a statistically significant increase in cells showing structural chromosome aberrations noted at the highest evaluable concentration of 60 µg/mL in the presence of metabolic activation. The structural aberrations occurred predominantly in the form of breaks and no chromatid exchange aberrations were observed. The number of cells showing numerical aberrations at this concentration was neither biologically relevant nor statistically significantly increased. The incidence of 4.5% was just outside the incidence of the historical controls (0 - 3.5%). However, the number of historical control experiments (N = 14) can be considered as relatively low. Moreover, this finding was only observed in one of the duplicate cultures. Thus, the biological relevance of this isolated finding at the highest and precipitating concentration is considered questionable. Furthermore, the weak response was not reproduced in the second experiment. It should be noted that CHO cells are recognised as having a relatively high and highly variable spontaneous frequency of cells with aberrations. Overall, this *in vitro* mammalian cell chromosomal aberration test performed in CHO cells is considered as negative and can be used as supportive information within a weight of evidence evaluation of AVO's mutagenic potential. As explained below, the presence of α-tocopherol is considered to have had no impact on the outcome of the study. Furthermore, the original conclusion of the Study Director of the sample being non-clastogenic is supported by the clear negative result of the study performed in human lymphocytes (Reference 85, discussed below).

***In vitro* mammalian cell chromosomal aberration test in Human peripheral blood lymphocytes (Ref: 85)**

SCCS comment - Submission II
AVO was tested with 1% of alpha-tocopherol. The Applicant did not explain why alpha-tocopherol was used. Alpha-tocopherol is a known antioxidant and can scavenge free radicals and prevent against the induction of mutation. Consequently this test has no value in the evaluation AVO mutagenicity.

Ref. 85 in Submission II

Submission III

No precipitation was observed in experiment 1 up to scorable concentrations of 60 µg/mL (without S9 mix) and 120 µg/mL (with S9 mix) or in experiment 2 up to scorable concentrations of 120 µg/mL (without S9 mix) and 80 µg/mL (with S9 mix). However, this does not mean that precipitation did not occur. In this study type whole blood cultures are used and the presence of erythrocytes makes it extremely difficult to observe precipitates. Normally, parallel cultures without the addition of blood are prepared as part of the range-finding experiment and these are used for the precipitate observations. In this study there was no range-finding experiment because the dose ranges were based on the CHO study. It can be reasonably assumed that precipitation would have been similar in this study to that observed in the CHO study.
As explained below, the presence of α-tocopherol is considered to have had no impact on the outcome of the study. Overall, this *in vitro* mammalian cell chromosomal aberration test performed in Human peripheral blood lymphocyte is considered as key information within a weight of evidence evaluation of AVO's mutagenic potential.

SCCS overall comment on mutagenicity / genotoxicity - Submission II
Overall, the genotoxicity of AVO was exclusively investigated in a gene mutation test in bacteria. This study was not finished. In the study reports No's 293M99, 361M99, 373MOO (batch: 9000360016) it is stated: "Vetiveryl Acetate has been evaluated for genotoxic activity using the Salmonella/mammalian microsome (Ames) test. Initially a batch (9000317035) with a degree of purity (ester component) of 65.9% was subjected to a range finder assay with strain TA100 (Study No. 293M99, GLP study). Since the batch was found to cause an increase

of the mutation frequency starting at a dose of 500 µg/plate, the experimentation with this batch was terminated. A series of further preparations were investigated in strain TA100 (Study No. 361M99; non-GLP study) to assess possible impurity or degradation-related effects. It was realised that addition of Tocopherol alpha was capable of abolishing the mutagenic activity of Vetiveryl Acetate. A new preparation of the test material containing Tocopherol alpha was, therefore, subjected to a complete Ames test (Study No373MOO)."
A full study report from this study was not provided.
All available studies were performed with AVO containing 1% alpha-tocopherol. The latter is known to have antibacterial properties as well as to be an antioxidant that can scavenge free radicals and as such prevent induction of gene mutations. Consequently, tests with AVO containing 1% alpha-tocopherol have no value in the evaluation of the genotoxic potential of AVO alone. Therefore, on the basis of the results from the study mentioned above AVO has to be considered genotoxic.
AVO containing 1% alpha-tocopherol was tested for mutagenicity/genotoxicity for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Exposure to AVO with alpha-tocopherol did not result in an increase in gene mutations in bacteria nor in mammalian cells. However, in the mammalian gene mutation test only a short term treatment protocol was used which may have been too short to discriminate a mutagenic potential. AVO containing 1% alpha-tocopherol did induce a slight but significant increase in cells with chromosome aberrations in CHO cells but not in human peripheral blood cells.
Based on the submitted studies, the mutagenic/genotoxic potential AVO cannot be evaluated as only partial and insufficient information on the composition of AVO on the market is reported and as the composition of the test substances used in the submitted mutagenic/genotoxic studies is not provided to the SCCS.

SCCS overall comment on *in vitro* mutagenicity/genotoxicity testing - Submission III
Based on available data and additional explanations provided by the Applicant the SCCS is of the following opinion:

1. Review of analytical data from 2007 and 2015 shows the constituents of AVO to be comparable over an extended period of time. As such, the composition of the 2003 test item can be considered equivalent to analytical data associated with 'Sample n' (2007) and 'Sample 18' (2015), all three samples coming from the same producer, with no intentional changes to the manufacturing process having taken place during this period.
2. AVO with 1% TP was tested in 4 GLP-compliant bacterial gene mutation studies with negative results (ref. 73-76-77-78 Submission II). The Applicant stated that another study reported in Submission II under ref. 75 showing negative result was conducted with AVO without TP.
3. AVO with 1% TP was tested in one GLP-compliant mammalian cells gene mutation study with negative result, which confirms the lack of gene mutation capability of AVO with 1% TP.
4. The Applicant did not provide any micronucleus test as preferred in the SCCS Notes of Guidance. Although equivocal result was observed in chromosomal aberration test on CHO cells with AVO with 1% TP, the chromosomal aberration test on human lymphocytes was negative.
5. Based on all data provided, the SCCS considers that AVO added with 1% TP, as used in the final products, is not likely to pose a risk of mutagenicity.

3.3.7.2 Mutagenicity / genotoxicity *in vivo*

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3.3.8 Carcinogenicity

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3.3.9 Photo-induced toxicity

3.3.9.1 Phototoxicity / photo-irritation and photosensitisation

In vitro

SCCS overall comment on phototoxicity *in vitro* - Submission II
A UV/vis absorption spectrum of the test item should be present.
Because of precipitation, the first study RIFM# 63844 (Ref. 90 in Submission II) using the NRU phototoxicity assay with Balb/c 3T3 mouse fibroblasts cannot be used to assess the phototoxicity of AVO. Likewise, the second follow-up study, RIFM# 63835 (Ref. 88 in Submission II) using an EpiDerm 3D skin model cannot be used either, because no positive control was included. Thus, based on the submitted data, the *in vitro* phototoxic potential of AVO cannot be evaluated.
In addition, only partial and insufficient information on the composition of AVO on the market is reported (see 3.1.4) and the composition of the test substances used in the submitted *in vitro* phototoxicity studies is also unknown to the SCCS.

Ref. 88 and 90 in Submission II

Submission III

First study
The UV/vis absorption spectra of current samples of AVO have been determined (Reference 10 in Ref 2 Submission III). The spectra demonstrate that the level of absorbance in the critical range is low and the potential for photoactivation is correspondingly low. The Applicant agrees that the results obtained with this NRU uptake phototoxicity assay in Balb/c 3T3 mouse fibroblasts *in vitro* are not robust. This is particularly due to the limited solubility of the test item and other observed limitations. Therefore, this information was mainly provided for sake of completeness and to aid an overall weight-of-evidence conclusion.

Ref. 90 in Submission II
Ref. 2

Second study
For information on composition of the tested sample and the UV spectrum, please see above. The UV/vis absorption spectra of current samples of AVO have been determined and are attached [Reference 10 in Ref 2 Submission III. The spectra demonstrate that the level of absorbance in the critical range is low and the potential for photoactivation is correspondingly low.
No specific guideline for photo-toxicity testing on the three dimensional human epidermis model (EpiDerm™) is available. However, the test using batch VE00196943 was technically correct and performed as the experimental design followed the MatTek Corporation phototoxicity protocol for use with EpiDerm™ under GLP conditions. Reporting and assessment can be considered as appropriate. As no guideline is available, there is no formal need to include a positive control. The MatTek protocol states 'For the present study, it is not necessary to include a positive control into each phototoxicity test as this reduces the number of concentrations of the test material. When the assay is newly established perform a full experiment with five concentrations of Chlorpromazine (dissolved in H2O) ranging from 0.001% to 0.1%. Repeat this test on a regular basis.' The laboratory performed internal validation phase positive control. The final study report is attached and the Applicant considers that the study is valid and demonstrates that Acetylated Vetiver Oil (AVO) has no phototoxic potential.

Ref. 88 in Submission II
Ref. 2

In vivo

SCCS overall comment on phototoxicity *in vivo* - Submission II
No information was provided on the composition of the test substance. The *in vivo* data on phototoxicity / photoirritation cannot be evaluated by the SCCS as the submitted reference only consists of 3 pages: a cover letter; a table summarising the results for nine compounds tested, including '5-vetiver acetylated 72-236'; and the last page featuring a spectrum for 'vetiver acetylated 72-236'.

Ref. 20 in Submission II

Submission III

The Applicant agrees that these data are of limited value and were supplied mainly for sake of completeness and to aid an overall weight-of-evidence conclusion.

SCCS comment on phototoxicity - Submission III
The SCCS noted the absence of a positive control in the second *in vitro* study with reconstructed human skin but has taken note of the internal validation with a positive control. The submitted data do not point towards phototoxicity.

Ref. 88 in Submission II

3.3.9.2 Photomutagenicity / photoclastogenicity

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3.3.10 Human data

SCCS overall comment on human data - Submission II

HRIPT
No information was provided on the composition of the test substances. The experimental detail is deficient in that the concentrations of the applied AVO are not stated in the report (RIFM # 54473) (Ref. 82 in Submission II). The tabulated data from show +/- reactions on challenge in 3 subjects out of 112 tested. The report does not identify exactly what are the constituents of the tested preparations labelled as H383-1, H373-2 and H373-3. The SCCS considers the HRIPT unethical.
No information was provided on the composition of the test substance in the Report RIFM # 63834. (Ref. 87 in Submission II)

Phototoxicity
The available test results do not indicate phototoxic potential.

Ref. 82 and 87 in Submission II

SCCS comment on human data - Submission III
The SCCS has noted the analyses of the different samples of AVO, and has considered that the range of this variability can be accepted for samples of natural origin. Therefore, the SCCS has accepted the results of the studies, indicating no sensitisation or phototoxic potential.
Furthermore, no report on photoxicity or photosensitisation could be identified in the public literature.

Ref. 82 and 87 in Submission II

3.3.11 Special investigations

Further assessment of toxicological hazard was carried out by the Applicant using *in silico* methods to provide additional supporting evidence for the safety of the identified components by dividing them into four chemical groups, which account for 93.1% of the total AVO constituents, acetates (44.2%), sesquiterpenes (32.6%), ketones (13.2%) and aldehydes (3.10%). The remaining 9 constituents represent <6% AVO. All constituents were treated as TTC Cramer Class III (worst case) using the Class III threshold value of 1.5 µg/kg/day. The Skin Absorption Model and the Skin Perm Model were used to calculate the maximum skin absorption over 24 hours exposure (worst case) for the three highest average percentage identified constituents from each of the four chemical groups. The resulting MOS for each product type alone, or when used together, indicated that the use of AVO at the intended concentrations in different product types as proposed by the Applicant is not likely to pose a health risk to the consumer.

Ref. 4

SCCS comment
The Applicant assessed AVO components according to TTC approach. However, a higher (7.9 µg/kg/day) than agreed threshold value (1.5 µg/kg/day) was proposed by the Applicant. The SCCS did not agree to the use of the higher threshold value in accordance with the SCCS Notes of Guidance (2016) and hence the TTC assessment provided by the Applicant was not taken into consideration by the SCCS.

3.4 EXPOSURE ASSESSMENT

Submission III

The total aggregated SED for the consumer, when calculated as described below, is a conservative but also realistic estimate of daily consumer exposure because it is based on real-life usage data of consumer products and experimentally measured exposures. The total 95th percentile systemic aggregate exposure to AVO, calculated from the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; 2017; Safford et al., 2015; 2017) is 71.1 µg/kg/day, based on the maximum product concentration limits provided in the SCCS mandate and assuming 100% skin absorption. A description of how this value is derived is included in Appendix 7 of Ref 4.
It is worth noting as a layer of conservatism in the Applicant's approach, that a lower estimate would have been obtained if variability in actual AVO concentrations reported from industry surveys of marketed products was incorporated in the calculation that has been performed with the Creme RIFM aggregate exposure model. The reported exposure to AVO based on use surveys, which reflect actual use levels of AVO in products performed routinely by the industry, is indeed lower. A survey was performed in July 2014 and an aggregated exposure value of 7.92 µg/kg/day was obtained. In a later survey of August 2016, a value of 3.73 µg/kg/day was obtained. The similarity of these two values provides a degree of confidence in the validity of the results. The value of 71.1 µg/kg/day represents the situation where every product that every user consumes contains the same, very high, concentration of AVO. In reality, the concentration of AVO is almost always lower than these values and has a wide variation between products; this variation can span several orders of magnitude.
One of the advantages of the Creme RIFM model is that it incorporates such variation in concentration into the exposure calculation. Individuals using products with low concentrations of AVO have a lower exposure to AVO and, given the variability of AVO

concentration, almost all individuals will be using such products. So, in reality, the estimated figure with the maximum limits stated in the mandate greatly overstates the aggregate exposure to AVO.

Ref. 4

SCCS comment

The Applicant used a more refined approach to assess the exposure to AVO in cosmetic products. Instead of using default values to estimate some parameters used to calculate the exposure, data-based values or modelled values using more realistic input variables were included in the assessment (Ref 4). The SCCS recognises the value of such a refined approach. However, the assumptions that are used as the basis for such calculations, as well as the input parameters and default variables, have to be justified. In particular, SCCS considers that the presence probability should not be considered for regulatory risk assessment as the trends in the market cannot be accurately predicted.

For MoS calculations, the SCCS used SED of 286.34 µg/kg/day derived from the classical deterministic approach using the following assumptions (Table 6):

1) AVO is present in all cosmetic categories which were considered by the Applicant to be a likely source of exposure to AVO,
2) each product contains the maximum industry use level of AVO ,
3) the exposure to the amount of product containing AVO including frequency of use is based on a maximised calculation (SCCS Notes of Guidance, 2016),
4) the aggregate exposure is based on a summation of individual product exposures, i.e. assumes that all the products under consideration are used at the same time at the highest concentration.
5) a default value of 50% skin absorption was used for AVO .

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

The SCCS applied a conservative approach to determine SED by applying a default 50% dermal absorption value as shown in Table 6 (cf. SCCS 10th revision of the Notes of Guidance (SCCS/1602/18)).

The deterministic aggregated systemic exposure dose for consumers (286.34 µg/kg/day) was used to calculate the Margin of Safety (MoS). For this, the NOAEL of 350 mg/kg bw, derived from the 28-day study, was extrapolated to a 90-day study by applying a safety factor of 3. The SCCS has used an oral bioavailability of 50%.

The resulting $NOAEL_{sys}$ of 58.33 mg/kg bw was used for the calculation of MoS. (Table 6)

Table 6. Margin of Exposure calculation

Categories of products	Concentration of Vetiver Oil (%)	SED (mg/kg bw/d)	$NOAEL_{sys}$ (mg/kg bw/day)	MoS
Hydroalcoholic-based fragrances (e.g. Eau de Toilette, Perfume, Aftershave, Cologne) §	0,90	0,0210	58,33	2778

Deodorants	0,05	0,0063	58,33	9333
Make up products (e.g. eye make-up, make-up remover, liquid foundation, mascara, eyeliner, lipstick)	0,05	0,0047	58,33	12499
Face cream	0,10	0,0128	58,33	4545
Hand cream	0,10	0,0180	58,33	3241
Body lotion	0,10	0,0652	58,33	895
Hair styling	0,10	0,0033	58,33	17499
Bath cleansing products (e.g. soaps, shower gel, rinse-off conditioner, shampoo)	0,20	0,0090	58,33	6481
Aggregated SED for consumer		0,140	58,33	416

§Amount of products applied on the skin (female 0.28 g or 4.67 mg/kg bw/day) Laboratoir d'Evaluation du Risque Chimique pour le Consummateur (LERCCo) 2017. Exposition de la Population Francaise aux Produits cosmetiques. Anne-Sophie Ficheuxand Alain-Claude Roudot.

The resulting MOS for each product type alone, or when used together, indicated that the use of AVO at the intended concentrations in different product types as proposed by the Applicant is not likely to pose a health risk to the consumer.

3.6 DISCUSSION

Physicochemical properties
AVO is the acetylated form of a natural fragrance (vetiver oil), which is composed of around 129 constituents. Data presented by Industry (13 May 2015) (Ref 2) concerned the analysis of 18 samples of different AVO batches produced by 10 manufacturers comparing analytical data from 2007 and 2015 shows that the range of variability of the constituents of Acetylated Vetiver, considered during an extended period of time, can be accepted for samples of natural origin. The SCCS has considered this variation acceptable for a plant-derived material of natural origin and on the basis of this presumption SCCS considered AVO as a single entity on which to assess the toxicity.

General toxicological evaluation
In view of the data provided, the SCCS confirms the evaluation performed in Submission II considering as adverse effects the variations of cholesterol, total protein and alanine transferase concentrations in females treated with 1000 mg/kg bw and the increase of absolute and relative liver weights. Based on these data, the NOAEL is set at 350 mg/kg bw.

Skin sensitisation
Based on the animal studies, AVO can be regarded as a moderate skin sensitiser. AVO did not induce skin sensitisation in human RIPT study. In the public literature there are no reports on sensitisation from AVO in humans.
Considering the results of the HRIPT study and the fact that AVO has been used for years in cosmetics without evidence of sensitising potential, it is unlikely that AVO would be causing contact allergy in humans.

Inhalation toxicity
No data have been provided on inhalation toxicity of AVO.

Mutagenicity / genotoxicity
AVO added with 1% tocopherol (TP) was tested in 4 GLP-compliant bacterial gene mutation studies with negative results. Additionally AVO without tocopherol was tested in one GLP-compliant study also with negative result. AVO added with 1% tocopherol (TP) was tested in 1 GLP-compliant mammalian cells gene mutation study with negative result.
The Applicant did not provide any micronucleus test as preferred in the SCCS Notes of Guidance. Although equivocal result was observed in chromosomal aberration test on CHO cells with AVO added with 1% TP, the chromosomal aberration test on human lymphocytes was negative.
The concentrations of AVO intended to be used in cosmetic products are very low. Additionally, in view of the likely low bioavailability of different AVO components, the SCCS considers that AVO added with 1% TP, as used in the final products, is not likely to pose a risk of mutagenicity.

Photo-induced toxicity
The submitted data do not point towards phototoxicity. In the public literature, there are no reports on phototoxicity from AVO in humans.

4. CONCLUSION

1. On the basis of currently available information, does the SCCS consider Acetylated Vetiver Oil (AVO) safe for use as fragrance ingredient in cosmetic leave-on and rinse-off type products in a concentration limit(s) according to the once set up by IFRA as reported above?

On the basis of the safety assessment carried out using a conservative approach, the SCCS considers the use of Acetylated Vetiver Oil (AVO, CAS 84082-84-8) with 1% alpha-tocopherol as a fragrance ingredient in cosmetic leave-on and rinse-off type products safe at the concentrations proposed by IFRA.

2. Does the SCCS have any further scientific concerns with regard to the use of Acetylated Vetiver Oil (AVO) as fragrance ingredient in cosmetic leave-on and rinse-off type products?

Acetylated Vetiver Oil (AVO) contains some constituents that belong to the chemical group of aldehydes and ketones that are known to be reactive towards biological entities, such as DNA and proteins. However, the overall health risk of such components is likely to be negligible at the concentrations intended to be used in cosmetics products.

The SCCS has noted that Acetylated Vetiver Oil (AVO) is a moderate skin sensitiser in test animals. Considering the results of the HRIPT study and the fact that AVO has been used for years in cosmetics without evidence of sensitising potential, it is unlikely that AVO would be causing contact allergy in humans.

Inhalation toxicity of Acetylated Vetiver Oil (AVO) was not assessed in this Opinion because no data were provided. Assessment of the inhalation risk would be needed if AVO was intended to be used in sprayable products.

5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

See SCCS/1602/18, 10th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 141

8. LIST OF ABBREVIATIONS

See SCCS/1602/18, 10th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 141

And the following additional Abbreviation:

AVO: Acetylated Vetiver Oil