Scientific Committee on Consumer Safety

SCCS

OPINION ON

fragrance ingredient

Acetylated Vetiver Oil (AVO)

Submission III

The SCCS adopted this document at its plenary meeting on 21-22 June 2018
ACKNOWLEDGMENTS

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http://ec.europa.eu/health/scientific_committees/experts/declarations/sccs_en.htm
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) added 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 based on animal studies.

In this Opinion SCCS did not assess aerosolised or sprayable application that could lead to exposure of the consumer’s lung by inhalation.

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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<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ABSTRACT .................................................................................................................. 3</td>
</tr>
<tr>
<td>2. MANDATE FROM THE EUROPEAN COMMISSION ............................................................... 6</td>
</tr>
<tr>
<td>3. OPINION .................................................................................................................... 8</td>
</tr>
<tr>
<td>3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS ............................................................ 8</td>
</tr>
<tr>
<td>3.1.1 Chemical identity .................................................................................................. 8</td>
</tr>
<tr>
<td>3.1.2 Physical form ........................................................................................................ 13</td>
</tr>
<tr>
<td>3.1.3 Molecular weight .................................................................................................. 13</td>
</tr>
<tr>
<td>3.1.4 Purity, composition and substance codes ............................................................. 13</td>
</tr>
<tr>
<td>3.1.5 Impurities / accompanying contaminants ............................................................ 17</td>
</tr>
<tr>
<td>3.1.6 Solubility ................................................................................................................ 17</td>
</tr>
<tr>
<td>3.1.7 Partition coefficient (Log P_{ow}) ........................................................................ 17</td>
</tr>
<tr>
<td>3.1.8 Additional physical and chemical specifications .................................................. 18</td>
</tr>
<tr>
<td>3.1.9 Homogeneity and Stability ................................................................................... 18</td>
</tr>
<tr>
<td>3.2 FUNCTION AND USES .............................................................................................. 19</td>
</tr>
<tr>
<td>3.3 TOXICOLOGICAL EVALUATION .............................................................................. 19</td>
</tr>
<tr>
<td>3.3.1 Acute toxicity ........................................................................................................ 19</td>
</tr>
<tr>
<td>3.3.2 Irritation and corrosivity ...................................................................................... 20</td>
</tr>
<tr>
<td>3.3.3 Skin sensitisation .................................................................................................. 23</td>
</tr>
<tr>
<td>3.3.4 Toxicokinetics ....................................................................................................... 24</td>
</tr>
<tr>
<td>3.3.5 Repeated dose toxicity ......................................................................................... 24</td>
</tr>
<tr>
<td>3.3.6 Reproductive toxicity ............................................................................................ 25</td>
</tr>
<tr>
<td>3.3.7 Mutagenicity / genotoxicity .................................................................................. 25</td>
</tr>
<tr>
<td>3.3.8 Carcinogenicity .................................................................................................... 34</td>
</tr>
<tr>
<td>3.3.9 Photo-induced toxicity .......................................................................................... 34</td>
</tr>
<tr>
<td>3.3.10 Human data ......................................................................................................... 36</td>
</tr>
<tr>
<td>3.4 EXPOSURE ASSESSMENT ......................................................................................... 37</td>
</tr>
<tr>
<td>3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS) .................... 38</td>
</tr>
<tr>
<td>3.6 DISCUSSION ............................................................................................................. 39</td>
</tr>
<tr>
<td>4. CONCLUSION ............................................................................................................. 41</td>
</tr>
<tr>
<td>5. MINORITY OPINION .................................................................................................. 41</td>
</tr>
<tr>
<td>6. REFERENCES .............................................................................................................. 42</td>
</tr>
<tr>
<td>7. GLOSSARY OF TERMS .............................................................................................. 45</td>
</tr>
<tr>
<td>8. LIST OF ABBREVIATIONS .......................................................................................... 45</td>
</tr>
</tbody>
</table>
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)\(^2\).

In December 2014, the Scientific Committee on Consumer Safety (SCCS) adopted an opinion on Vetiveryl acetate (SCCS/1541/14)\(^3\). 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.


Table with concentration limits for Acetylated Vetiver Oil (AVO)

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

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

From submission II

SCCS comment

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).

In reply to the SCCS comments, the Applicant stated that ‘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) CAS 84082-84-8

As for submission of May 13, 2015 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

### From submission II

In reply to the SCCS comments on missing/wrong citation of references, the Applicant stated that the citation of Reference 94 by SCCS on page 7 should have read Reference 95 in submission II (Vetiveryl acetate industry consortia (2012)). The information provided in Ref. 95 in submission II was based on the Applicant’s knowledge at the time of submission, yet more detailed and robust information are now provided.

Ref. 95 in submission II

### 3.1.1.4 CAS / EC number

Acetylated Vetiver Oil (AVO) CAS 84082-84-8
CAS: 62563-80-8
EINECS: 263-597-9
CAS: 68917-34-0
CAS: 73246-97-6
CAS: 84082-84-8
Opinion on fragrance ingredient Acetylated Vetiver Oil (AVO) - submission III

SCCS/1599/18

Preliminary Opinion

EINECS: 282-031-1

From submission I

SCCS comment

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 the description (see 3.1.4), Vetiveryl acetate is a mixture of many constituents. The rational for reporting up to six CAS and/or EC No. of Vetiveryl acetate is not given. According to the Reference AR1 in submission II, Vetiveryl acetate has CAS No. 68917-34-0. According to the Reference AR2 in submission II, Vetiveryl acetate has CAS No. 62563-80-8. Reference 13 in submission II, NCI (National Chemical Inventories database, 2012) Issue 1. American Chemical 36 Society, was not submitted.

In reply to the SCCS comment, the Applicant agreed that the number of CAS numbers that are available 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.

SCCS Comment on chemical identity, names and CAS numbers

It was 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.

SCCS/1599/18

Preliminary Opinion
Since the focus of safety assessment is on Acetylated Vetiver Oil (AVO) (acetylated extract of Vetiveria zizanoides, CAS 84082-84-8), the SCCS asked the Applicant to provide an unambiguous clarification on the apparent use of test substances belonging to different CAS numbers in the toxicological studies.

In reply, (Ref 2), 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).

A summary of the explanation provided by the Applicant is as follows:

'Acetylated Vetiver Oil (AVO) used by the industry in the supply chain. However, this was incorrect, and we would like to reiterate the fact that Acetylated Vetiver Oil (AVO) is truly a mixture, i.e. reaction mass, obtained from a naturally occurring Natural Complex Substance, of various constituents as outlined in our detailed analytical work from 2015 (Ref 2). Other related CAS numbers, e.g. 62563-80-8, 68917-34-0 and 73246-97-6, are relics from the historical process of substance registrations around the globe and may still be used to describe the exact same material in other regions of the world. As per our submission of May 13, 2015 (Ref 2) and the recent EU REACH submission (see link to the ECHA dissemination website, http://echa.europa.eu/information-on-chemicals), the industry has agreed to use CAS 84082-84-8 to represent the product in Europe. The only difference among the materials related to CAS 84082-84-8, and as described in pages 5-8 of Annex I of our 2015 reply (13 May 2015, Industry reply to SCCS Opinion SCCS/1541/141 – Ref 25), stems from the sequence of production processes. That is, whether first the so-called alcoholic fraction of the starting material, the Essential Oil of Vetiver, is concentrated by distillation and then acetylated, or whether the acetylation takes place on the entire Essential Oil and then the relevant components of the commercial grades are concentrated by distillation. With our detailed overview of analyses from 2015, which includes oils sourced from different regions in the world, we hope to have made visible to you that this does not alter the constituents in a way that would justify separate assessments.

We would also like to highlight the fact that the fragrance material Acetylated Vetiver Oil (AVO) has recently been registered under REACH with exactly the same prerogative, i.e. CAS 84082-84-8.'

Hence, we would also recommend using this CAS number 84082-84-8 and its description as the sole entry in the CosIng Database.

Each and every test carried out on the commercial fragrance material Acetylated Vetiver Oil (AVO) and included in the overview table of the studies presented in Submission II Dossier of 11 June 2013 (Ref 11), has been conducted on the mixture of many constituents described in our previous submissions. Although the study reports state Vetiveryl Acetate, and many of them identify CAS 117-98-6, in reality the identity of the test article used in the studies was what we describe now 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

From submission II

SCCS comment
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:

\[ \text{Structural formula} \]

In reply to the SCCS comment, the Applicant stated that 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

From submission II

SCCS comment
According to IFRA standard (44th Amendment), Vetiveryl acetate, CAS 117-98-6, has the empirical formula C17H26O2.

Empirical formula of a mixture of many constituents, (see 3.1.4) is not possible.

Applicant reply
The Applicant agrees with the SCCS comment and 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

From submission II

SCCS comment

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.

In reply to the SCCS comments, 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.
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.

According to the Applicant, the SCCS Notes of Guidance (2016) are not that detailed in regard to providing a framework for the assessment of materials with complex and variable composition, and the Industry has drawn upon its experience of REACH in order to better characterise the material. 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

<table>
<thead>
<tr>
<th>ID</th>
<th>Constituent</th>
<th>Class</th>
<th>Av %</th>
<th>Min %</th>
<th>Max %</th>
</tr>
</thead>
<tbody>
<tr>
<td>97</td>
<td>Khusimyl acetate</td>
<td>Acetate</td>
<td>13.99</td>
<td>9.57</td>
<td>24.01</td>
</tr>
<tr>
<td>105</td>
<td>(E)-Isovalencenyl acetate</td>
<td>Acetate</td>
<td>13.84</td>
<td>1.81</td>
<td>24.29</td>
</tr>
<tr>
<td>94</td>
<td>Vetiselinenyl acetate</td>
<td>Acetate</td>
<td>6.99</td>
<td>2.89</td>
<td>11.98</td>
</tr>
<tr>
<td>89</td>
<td>beta-Vetivone</td>
<td>Ketone</td>
<td>4.78</td>
<td>3.20</td>
<td>6.58</td>
</tr>
<tr>
<td>37</td>
<td>beta-Vetivenene</td>
<td>Sesquiterpene</td>
<td>2.99</td>
<td>0.00</td>
<td>8.52</td>
</tr>
<tr>
<td>83</td>
<td>Khusian-2-yl acetate</td>
<td>Acetate</td>
<td>2.90</td>
<td>2.10</td>
<td>4.29</td>
</tr>
<tr>
<td>82</td>
<td>Cyclocopacamphanyl acetate B</td>
<td>Acetate</td>
<td>2.69</td>
<td>1.75</td>
<td>3.98</td>
</tr>
<tr>
<td>95</td>
<td>alpha-Vetivone</td>
<td>Ketone</td>
<td>2.42</td>
<td>0.00</td>
<td>4.87</td>
</tr>
<tr>
<td>86</td>
<td>Ziza-6(13)-en-3a-yl acetate</td>
<td>Acetate</td>
<td>2.29</td>
<td>1.78</td>
<td>3.32</td>
</tr>
<tr>
<td>78</td>
<td>Ester SQ m/z 159(100), 91(40), 105(40), 131(35), 187(35), 202(30), 262(5)</td>
<td>Acetate</td>
<td>2.09</td>
<td>1.10</td>
<td>7.97</td>
</tr>
<tr>
<td>79</td>
<td>Cyclocopacamphanyl acetate A</td>
<td>Acetate</td>
<td>1.99</td>
<td>1.31</td>
<td>3.26</td>
</tr>
<tr>
<td>98</td>
<td>Unknown structure MW 262 &amp; 264</td>
<td>Acetate</td>
<td>1.89</td>
<td>1.34</td>
<td>2.91</td>
</tr>
<tr>
<td>52</td>
<td>Unknown mixture MW 200, 202</td>
<td>Ketone</td>
<td>1.66</td>
<td>0.00</td>
<td>4.08</td>
</tr>
<tr>
<td>93</td>
<td>Isokhusimyl acetate</td>
<td>Acetate</td>
<td>1.58</td>
<td>0.00</td>
<td>5.20</td>
</tr>
<tr>
<td>58</td>
<td>13-nor-7,8-Epoxyeremophil-1(10)en-11-one</td>
<td>Ketone</td>
<td>1.55</td>
<td>0.00</td>
<td>4.25</td>
</tr>
<tr>
<td>92</td>
<td>Unknown structure m/z 159(100), 218(20), 202(20)</td>
<td>Ketone</td>
<td>1.30</td>
<td>0.00</td>
<td>2.52</td>
</tr>
<tr>
<td>103</td>
<td>Unknown structure MW 262 m/z 187(100), 202(90) 131(30)</td>
<td>Acetate</td>
<td>1.29</td>
<td>0.00</td>
<td>4.03</td>
</tr>
<tr>
<td>81</td>
<td>Ester SQ m/z 187(100), 159(70), 105(30), 174(30), 202(30)</td>
<td>Acetate</td>
<td>1.11</td>
<td>0.00</td>
<td>4.77</td>
</tr>
<tr>
<td>108</td>
<td>Unknown structure 218(100), 203(60), 176(30), 260(20)</td>
<td>Acetate</td>
<td>1.10</td>
<td>0.00</td>
<td>5.17</td>
</tr>
<tr>
<td>60</td>
<td>Unknown / Mixture</td>
<td>Unidentified</td>
<td>1.03</td>
<td>0.09</td>
<td>1.78</td>
</tr>
<tr>
<td>25</td>
<td>beta-Vetispirene</td>
<td>Sesquiterpene</td>
<td>1.00</td>
<td>0.00</td>
<td>2.79</td>
</tr>
<tr>
<td>28</td>
<td>delta-Amorphone</td>
<td>Sesquiterpene</td>
<td>1.00</td>
<td>0.00</td>
<td>4.11</td>
</tr>
</tbody>
</table>

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:
1. ‘Sample 1’ analysed in 2015 equates to ‘Sample a’ analysed in 2007 (when it was used for testing of sensitisation).
2. ‘Sample 12’ analysed in 2015 equates to ‘Sample g’ analysed in 2007 (when it was used for testing of sensitisation).
3. ‘Sample 18’ analysed in 2015 equates to ‘Sample n’ analysed in 2007 (when it was used as the test material for several endpoints).

Compositions of the three pairs of samples mentioned above were examined thoroughly and the constituents found to be equivalent in 2007 and 2015. 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>
<thead>
<tr>
<th>ID</th>
<th>Constituent</th>
<th>Average from all 2007</th>
<th>Average from all 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>97</td>
<td>Khusimyl acetate</td>
<td>15.37</td>
<td>13.99</td>
</tr>
<tr>
<td>105</td>
<td>(E)-Isovalencenyl acetate</td>
<td>14.80</td>
<td>13.84</td>
</tr>
<tr>
<td>94</td>
<td>Vetiselinenyl acetate</td>
<td>4.44</td>
<td>6.99</td>
</tr>
<tr>
<td>89</td>
<td>beta-Vetivone</td>
<td>4.24</td>
<td>4.78</td>
</tr>
<tr>
<td>82</td>
<td>Cyclocopacamphanyl acetate B</td>
<td>4.06</td>
<td>2.69</td>
</tr>
<tr>
<td>79</td>
<td>Cyclocopacamphanyl acetate A</td>
<td>3.08</td>
<td>1.99</td>
</tr>
<tr>
<td>83</td>
<td>Khusian-2-yl acetate</td>
<td>2.29</td>
<td>2.90</td>
</tr>
<tr>
<td>93</td>
<td>Isokhusimyl acetate</td>
<td>2.23</td>
<td>1.58</td>
</tr>
<tr>
<td>37</td>
<td>beta-Vetivenene</td>
<td>1.87</td>
<td>2.99</td>
</tr>
<tr>
<td>101</td>
<td>Isonootkatyl acetate</td>
<td>1.71</td>
<td>0.40</td>
</tr>
<tr>
<td>59</td>
<td>Ziza-6(13)-en-3-one</td>
<td>1.69</td>
<td>0.72</td>
</tr>
<tr>
<td>95</td>
<td>alpha-Vetivone</td>
<td>1.48</td>
<td>2.42</td>
</tr>
</tbody>
</table>

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.

According to the Applicant, the toxicity profile of AVO as a whole is of importance, as this is the material the consumer will be exposed to via fragrance compounds in cosmetic products. The toxicity of a given single constituent of AVO however is virtually irrelevant, as a multi-constituent substance cannot be adequately characterised in this way. For this reason, emphasis is placed on test results obtained using the characterised multi-constituent substance.

Three additional qualities of AVO (no longer produced by Givaudan) have been analysed in 2007 (origins: Java, Haití 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.
SCCS comment
SCCS agrees that the variability in the composition of the samples from different geographical origins is within acceptable ranges.

3.1.5 Impurities / accompanying contaminants
Presence of residual process chemicals was investigated during analysis of 18 samples in 2015. 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.

SCCS comment
There is no mention of whether there were any leftover concentrations of acetic anhydride/acid, phosphoric acid etc. that were used in the acetylation process.

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

3.1.7 Partition coefficient (Log P_{ow})
SCCS comment - submission II
Not submitted
Applicant reply:
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.
SCCS comment – submission III

As a minimum, calculated LogP values for the known chemical compounds should be provided.

3.1.8 Additional physical and chemical specifications

<table>
<thead>
<tr>
<th>Boiling point: 285 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity: 1</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Nominal concentration (mg/mL)</th>
<th>Concentration found initially (mg/mL)</th>
<th>Concentration found after storage for 23 days (mg/mL) (expressed as % of initial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.75</td>
<td>4.098</td>
<td>4.812 117</td>
</tr>
<tr>
<td>250</td>
<td>284</td>
<td>288 101</td>
</tr>
</tbody>
</table>

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 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 (2016), 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% |
| 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
Based on the submitted studies the acute toxicity of Acetylated Vetiver Oil (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 acute toxicity studies is unknown to the SCCS.

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 unknown to the SCCS.
Ref. 16 in submission II

3.3.1.3 Acute inhalation toxicity
/

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 unknown to the SCCS.

Applicant reply (first study)
The test material used for this investigation was produced prior to the 1999 study date. However, comparison of analytical data from 2007 and 2015 shows the constituents of AVO to be analogous over an extended period of time. As such, the composition of the 1999 test item can be considered equivalent to analytical data associated with 2007 ‘Sample n’ and 2015 ‘Sample 18’.

Please refer to Reference 2 for further information. This animal study should be considered as reliable as it was technically correctly performed according to guideline requirements under GLP conditions and reporting and assessment was appropriate. The ester content of the tested batch 9000317035 as key ingredient was specified as 65.9%, which is in line with commercial products and can be considered as representative. Although the composition was not specified in the test report this animal test should be considered as reliable. Moreover, with regards to the weight of evidence, this result is in line with other study results. Finally, this study should be considered as key study.
Ref: 71 in submission II
Ref. 2
Applicant reply (second study)
This study was reported and submitted as supportive information for sake of completeness. With regards to the weight of evidence, the result is in line with other study results. Pages 1-13 of the document contain the study report and pages 14-31 are copies of the study raw data. As such, these are the best available copies from the study report dating back to 1982 and common for study reports of the pre-GLP era. The ester content of batch PPL is 46% and the general question of AVO composition is addressed in the Applicant response to SCCS comments in Reference 2.

Applicant reply (to SCCS overall comments)
The information of the first study (RIFM 1999b / Reference 71 in Submission II; equivalent to 2007 ‘Sample n’ and 2015 ‘Sample 18’) should be considered reliable as it was technically correctly performed under GLP conditions and parameters comply with guideline requirements. Key information on experimental outline and results were provided. The second study (Ref: 49 in submission II) was supplied as supportive information. All of the study data, whether GLP or non-GLP, support the conclusion submitted by the consortium and it is considered that the skin irritation properties of AVO have been identified. The general question of composition is addressed in the Applicant response to SCCS comments in Reference 2.

At the low level of consumer exposure through the presence of this substance in fragrances as detailed in the original submission, AVO would not be expected to present a risk of skin irritation to consumers.

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.

Applicant reply (first study)
The test material used for this investigation was produced prior to the 1999 study date. However, comparison of analytical data from 2007 and 2015 shows the constituents of AVO to be equivalent over an extended period of time. As such, the composition of the 1999 test item can
Preliminary Opinion

Opinion on fragrance ingredient Acetylated Vetiver Oil (AVO) - submission III

be considered to be in line with analytical data associated with 2007 ‘Sample n’ and 2015 ‘Sample 18’. Please refer to Reference 2.

This animal study should be considered as reliable as it was technically correctly performed according to guideline requirements under GLP conditions and reporting and assessment was appropriate. The ester content of the tested batch 9000317035 as identifying parameter was specified as 65.9\%, which is in line with commercial products and can be considered as representative. The question of composition is addressed in the Applicant response to SCCS comments in Ref: 2. Although the composition was not specified, this animal test should be considered reliable. Moreover, with regards to the weight of evidence, this result is in line with other study results. The neat substance was only initially and slightly irritating. All findings were reversible and the primary irritation index was calculated as 0.00. Thus, the substance can only be considered as slightly irritating. The incidence and severity of findings will not trigger classification.

Ref. 72 in submission II
Ref. 2

**Applicant reply (second study)**

The test material used for this investigation was produced prior to the 2000 study date. However, comparison of analytical data from 2007 and 2015 shows that constituents of Acetylated Vetiver Oil (AVO) are comparable over an extended period of time. As such, the composition of the 2000 test item can be considered to be equivalent to the 2007 analytical data associated with ‘Sample c’. Please refer to Reference 2 for further information.

This animal study should be considered as reliable as it was technically correctly performed according to guideline requirements under GLP conditions and reporting and assessment was appropriate. The ester content of the test item (batch 20070028) as identifying parameter was specified as 85.7\%, which is in line with commercial products and can be considered as representative. Although the exact chemical composition was not specified in the report, this animal test should be considered as reliable. Further information on the general question of AVO composition is available from the Applicant response to SCCS comments in Reference 2. Moreover, with regards to the weight of evidence, this result is in line with other study results. The neat substance can only be considered as moderately irritating but in any case the incidence and severity of findings will not trigger classification.

Ref. 74 in submission II
Ref. 2

**Applicant reply (third study)**

This study was reported and submitted as supportive information for sake of completeness. With regards to the weight of evidence, the result is in line with the study results. The ester content of batch PPL is 46\%. The question of AVO composition is further addressed in the Applicant response to SCCS comments in Reference 2.

Ref. 59 in submission II
Ref. 2

**Applicant reply (fourth study)**

This study was reported and submitted as supportive information for sake of completeness. With regards to the weight of evidence, the result is in line with other study results. The ester content
of batch PPL is 46%. The question of AVO composition is further addressed in the Applicant response to SCCS comments in Reference 2.

Applicant reply (to SCCS overall comments)
The information of the first and second studies (Reference 72 in Submission II, which equates to 2007 ‘Sample n’ and 2015 ‘Sample 18’ (contains 1% α-tocopherol), and Reference 74, which equates to 2007 ‘Sample c’ (contains no α-tocopherol)) should be considered reliable as they were technically correctly performed under GLP conditions and parameters comply with guideline requirements. The results of both studies concur, which provides evidence that the addition of α-tocopherol does not impact the eye irritation potential of AVO. Key information on experimental outline and results were provided. The third and fourth studies (References 58 and 59 in Submission II) were supplied as supportive information.

Overall, the neat substance can only be considered as slightly to moderately irritating but in any case the incidence and severity of findings will not trigger classification.

At the low level of consumer exposure through the presence of this substance in fragrances as detailed in the original submission, Vetiveryl acetate 112 Extra (AVO) would not be expected to present a risk of eye irritation to consumers.

SCCS comment on eye irritation - 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.

Applicant reply
Review of analytical data from 2007 and 2015 shows that constituents of AVO are comparable over an extended period of time. As such, it can be concluded that test materials used during four investigations of skin sensitisation in 2008 were equivalent to qualities analysed in 2007 (Samples a, g, f and n). Furthermore, it can be concluded that material analysed in 2015 (Samples 1, 11 and 18) also represent the test materials used in 2008 to investigate skin sensitisation. Please refer to Reference 2 for further information.
All available local lymph node assays should be considered as reliable as they were technically correctly performed according to guideline requirements under GLP conditions. Key information on experimental outline and results were provided. From an overall assessment point of view, the local lymph node assays should be considered as key information. The weight of evidence of all studies supports the conclusion that AVO has a moderate skin sensitising potential.

**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**

/ 

**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 synthesise 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 monoterpen, 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 on 28 day oral toxicity study - 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

3.3.5.3 Chronic (> 12 months) toxicity

3.3.6 Reproductive toxicity

3.3.7 Mutagenicity / genotoxicity

3.3.7.1 Mutagenicity / genotoxicity in vitro

From submission II

First Ames study (Ref: 36)

SCCS comment
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 suggests ‘stab’ means stabilised with alpha-tocopherol. Consequently this test has no value in the evaluation of AVO mutagenicity.

Ref. 36 in Submission II
Applicant reply

The test material used for this investigation was produced prior to the 2003 study date. However, review of analytical data from 2007 and 2015 shows the constituents of Acetylated Vetiver oil (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. Please refer to Reference 3 for further information.

This Ames test on batch 9000429043 should be considered as reliable as it was technically correctly performed according to guideline requirements under GLP conditions and reporting and assessment was appropriate. 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].

In the context of this study, ‘stab’ means that the sample was stabilised with 1 % α-tocopherol and the sample name ‘Vetiveryl acetate stab. (18 month)’ indicates that it was tested after storage for 18 months. Overall, this Ames test can be considered as key information within the evaluation of Acetylated Vetiver oil’s mutagenic potential.

Second Ames study (Ref: 77)

SCCS comment

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 suggests ‘stab’ means stabilised with alpha-tocopherol. Consequently this test has no value in the evaluation of AVO mutagenicity.

Applicant reply

The test material used for this investigation was produced prior to the 2003 study date. However, review of analytical data from 2007 and 2015 shows that constituents of AVO are equivalent over an extended period of time. As such, the composition of the 2003 test item can be considered to be in line with analytical data from 2007 ‘Sample n’ and 201 ‘Sample 18’. Please refer to Reference 3 for further information.
This Ames test on batch 9000429043 should be considered as reliable as it was technically correct, performed according to guideline requirements under GLP conditions and reporting, and assessment was appropriate. 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.

In the context of this study, ‘stab’ means that the sample was stabilised with 1 % α-tocopherol and the sample name ‘Vetiveryl acetate stab. (24 month)’ indicates that it was tested after storage for 24 months. Overall, this Ames test can be considered as key information within the evaluation of Acetylated Vetiver oil’s mutagenic potential.

Third Ames study (Ref: 78)

SCCS comment
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 suggests ‘stab’ means stabilised with alpha-tocopherol. Consequently this test has no value in the evaluation of AVO mutagenicity.

Ref. 78 in submission II

Applicant reply
The test material used for this investigation was produced prior to the 2003 study date. However, comparison of analytical data from 2007 and 2015 shows the constituents of AVO to be analogous over an extended period of time. As such, the composition of the 2003 test item can be considered equivalent to analytical data associated with 2007 ‘Sample n’ and 2015 ‘Sample 18’. Please refer to Reference 3 for further information.

This Ames test on batch 9000428765 should be considered as reliable as it was technically correct performed according to guideline requirements under GLP conditions and reporting and assessment was appropriate. 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.

Typically the term ‘extra’ in the sample name refers to a higher level of acetates in the substance composition and, in the context of this study, ‘stab’ means that the sample was stabilised with 1 % α-tocopherol. The sample name ‘Vetiveryl acetate extra stab. (24 month)’ indicates that it was tested after storage for 24 months. Overall, this Ames test can be considered as key information within the evaluation of AVO’s mutagenic potential.

Fourth Ames study (Ref: 75)

SCCS comment
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 suggests ‘extra’ means stabilised with alpha-tocopherol. Consequently this test has no value in the evaluation of AVO mutagenicity.

Ref. 75 in submission II
Applicant reply:
The test material used for this investigation was produced prior to the 2001 study date. However, the review of analytical data from 2007 and 2015 shows that constituents of AVO are comparable over an extended period of time. As such, the composition of the 2001 test item can be considered equivalent to the 2007 analytical data associated with ‘Sample c’ coming from the same supplier and with no indications that the manufacturing process had changed in the interim. Please refer to Reference 3 for further information.

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.

This Ames test should be considered reliable as it was technically correctly performed according to guideline requirements under GLP conditions and reporting and assessment was appropriate. ‘Extra’ did not mean that it was stabilised with α-tocopherol and the manufacturer of this sample has confirmed that ‘Extra’ simply means a relatively high ester content (85.7%) of the tested quality. 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
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

Applicant reply
The test material used for this investigation was produced prior to the 2000 study date. However, review of analytical data from 2007 and 2015 shows that constituents of AVO are equivalent over an extended period of time. As such, the composition of the 2000 test item can be considered to be in line with analytical data from 2007 ‘Sample n’ and 2015 ‘Sample 18’, all three samples coming from the same producer with no known changes in the manufacturing process during that period of time. Please refer to Reference 3 for further information.

This Ames test on batch 9000360016 should be considered reliable as it was technically correctly performed according to guideline requirements under GLP conditions and reporting and assessment was appropriate. 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
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

Applicant reply
The test material used for this investigation was produced prior to the 2013 study date. However, comparison of analytical data from 2007 and 2015 shows the constituents of AVO to be equivalent over an extended period of time. As such, the composition of the 2013 test item can be considered to be equivalent to analytical data associated with 2007 ‘Sample n’ and 2015 ‘Sample 18’, all three samples coming from the same producer and with no known changes to the manufacturing process during that period of time. Please refer to Reference 3 for further information. This in vitro mammalian cell gene mutation test on batch VE00231600 should be considered as reliable as it was technically correctly performed according to guideline requirements under GLP conditions and reporting and assessment was appropriate. 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)’.

Overall, this in vitro mammalian cell gene mutation test can be considered as key information within the evaluation of AVO’s mutagenic potential.

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

SCCS comment
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

Applicant reply

The test material used for this investigation was produced prior to the 2010 study date. However, 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 2010 test item can be considered equivalent to analytical data associated with 2007 ‘Sample n’ and 2015 ‘Sample 18’, all three samples coming from the same producer, and no changes to the manufacturing processes during that time are known. Please refer to Reference 3 for further information.

This in vitro mammalian cell chromosomal aberration test in CHO cells using test item batch VE00034228 should be considered as reliable as it was technically correct, performed according to guideline requirements under GLP conditions, and reporting and assessment was appropriate. 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

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

Applicant reply
The test material used for this investigation was produced prior to the 2011 study date. However,
comparison of analytical data from 2007 and 2015 shows the constituents of AVO to be
equivalent over an extended period of time. As such, the composition of the 2011 test item can
be considered to be in line with analytical data associated with 2007 ‘Sample n’ and 2015
‘Sample 18’, all three samples coming from the same producer, with no intentional changes to
the manufacturing processes having taken place during that period of time. Please refer to
Reference 3 for further information.

This in vitro mammalian cell chromosomal aberration test using batch VE00085543 in Human
peripheral blood lymphocytes should be considered as reliable as it was technically correct,
performed according to guideline requirements under GLP conditions, and reporting and
assessment was appropriate.

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
erthrocytes 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 experiment 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% α-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 unknown to the SCCS.

**Overall Applicant reply on submission II**
A full report and the raw data of study number 373MOO are not available to the Applicant, as it represents a preliminary test, not a full study. This study is considered to be not relevant to the question of the potential mutagenicity of marketed samples of AVO today. There is no information available about the quality or source of the sample used in that study and the comment attached to the report is believed to be erroneous, even though given in good faith. The note attached to research report No. 171518 (Reference 3) indicates that batch 9000317035 caused an increase in mutation frequency at 500 μg/plate and that no further work was performed on that batch. It is not clear from the note whether those results were ever duplicated because it simply states ‘...the addition of Tocopherol alpha was capable of abolishing the mutagenic activity of Acetylated Vetiver oil,’ but in this case it was a different batch that was used (9000360016), which could well reflect a different manufacturing process, given the investigative nature of this preliminary study. Therefore, it cannot be determined for certain whether it was the batch difference, the presence of α-tocopherol or in fact a technical error that caused the results seen in the terminated study. However, full GLP and OECD 471 compliant studies are available on samples both containing and not containing α-tocopherol and all provide non-mutagenic results.

α-tocopherol has been shown to be very inefficient at inhibiting the mutagenicity of mutagens, even those that act via reactive oxygen species [Reference 5; Ajith et al, 2008]. A sample of AVO that contains α-tocopherol at 1%, when dosed at 500 μg/plate, would contain 5 μg/plate of α-tocopherol, which is considered to be insufficient to have a significant impact on the mutagenicity of any mutagen, based on the data of Ajith et al, who used it at 5, 10 and 15 mg/plate and achieved only 10.1, 13.9 and 14.4% inhibition of NaN3 mutagenicity in strain TA100. The mutagenicity of MMNG was inhibited by a similar amount only and that of 4-nitro-o-phenylenediamine was not inhibited at all. The level of α-tocopherol used by Ajith et al was 100 to 300 times greater than that present in the Ames test on AVO stabilised with 1% α-Tocopherol. Very similar results on the weak or ineffective anti-mutagenicity of α-tocopherol against the mutagenicity of various extracts of environmental and dietary mutagens have been reported [Reference 6; Ong et al, 1988]. Furthermore, in support of this Opinion, the dossier contains GLP, OECD 471 compliant studies on samples of AVO, both containing and not containing 1% α-tocopherol, and all have given clear negative results for mutagenicity. Note that α-tocopherol, even at levels 300 times higher than used in the AVO sample, caused no toxicity to the Salmonella strains of bacteria used in the work reported by Ajith and Ong. Consequently, if α-tocopherol has got anti-bacterial properties then the activity is very weak and not expressed at low to moderate concentrations and was not relevant to the testing for bacterial mutagenicity.

It is not correct to assume 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)’. The Applicant agrees that the chromosome aberration study in CHO cells is of lower quality, but it was included for the sake of completeness and openness. Whilst a weak response was observed in one of two duplicate cultures at the highest dose level in Experiment 1, the response was not reproduced in Experiment 2. Furthermore, the original non-clastogenic conclusion of the Study Director is supported by the clear negative result of the study performed in human lymphocytes (Reference 85, discussed above).

All submitted in vitro mutagenicity studies should be considered as reliable as they were technically correctly performed according to guideline requirements under GLP conditions. Reporting and assessment was appropriate. The obtained results reflect normal biological variations and normal differences in susceptibility. With five preparations of AVO, consistent negative Ames test results were obtained, when tested with and without metabolic activation up to cytotoxic concentrations. Whilst some of the tested preparations contained α-tocopherol others did not, and in all cases the studies generated clear negative results.

The negative in vitro mammalian cell gene mutation test can be technically considered as key information within the evaluation of AVO’s mutagenic potential. The weight of evidence regarding both technically correct in vitro mammalian cell chromosomal aberration tests indicated no clastogenic potential of AVO. Please consider further arguments supplied above under the specific sections of in vitro mutagenicity. Taking all results from the assays provided on various samples together, the Applicant is of the firm belief that AVO does not have a mutagenic/genotoxic potential, regardless of whether it is stabilised with 1% α-Tocopherol or not.

SCCS comment on in vitro mutagenicity/genotoxicity testing provided in 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. 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.

3.3.7.2 Mutagenicity / genotoxicity in vivo

3.3.8 Carcinogenicity

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.

Applicant reply

First study

The test material used for this investigation was produced prior to the 2012 study date. However, comparison of analytical data from 2007 and 2015 shows constituents of AVO to be equivalent over an extended period of time. As such, the composition of the 2012 test item can be considered to be analogous to analytical data associated with 2007 ‘Sample n’ and 2015 ‘Sample 18’, all three samples coming from the same producer and with no intentional changes to the production process having taken place during that period of time. Please refer to Reference

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

Applicant reply
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
/
3.3.10 Human data

SCCS overall comment on human data - submission II

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)

The available test results do not indicate a phototoxic potential.

Ref. 82 and 87 in submission II

Applicant reply

Irritation and sensitisation

The Applicant agrees that the concentrations of the applied AVO were not stated in the 2008 report and no information on the composition of the test substance was provided. Analytical evidence from 2007 and 2015 shows that the composition of the test item can be considered to be analogous to ‘Sample g’ analysed in 2007 and ‘Sample 12’ analysed in 2015 (see 3.1.4).

For clarification, H373-1 was a 2 % solution of AVO in 1:3 ethanol/diethyl phthalate, H373-2 was a saline solution and H373-3 was the vehicle - 1:3 ethanol/diethyl phthalate.

This repeated insult patch test (RIPT) should be considered as reliable as it was technically correctly performed according to an approved study protocol on a panel of 120 volunteers (40 males, 79 females) under GLP conditions. Assessment was appropriate. Under the conditions of the study, 2.0 % (2362 pg/cm2) of AVO in 1:3 ethanol/diethyl phthalate did not induce dermal sensitisation any of the subjects tested.

Phototoxicity

The Applicant agrees that the concentrations of the applied AVO were not stated in the 2012 report and no information on the composition of the test substance was provided. However, analytical evidence from 2007 and 2015 shows that the composition of the test item can be considered to be analogous to ‘Sample n’ analysed in 2007 and ‘Sample 18’ analysed in 2015 (see 3.1.4). This modified photo-toxicity test on human volunteers using test items named as VA-1, VA-2, VA-3, VA-4 and VA-5 should be considered as reliable. Batch numbers relating to the test items have been subsequently reported by the supplier as VE00215430 and VE00181147 (VA-1), VE00215430 and VE00181147 (VA-2) and VE00196943 (VA-3). The investigation was technically correctly performed according to an approved study protocol on a panel of 27 volunteers (13 males/14 females, age range: 18- 64) under GLP conditions. Reporting and assessment was appropriate and the general question of AVO composition is addressed in the Applicant response to SCCS comments in 3.1.4.

Finally, this study should be considered as key study and indicated that UV-B and UV-A irradiation led to no serious adverse and did not induce a photo-toxic response on the skin of Human volunteers.
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 was not taken into consideration by the SCCS.

3.4 EXPOSURE ASSESSMENT

From 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.

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 resulting corrected NOAEL of 58.33 mg/kg bw was used for the calculation of MoS. (Table 6)
Table 6 Margin of Exposure calculation

<table>
<thead>
<tr>
<th>Product type</th>
<th>% Acetylated Vetiver Oil (AVO) in consumer product</th>
<th>SED (µg/kg/day)</th>
<th>MoS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic-based fragrances (e.g. Eau de Toilette, Perfume, Aftershave, Cologne)</td>
<td>0.90</td>
<td>64.53</td>
<td>904</td>
</tr>
<tr>
<td>Deodorants</td>
<td>0.05</td>
<td>11.04</td>
<td>5284</td>
</tr>
<tr>
<td>Make up products (e.g. eye make-up, make-up remover, liquid foundation, mascara, eyeliner, lipstick)</td>
<td>0.05</td>
<td>8.53</td>
<td>6839</td>
</tr>
<tr>
<td>Face cream</td>
<td>0.10</td>
<td>24.24</td>
<td>2416</td>
</tr>
<tr>
<td>Hand cream</td>
<td>0.10</td>
<td>32.70</td>
<td>1784</td>
</tr>
<tr>
<td>Body lotion</td>
<td>0.10</td>
<td>123.20</td>
<td>474</td>
</tr>
<tr>
<td>Hair styling</td>
<td>0.10</td>
<td>5.74</td>
<td>10163</td>
</tr>
<tr>
<td>Bath cleansing products (e.g. soaps, shower gel, rinse-off conditioner, shampoo)</td>
<td>0.20</td>
<td>16.46</td>
<td>3544</td>
</tr>
<tr>
<td>Aggregated SED for consumer</td>
<td></td>
<td>286.34</td>
<td>204</td>
</tr>
</tbody>
</table>

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.

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) added 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 based on animal studies.

In this Opinion SCCS did not assess aerosolised or sprayable application that could lead to exposure of the consumer’s lung by inhalation.

5. MINORITY OPINION
6. REFERENCES

References from the Submission III

1. Dossier on the Safety of Vetiveryl Acetate (CAS 117-98-6) in Cosmetic Products
   Submission II – Update – 11 June 2013 and its Reference folder

2. Industry Reply To the Opinion Of the Scientific Committee on Consumer Safety (SCCS) on
   Vetiveryl acetate (fragrance ingredient) SCCS opinion SCCS/1541/14 adopted on 16
   December 2014) – 13 May 2015 and its Reference folder

3. Givaudan: Comments on the Scientific Committee on Consumer Safety (SCCS) opinion on
   Vetiveryl Acetate (Fragrance Ingredient)-Givaudan International SA -18 May 2015

4. Industry Reply To the Request of the Scientific Committee on Consumer Safety (SCCS) for
   more Information on Acetylated Vetiver Oil (AVO) (fragrance ingredient) (SCCS
   Communication on 10th May 2017) 20 October 2017

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

References from the Submission II (see next page)
6. RIVM (Research Institute for Fragrance Materials, Inc., 1972) Acute toxicity studies in mice, rats and rabbits. RIFM# 2536, 19 February 1971 (RIFM, Woodcliff Lake, NJ, USA) Ref. 16 in Subm II

7. RIFM (Research Institute for Fragrance Materials, Inc., 1972d) Phototoxicity and irritation tests of fragrance materials in the hairless mice and miniature swine. RIFM# 2035, 19 December 1972 (RIFM, Woodcliff Lake, NJ, USA) Ref. 20 in Subm II


9. RIFM (Research Institute for Fragrance Materials, Inc., 1982a) Rabbit covered patch skin irritation test with AVO. RIFM# 49323, 03 March 1982 (RIFM, Woodcliff Lake, NJ, USA) Ref. 49 in Subm II

10. RIFM (Research Institute for Fragrance Materials, Inc., 1982j) Rabbit eye irritation test with AVO. RIFM# 49325, 02 August 1982 (RIFM, Woodcliff Lake, NJ, USA) Ref. 58 in Subm II

11. RIFM (Research Institute for Fragrance Materials, Inc., 1982k) Rabbit eye irritation test with AVO. RIFM# 49326, 03 March 1982 (RIFM, Woodcliff Lake, NJ, USA) Ref. 59 in Subm II


report from Givaudan, 14 March 2003, RIFM# 43187 (RIFM, Woodcliff Lake, NJ, USA) Ref. 76 in Subm II


25. RIFM (Research Institute for Fragrance Materials, Inc., 2010a) AVO: Chromosome aberration test in CHO cells: In vitro. RIFM# 59006, 16 April 2010 (RIFM, Woodcliff Lake, NJ, USA) Ref. 83 in Subm II

26. RIFM (Research Institute for Fragrance Materials, Inc., 2011a) AVO: Twenty-eight day repeated dose oral (Gavage) toxicity study in the rat. RIFM# 62943, 16 November 2011 (RIFM, Woodcliff Lake, NJ, USA) Ref. 84 in Subm II


29. RIFM (Research Institute for Fragrance Materials, Inc., 2012a) Modified phototoxicity test (MPT) with AVO in human. 24 September 2012, RIFM# 63834 (RIFM, Woodcliff Lake, NJ, USA) Ref. 87 in Subm II

with artificial sunlight. 17 September 2012 (draft), RIFM# 63835 (RIFM, Woodcliff Lake, NJ, USA) Ref. 88 in Subm II

31. RIFM (Research Institute for Fragrance Materials, Inc., 2012d) AVO: Neutral red uptake phototoxicity assay in BALB/c 3T3 mouse fibroblast. 25 September 2012, RIFM# 63844 (RIFM, Woodcliff Lake, NJ, USA) Ref. 90 in Subm II

32. RIFM (Research Institute for Fragrance Materials, Inc., 2013) AVO: Mutation at the hprt locus of mouse lymphoma L5178Y cells (MLA) using the MicrotitreR fluctuation technique. June 2013, RIFM# 65094 (RIFM, Woodcliff Lake, NJ, USA) Ref. 91 in Subm II

33. Vetiveryl acetate industry consortia (2012) Ref. 95 in Subm II

7. GLOSSARY OF TERMS

See SCCS/1564/15, 9th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 144

8. LIST OF ABBREVIATIONS

See SCCS/1564/15, 9th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 144

And the following additional Abbreviation:

AVO: Acetylated Vetiver Oil