Scientific Committee on Consumer Safety

SCCS

OPINION ON

the inhalation toxicity of the fragrance ingredient Acetylated Vetiver Oil – AVO (CAS No 84082-84-8, EC No 282-031-1) in sprayable cosmetic products

Submission IV

The SCCS adopted this Opinion
by written procedure on 28 February 2024

ACKNOWLEDGMENTS

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

1. ABSTRACT

The SCCS concludes the following:

(1) In light of the data provided concerning inhalation toxicity, does the SCCS consider Acetylated Vetiver Oil (AVO) safe when used in sprayable cosmetic products with intended maximum concentrations (IMCs) of 0.9% (w/w) in fragrance pump sprays, 0.05% (w/w) in deodorant sprays and 0.1% (w/w) in hairsprays and body lotion sprays?

Having considered the data provided concerning inhalation toxicity and aggregate exposure, the SCCS considers Acetylated Vetiver Oil (AVO) (with 1% alpha-tocopherol) safe when used at the intended maximum concentrations (IMCs) of 0.9% (w/w) in fragrance pump sprays, 0.05% (w/w) in deodorant sprays and 0.1% (w/w) in hairsprays and body lotion sprays. The findings of an *in vitro* study using Mucilair™ also support this conclusion.

(2) Does the SCCS have any further scientific concerns regarding the use of Acetylated Vetiver Oil (AVO) in cosmetic products?

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Keywords: SCCS, scientific opinion, Acetylated Vetiver Oil (AVO), Regulation 1223/2009, Acetylated Vetiver oil – AVO, CAS No 84082-84-8, EC No 282-031-1, SCCS/1663/24

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

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 subject to further processing to obtain Acetylated Vetiver oil – AVO (CAS No 84082-84-8, EC No 282-031-1).

In June 2019, the Scientific Committee on Consumer Safety (SCCS) adopted a corrigendum to its opinion on Acetylated Vetiver Oil – AVO (SCCS/1599/18)[1]. More specifically, the SCCS considered the use of Acetylated Vetiver Oil with 1% alpha-tocopherol as a fragrance ingredient in cosmetic leave-on and rinse-off type products as safe (at the concentrations proposed by IFRA). However, the SCCS noted that '*Inhalation toxicity of Acetylated Vetiver Oil (AVO) was not assessed in this Opinion because no data were provided. Assessment of the inhalation risk would be needed if AVO was intended to be used in sprayable products*'.

On 31 March 2023, industry submitted a new safety dossier focusing on the inhalation toxicity of AVO in sprayable cosmetic products to address the SCCS concerns. According to industry, typical cosmetic applications of AVO that may lead to inhalation exposure include fine fragrance pump sprays, deodorant sprays, hairsprays, and body lotion sprays with Intended Maximum Concentrations (IMCs) of AVO being up to 0.9% (w/w) in fine fragrance sprays, 0.05% (w/w) in deodorant sprays and 0.1% (w/w) in hairsprays and body lotion sprays. The Commission requests the SCCS to carry out a safety assessment of AVO in sprayable cosmetic products in view of the new information provided for inhalation toxicity.

Terms of reference

(1) In light of the data provided concerning inhalation toxicity, does the SCCS consider Acetylated Vetiver Oil (AVO) safe when used in sprayable cosmetic products with intended maximum concentrations (IMCs) of 0.9% (w/w) in fragrance pump sprays, 0.05% (w/w) in deodorant sprays and 0.1% (w/w) in hairsprays and body lotion sprays?

(2) Does the SCCS have any further scientific concerns regarding the use of Acetylated Vetiver Oil (AVO) in cosmetic products?

[1] https://health.ec.europa.eu/system/files/2021-08/sccs_o_221_0.pdf

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

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

A) repeated distillation (rectification) to yield 'Vetiverol' (Vetiver oil fraction rich in sesquiterpene alcohols), which is then followed by acetylation, purification and rectification,

B) acetylation (the generally applied method requiring acetic anhydride and phosphoric acid as process materials plus a temperature of 100–120 °C) to yield raw Acetylated Vetiver oil, which is then purified by neutralisation, washing steps and rectification(s)

Previously, a third manufacturing process was also used:

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

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

3.1.1.1 Primary name and/or INCI name

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

3.1.1.2 Chemical names

SCCS comment (from SCCS/1599/18)
According to the Applicant, 'Vetiveryl acetate' would be better described as AVO. A description of the production method used by fragrance industry was provided, according to which Vetiver oil is produced by distillation of fresh or dried roots of Vetiveria (Chrysopogen) zizanoides originating from various geographical areas as a UVCB substance (Unknown or Variable composition, Complex reaction products or Biological materials). The oil is then subjected to further processing (see 3.1.1 above).

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

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

3.1.1.3 Trade names and abbreviations

Acetylated Vetiver Oil (AVO)

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

Vetiver acetate

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

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

Ref. 34

3.1.1.4 CAS / EC number

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

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

CAS: 68917-34-0

CAS: 73246-97-6

SCCS comment (from SCCS/1599/18)
The Applicant agreed that the available CAS numbers for substances derived from natural sources such as Acetylated Vetiver Oil (AVO) is highly confusing, and that registrations within the Chemical Abstract Survey register relate to global differences in requirements for assigning specificity around UVCB regarding plant sections in certain regions of the world, such as the USA.

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

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

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

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

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

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

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

3.1.1.5 Structural formula

SCCS comment (from SCCS/1599/18)
According to the Applicant, supply of structural formulas for AVO, being a complex natural substance, is not appropriate. However, structural information is supplied where available for the 129 constituents of AVO recorded during an analysis in 2015 (Ref. 2 and 3.1.4 below).

3.1.1.6 Empirical formula

SCCS comment (from SCCS/1599/18)
According to the Applicant, this will be addressed in the next Amendment to the IFRA Standard. It is not possible to provide an empirical formula for a complex natural substance like Acetylated Vetiver Oil (AVO). In this respect, reference is made to the Industry dossier (mixture of many constituents, see 3.1.4).

3.1.2 Physical form

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

Ref. 5

3.1.3 Molecular weight

Not applicable (mixture of many constituents, see 3.1.4)

3.1.4 Purity, composition and substance codes

SCCS comment (from SCCS/1599/18)
The Applicant provided an overview of constituents from an analysis of Acetylated Vetiver Oil (AVO) dating from 2015 (Table 1). In addition, full details of constituents identified during analyses of AVO conducted in 2007 and 2015 were provided separately.

Ref: 2

Table 1: Constituents of Acetylated Vetiver Oil (AVO)

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

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

A multi-constituent substance has, as a general rule in accordance with Regulation EC 1907/2006 (REACH), a composition in which several main constituents are present at a

concentration ≥ 10 % (w/w) and < 80 % (w/w). It is considered normal by the Applicant for constituents present at ≥ 1% to be specified, together with any known impurities present at lower concentration, that contribute to the Classification and Labelling according to Regulation EC 1272/2008 (CLP) of the material.
Each of the 129 listed constituents has a determined concentration range, 97.55 % of AVO composition is known in terms of chemical class, and 74.93 % of AVO constituents have been identified.
According to the Applicant, consideration of minimum, maximum and percentage range values relating to the 18 samples analysed in 2015, plus ECHA guidance on REACH registration, leads to the conclusion that it is correct to consider the AVO submitted for analysis as one multi-constituent substance, *i.e.* geographical origin of the AVO and use of production processes A or B do not affect the range of constituents present. A total of 22 constituents were listed as present at an average concentration ≥ 1 % during the 2015 analytical procedure (Table 2).

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

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

The Applicant has concluded that the processed materials referred to collectively by the fragrance industry as AVO can be considered equivalent and should be treated as one multi-constituent substance during the discussion of the toxicological profile.
Results of the 2015 analytical procedure were compared with data from seventeen representative samples of AVO analysed during 2007. Chemical constituents were considered

to be characteristic of AVO, notably the main constituents Khusimyl acetate and (E)-Isovalencenyl acetate. Although the groups of companies submitting samples of AVO for analysis were different in 2007 and 2015, three of the samples refer to the same commercial qualities (Sample 1 and 12 used for testing of sensitisation, and 18 used for several endpoints).

Expansion of the data review to include all samples from 2007 and 2015 showed twelve constituents present at an average concentration of ≥ 1% in 17 samples analysed during 2007 (Ref. 2). The same twelve constituents were present in 18 samples characterised during 2015 (Table 3).

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

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

In summary, following detailed analysis of the compositional data, the Applicant found no relationship between either the geographical origin of the Vetiver Oil or the order in which the acetylation and distillation process were performed and the composition of the final AVO. In common with many other substances derived from natural sources, such variations in composition are to be expected as factors such as time of harvest, soil composition in the fields and variations in weather conditions from growing season to growing season will affect the composition of the Vetiver oil used as the starting material.

Three additional qualities of AVO (no longer produced by Givaudan) have been analysed in 2007 (origins: Java, Haiti and combined origins) and compared with Givaudan's quality of AVO (Vetiveryl acetate 112 Extra) (Table 4). These qualities were all produced following "Process B", acetylation of vetiver oil and subsequent purification.

Table 4: Analysis of 17 samples of Acetylated Vetiver Oil (AVO) in 2007 compared to 2015

Substances	Vetiveryl acetate Haïti	Vetiveryl acetate Bourbon	Vetiveryl acetate Java DM	Vetiveryl acetate 112 Extra
Year of analysis	2007	2007	2007	Current quality
Sesquiterpenes	16%	10%	12%	16.04% (13.94%)
Ketones	24%	15%	21%	14.74% (16.80%)
Acetates	54%	65%	57%	65.45% (65.41%)

Unknowns	6%	10%	10%	3.77%

Ref: 2

SCCS comment (from SCCS/1599/18)

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, and shows the range of variability of the constituents of Acetylated Vetiver, considered during an extended period of time. The SCCS has considered this variation acceptable for a plant-derived material of natural origin and, on the basis of this presumption, the SCCS considered AVO as a single entity on which to base the safety assessment.

3.1.5 Impurities / accompanying contaminants

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

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

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

3.1.6 Solubility

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

3.1.7 Partition coefficient (Log P_{ow})

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

SCCS comment (from SCCS/1599/18)

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

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

3.1.8 Additional physical and chemical specifications

Boiling point: 285 °C
Specific gravity: 1

Ref. 6

3.1.9 Homogeneity and Stability

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

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

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

Stability of the test solutions was not assessed in any of the other studies where a solvent was used. However, based on the functional groups identified in AVO, the nature of the solvents used and the short time period between preparation and use of the solutions it is expected that they would be stable.
The shelf life of AVO claimed by manufacturers varies between one and two years when stored in full, sealed containers.
Typically, product shelf-life is determined after a series of analytical investigations over the time period claimed. Samples are checked regularly following the same initial control plan used for reception/manufacture.
The main investigations concern the physicochemical 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. 6

SCCS comment (from SCCS/1599/18)
Stability data provided by the Applicant contain only raw data without any interpretation of the results. Based on the SCCS Notes of Guidance (SCCS/1647/22), 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 (SCCS/1647/22) 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 (Ref. 4)

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

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

SCCS comment (from SCCS/1599/18)
The SCCS has noted the analyses of the different samples of AVO and has considered that the range of this variability can be accepted because the samples are 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.

Ref. 7, 9 and 13

3.3.1.2 Acute dermal toxicity

SCCS overall comment on acute dermal toxicity (from SCCS/1541/14)

The study could not be evaluated by the SCCS as the submitted original report only consisted of two pages in addition to the front page. The composition of the test substance is not known to the SCCS.

Ref. 7

3.3.1.3 Acute inhalation toxicity

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3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

SCCS comment (from SCCS/1599/18)
The SCCS has noted the analyses of the different samples of AVO and has considered that the range of this variability is acceptable because the samples are 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.

Ref. 7, 10 and 14

3.3.2.2 Mucous membrane irritation / eye irritation

SCCS comment (from SCCS/1599/18)
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.

Ref. 11, 12, 15 and 17

3.3.3 Skin sensitisation

SCCS comment (from SCCS/1599/18)
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.

Ref. 22, 23, 24 and 29

3.3.4 Toxicokinetics

/

3.3.5 Repeated dose toxicity

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

SCCS comment (from SCCS/1599/18)
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.

Ref. 27

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*

SCCS overall comment on *in vitro* mutagenicity/genotoxicity testing (from SCCS/1599/18)
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% alpha- tocopherol (TP) was tested in 4 GLP-compliant bacterial gene mutation studies with negative results (ref. 16-19-20-21 Submission II). The Applicant stated that another study reported in Submission II under ref. 19 showing a 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 (SCCS/1647/22). Although equivocal result was observed in chromosomal aberration test on CHO cells with AVO with 1% TP (Ref. 26), the chromosomal aberration test on human lymphocytes was negative (Ref. 28).
5. Based on all data provided, the SCCS considers that AVO added with 1% TP, as used in the final products, is not likely to pose a risk of mutagenicity.

3.3.7.2 Mutagenicity / genotoxicity *in vivo*

/

3.3.8 Carcinogenicity

/

3.3.9 Photo-induced toxicity

3.3.9.1 Phototoxicity / photo-irritation and photosensitisation

In vitro

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 (from SCCS/1599/18)
The SCCS noted the absence of a positive control in the second *in vitro* study with reconstructed human skin but has also taken note of the internal validation with a positive control. The submitted data do not point towards phototoxicity.

Ref. 8, 31, 32

3.3.9.2 Photomutagenicity / photoclastogenicity

/

3.3.10 Human data

SCCS comment on human data (from SCCS/1599/18)
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 phototoxicity or photosensitisation could be identified in the public literature.

Ref. 25, 30

3.3.11 Special investigations

3.3.11.1 Data from new approach methodology (NAM)

From SCCS/1599/18

3.3.11.1.1 Threshold of Toxicological Concern

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. 3

SCCS comment (from SCCS/1599/18)
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 (SCCS/1647/22) and hence the TTC assessment provided by the Applicant was not taken into consideration by the SCCS.

Submission IV

The Applicant proposed the use of Threshold of Toxicological concern tool for local respiratory effects. In 2009, Carthew et al. published an exposure based waiving approach that included the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products (Carthew et al., 2009). Their evaluation resulted in a TTC for local effects upon inhalation exposure for a material belonging to either Cramer Class I (1.4 mg/day) or III (0.47 mg/day) assuming a human lung weight of 650g. In order to derive these values, a group of 92 chemicals used primarily in consumer products was evaluated for both systemic and site of contact effects. The authors established NOAECs for site contact effects and assigned a Cramer class for each chemical. Over the years other authors have also reported separate values to address inhalation TTC (Escher et al., 2010 and later publication by the same group, Tluczkiewicz et al., 2016). A summary of strengths and weakness of both approaches is presented in ANNEX 1 Inhalation TTC for Local Effects: Strengths and Weaknesses. The Research Institute for Fragrance Material (RIFM) applies the values reported by Carthew et al. (2009) in the risk assessment of fragrance materials (Api et al., 2015), following a review and acceptance by their Expert Panel of Fragrance Safety of these values in preference to those by the other authors.
In order to apply a worst-case scenario in the application of the TTC for respiratory local effects to the assessment of AVO, it is assumed that all components in AVO are of Cramer Class III structure. Therefore, the TTC value of 470 µg/d or 7.8 µg/kg/d (for 60 kg body weight) is used.

Ref 35,36,37 and 38

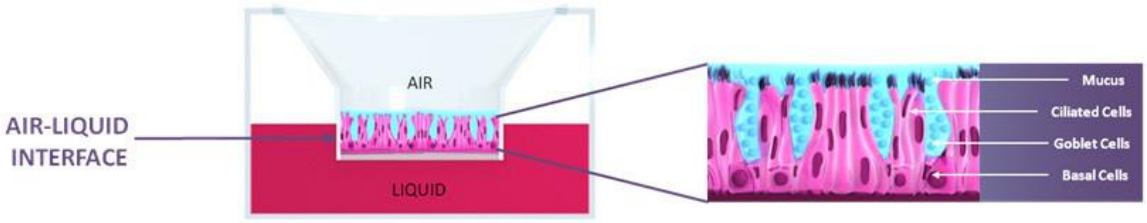
SCCS comment
The SCCS is of the view that the proposal is not acceptable as it is based either on an insufficiently robust dataset (92 compounds), or on the definition of thresholds NOEC values covering a very wide range from 0.001 to 100,000 ppm.

3.3.11.1.2 In vitro respiratory assessment.

According to the Applicant, for AVO no *in vivo* inhalation study is available, and there are no OECD approved or validated *in vitro* inhalation toxicity models available.
Based on previous SCCS Opinion, AVO has been shown to be mildly irritant or irritating to the skin and eye depending on the method used, including to rabbit eyes. In addition, it is classified as a moderate sensitiser (EC3 value of AVO is in the range of 9.3%-13.3%).

MucilAir™ is an *in vitro* cell model of the human airway epithelium cultured at the air liquid interface. It is a 3-D *in vitro* model comprising human basal, goblet and ciliated cells, represents a fully differentiated respiratory epithelium. This model reflects the upper respiratory tract (morphology and functions mirroring the tracheo-bronchial epithelium), being characterised by:

- Production of mucus
- Active cilia-beating



The model shows active ion transport, tight junctions, metabolic activity, cytokines, chemokines, metalloproteinases release.
The model does not reflect the lower respiratory tract of the lung. However, study showed a good predictive capacity of respiratory toxicity of inhaled drugs, with data showing that in vivo toxicity can be predicted in vitro by studying cell barrier integrity by transepithelial electrical resistance (TEER), and cell viability determined by the Resazurin method (88% sensitivity and 100% specificity). MucilAirTM tissues were exposed to 3 concentrations of AVO (0.1, 1 and 5%) for 6 and 24 h. SDS (1 and 2.5 mM) was used as positive control, beta-lactose (3 mg/ml) as negative control, and mineral oil as vehicle control. AVO at all concentrations did not reduce TEER or increase LDH leakage or IL-6 release compared to vehicle and untreated groups. There was a dose- and time-dependent increase in IL-8 release, which was also observed with the negative control. Histology showed some microscopic findings at 5% AVO which were absent or minimal in vehicle, negative and untreated tissues. Overall results show a minor injurious effect at the concentration of 5%. This result is consistent with the mild ocular irritation (eye irritation is believed to be closer analogue to respiratory irritation). Compared to total local inhalation exposure from sprayable products, the doses applied to the MucilAirTM is considerable higher: for the 1%, the dose applied *in vitro* (10 µl) is 330.000 higher of the expected total local inhalation exposure.

Ref. 39 and 40

SCCS comment
The SCCS has considered that AVO has a relatively low volatility, and that the respiratory exposure would mostly result from sprayed droplets that are likely to deposit in the nose, mouth and throat with a minimal exposure of the consumer's lung. Therefore, AVO used in the cosmetic sprayable products at concentrations <1% can be considered of no concern regarding local respiratory irritation.

3.4 EXPOSURE ASSESSMENT

General considerations
The exposure assessment described here includes both inhalation and dermal exposure routes to AVO when present at the IMCs of 0.9% (w/w) in fine fragrance sprays, 0.05% (w/w) in deodorant sprays and 0.1% (w/w) in hairsprays.

According to the Applicant, for AVO used in sprayable products, the potential for exposure to the respiratory tract can be via either volatilization of AVO or via inhalation of an aerosol of sprayable products that contains AVO.

Fragrance materials by their nature can volatilize, however this does not imply that such substances have high volatility. Indeed, the opposite is usually the case, a lot of fragrance substances have low volatility, so that they can generate an odour for an extended period of time. AVO has a vapour pressure in the range of 0.01-0.1 Pa (0.1 Pa at 20°C in the REACH dossier: (https://echa.europa.eu/registration-dossier/-/registered-dossier/22147/4/7) placing it amongst materials of low volatility (ECHA Guidance on Information Requirements and chemical safety assessment Chapter 15: Consumer exposure assessment Version 3.0 – _July 2019). Consequently, rapid volatilization of AVO after spraying the product is not likely to occur and respiratory exposure would mostly result from sprayed droplets.

SCCS comment
With the described vapour pressure, AVO can be considered as semi-volatile.

The Applicant has presented two exposure models for inhalatiAlphaon, the one-box model and two-box model, respectively.
A one-box model assumes that the chemical emitted from a spray becomes instantaneously homogenously distributed in an exposure room of known volume. In the more common one-box model, absence of air exchange ("sealed room") is used. However, in some mathematical models (*e.g.* ConsExpo) air exchange is also integrated which reduces the concentration in the air (Ref. 41 and Ref 42). Importantly, one-box modelling is considered more relevant for products sprayed into the air, and not directly at the user (Ref. 43). In the present assessment, where spray products are directed at the user, a more complex two-box model which models air circulating in a room would provide a more appropriate exposure assessment. The two-box model describes the time-dependent change in chemical concentration emitted from a spray in a volume around the user ('breathing zone'), the air flow between the surrounding room volume (*e.g.* bathroom) and the decline of overall concentration due to air exchange (room ventilation). Moreover, the two-box model allows modelling of inhalation exposure for situations where the subject leaves the breathing zone and moves into the surrounding room after a specific time. The two-box model is also relevant for products sprayed at the body due to the inclusion of a near-field breathing zone (Ref. 43).

As a first-tier exposure assessment by inhalation, because it is often more conservative, the common one-box model is usually used. Indeed, the calculation is simple to pursue and does not require any mathematical modelisation in contrast to the two-box model. In this exposure assessment, we decided to run the two models to compare their results even if the two-box model is considered more relevant for products sprayed at the body like fine fragrance spray, deodorant spray and hairspray.
Based on this information, the exposure scenario has been performed by using two models, briefly described as:

- One-box-model based on the room volume, the time spent in the room, *i.e.*, time elapsed from the start of the emission and staying in this room and the respiration rate of an adult. A more conservative assumption of absence of air exchange ("sealed room") is used in the scenario.
- Two-box model based on the volume and the time spent in each box of the room, as well as air exchange between these different "boxes" and the respiration rate of an adult. The Research Institute of Fragrance Materials (RIFM) two-box model with adjustments has been used (see description in 1.1.2.2).
-

In the development of these two models for inhalation exposure from sprayed products for AVO, the source of the parameters used has been carefully considered and are described in detail herein.

SCCS comment
The SCCS agrees that for sprays directed to the body, the one-box model is not appropriate, and a two-box model should be used.

3.4.2. Parameters

Regarding the **daily amount use (g/d)** for fine fragrance spray, this is taken from AVO Opinion SCCS/1599/18, that is 0.28 g/day reported as the weight loss after use (Ref. 44). For deodorant sprays the amount that is used in the assessment is 6.1 g/d reported as the weight loss after use of spray (Ref. 46). Regarding the daily amount of product use for hairspray, (Ref. 43) reported 6.8 g/d for hairspray (aerosol) (Ref. 41) and 3.6 g/d for hairspray (pump spray) (Ref. 47). In the AVO assessment, the worst-case amount for hairspray is used for aerosol and pump sprays which is the one corresponding to aerosol, *i.e.* 6.8 g/d.
The **room volume** of the bathroom, *i.e.* 10 m^3, has been chosen (Ref. 41 and Ref. 42).
The **duration of inhalation exposure** may be assumed to be 10–20 min, in a worst-case scenario (Ref. 48). The default parameters of the RIFM two-box model are 1 min, in the first box and 20 min in the second box (the room). Therefore, the time spent in the room applied is 21 min in the two models.
For **the respiration rate,** 9 L/min is used assuming rest to light activity as documented in US EPA, 2011 and also similar to data reported in Ref. 42.

In order to calculate the full systemic exposure dose following the use of the sprayable product, in addition to the exposure to AVO through the inhalation route, the dermal contact/uptake also needs to be considered.

In this regard, an experimental study conducted (Ref. 49) showed the fraction of the dose in ethanol-based deodorant sprays to be 23.5% for dermal exposure and consequently, 76.5% available for inhalation, whereas for non-ethanol based deodorant sprays it was 11.4% for dermal exposure and 88.6% available for inhalation. In order to consider the worst-case scenario for inhalation exposure, the fraction of 88.6% available for inhalation has been applied. This is highly conservative since a significant fraction of the 88.6% fraction will be deposited on surfaces and will not be available for inhalation.
For hairspray and fine fragrance products, as a first-tier exposure assessment and as a worst-case scenario, 100% of the sprayable product has been considered as available for inhalation. In reality only a fraction of this is available for inhalation. For example, in Ref. 41 the assumption for dermal exposure is that 85% is retained on the skin and the product fraction available for inhalation is assumed to be similar (15%) for all the sprayed products. For the dermal exposure, the assumption made (Ref. 41) that 85% is retained on the skin is used for fine fragrance sprays and hairsprays.

The pulmonary exposure would mostly result from inhalation of product droplets <10 µm that can reach the airways, (with added conservatism since only respirable particles <5 µm can reach the gas exchange region) and of which the delivered fraction is very low with pump sprays (≈ 1% for hydro alcoholic-based fragrances and hair styling pump sprays) and rather low (≈10%) with aerosol hairsprays and of about 20% for aerosolized deodorants (Ref. 50). Aerosol droplets will also settle on to clothes and furniture, which will reduce the amount available for inhalation. Furthermore, the nature or use of the product will also have an impact on the level of exposure; whether it is sprayed close to the face or on the body distant from the face, for example. However, in the AVO exposure assessment, as a first-tier exposure assessment and as a worst case scenario, it is assumed that for fine fragrance sprays, deodorant sprays, and hairsprays, the fraction of the sprayed product available for inhalation (88.6% for deodorant sprays and 100% for fine fragrance sprays and hairsprays)

is entirely inhaled (*i.e.*, all the droplets are considered with a size < 10 µm) and it reaches the deep lung where AVO content is totally (100%) absorbed.

SCCS comment

The study (Ref. 43) derived worst cases for dermal exposure, not for inhalation exposure. Therefore, the SCCS, in the absence of better data, assumes an availability of 100% for inhalation of the deodorant. Likewise, the inhalation rate should be 13 L/min instead of 9 L/min (SCCS Notes of Guidance – SCCS/1647/22).

3.4.3 One-box model and dermal exposure

The one-box model is based on the assumption that particles/droplets are homogeneously distributed in an exposure room of known volume. Concentrations are calculated as a function of the sprayed amount, the room volume and the respiration rate as well as the time spent in the room. Absence of air exchange ("sealed room") is considered in the scenario.

Table 6 . Total exposure by dermal and inhalation from sprayed products with one-box inhalation model

PRODUCT TYPE	AVO maximum concentration in finished product type % (w/w)	Daily amount of product use (g/d)[1]	SED by dermal (µg/kg/d)[2]	Respiratory tract and SED by inhalation (µg/kg/d)[3]	TOTAL SED (µg/kg/d)
Fine fragrance spray	0.9	0.28	= 0.28g/d x 1000/60kg = 4.7mg/kg/d 4.7 mg/kg/d x 1 x 0.9% x 85% x 1000 x 50% = **17.9**	= (0.9% x 0.28g/d) / 10 m3 = 0.252 mg/m3 inhaled = 0.252 mg/m3 x 21 min x 0.009 m3/min = 47.63 µg/d = **0.794**	17.9 + 0.794 = **18.6**
Deodorant spray	0.05	6.1	= 6.1g/d x 1000/60kg = 101.7 mg/kg/d 101.7 mg/kg/d x 1 x 0.05% x (100-88.6)% x 1000 x 50% = **2.90**	= (0.05% x 6.1g/d x 88.6%) / 10 m3 = 0.270 mg/m3 inhaled = 0.270 mg/m3 x 21 min x 0.009 m3/min = 51.07 µg/d = **0.851**	2.90 + 0.851 = **3.75**
Hairspray	0.1	6.8	= 6.8g/d x 1000/60kg = 113.3 mg/kg/d 113.3 mg/kg/d x 0.1 x 0.1% x 85% x 1000 x 50% = **4.82**	= (0.1% x 6.8g/d) / 10 m3 = 0.68 mg/m3 inhaled = 0.68 mg/m3 x 21 min x 0.009 m3/min = 128.5 µg/d = **2.14**	4.82 + 2.14 = **6.96**
TOTAL			**25.6**	**3.79**	**29.4**

[1] Fine fragrance spray (0.28 g/d = 4.67 mg/kg/d (Ficheux and Roudot, 2017, SCCS/1599/18). Deodorant spray (6.1 g/d sprayed; Hall et al. 2007). Hairspray (6.8 g/d; Steiling et al., 2014 / Bremmer et al., 2006).

[2] 85% of total amount of the sprayed product is considered available for dermal exposure (as per Bremmer 2006). 50% dermal bioavailability as per AVO Opinion SCCS/1599/18; dermal retention factor 1.0 for fine fragrance spray and deodorant spray, and 0.1 for hairspray as per SCCS Notes of Guidance Revision 10.

[3] Respiratory tract exposure is equal to SED as 100% of lung absorption is considered. Parameters used: room volume = 10 m³; time in the room = 21 min; ventilation = sealed room = 0. Respiration rate 9 L/min (US EPA, 2011; Biesebeek et al 2014). Deodorant spray: inhalable fraction 88.6% (Steiling et al. 2012). Fine fragrance spray and hairspray: 100% product available for inhalation. 60 kg body weight.

3.4.3. Two-Box Model

The RIFM two-box model is a conservative approach, yet more realistic as compared to the one-box model, using the concept of "room within a room" model to calculate peak air concentrations from multiple sources under typical consumer use conditions. There are two types depending on the product:

1) Far-field model which is used to model the concentration levels of a chemical between two zones (example: room within a residence), and
2) Near-field model, which calculates the exposure concentration of a compound between a smaller zone around a user's body/head and a larger zone.

The current assessment of AVO exposure from sprayable products is based on the use of near-field RIFM 2-box model (Ref. 43 and Ref. 50) including some modifications related to the exposure of the sprayable products taken into account in this risk assessment.
The **daily amounts** of product use are the same as those described above in the 1-box model. The **spray rate** has been determined for each product type according to daily amount used, frequency of application from SCCS Notes of Guidance 10th Revision and the emission duration described in Ref. 41. The spray rates have been calculated with the following formula and results are detailed below:

$$\text{Spray rate (mg/min)} = \frac{\text{amount used per day (mg)} / \text{frequency of application}}{\text{emission duration (min)}}$$

Table 7 : Exposure parameters used in the modified RIFM two-box model for each finished product category type.

	Fine fragrance spray	Deodorant spray	Hairspray
daily amount used (g) [1]	0.28	6.1	6.8
frequency of application / day [2]	1	2	1.14
sprayed amount / application (g)	0.28	3.05	5.96
emission duration (min) [3]	0.08	0.17	0.24
spray rate (mg/min) ***calculated***	3500	17941	24854
% available for inhalation [4]	100	88.6	100

[1] Fine fragrance spray (0.28 g/d = 4.67 mg/kg/d (Ficheux and Roudot, 2017, SCCS/1599/18). Deodorant spray (6.1 g/d sprayed; Hall et al. 2007). Hairspray (6.8 g/d; Steiling et al., 2014 / Bremmer et al., 2006).
[2] SCCS Notes of Guidance Revision 10.
[3] ConsExpo model, Bremmer et al., 2006
[4] Deodorant spray inhalable fraction 88.6% (Steiling et al. 2012). Fine fragrance spray and Hairspray 100% product available for inhalation. 100% lung absorption. 60 kg body weight.

Table 8 . Summary of the two-box model parameters

Two-Box Model Parameters			References
Zone 1 Volume	m^3	1	Sahmel, 2009; Nicas, 1996
Zone 2 Volume	m^3	10	Bathroom, Bremmer et al., 2006
Air Flow (1 -> Outside)	m^3/min	0	There is no air flow from the cloud (zone 1) to the outdoors. (FEA, 2013)
Air Flow (2 -> Outside)	m^3/min	0,1	Bremmer et al, 2006; Dimitroulopoulou, 2012
Air Flow (1 -> 2)	m^3/min	7,24	Institute of Medicine, 2000; Nicas, 1996; FEA, 2013
Time in Zone 1	min	1	Rothe et al., 2011
Time in Zone 2	min	20	Rothe et al., 2011
Inhalation Model Info			
Body Weight	kg	60	Average ideal bodyweight for an adult.
Inhalation Rate	L/min	9	Assuming rest to light activity US EPA, 2011; Biesebeek et al., 2014
Computational Settings			
Initial Zone 1 Concentration	mg/m^3	0	
Initial Zone 2 Concentration	mg/m^3	0	

SCCS comment

Since these products are directed towards the user's body when used, a one-box model is not appropriate. The SCCS has therefore considered only exposure estimates from the 2-Box-Model. However, the presented calculations with the 2-Box model use an air flow of 0.1 m^3/min, which is not a conservative assumption (SCCS Notes of Guidance – SCCS/1647/22).

The SCCS has recalculated the exposure estimate using a 2-Box model presented in the SCCS Notes of Guidance (SCCS/1647/22), Appendix 11. Based on Ref. 46 the daily use amounts were adapted for deodorant spray (6.54 g/day; P90 for entire exposed population).

3.4.4 Exposure estimates (SEDs)

Table 9: Total exposure by dermal and inhalation from sprayed products with the modified RIFM two-box inhalation model

FINISH PRODUCT TYPE		Fine Fragrance spray	Deodorant spray	Hairspray	ALL sprayed products
Amount used per day	g	0.28	6.1	6.8	
Frequency of application per day		1	2	1.14	
% of sprayed product available for inhalation	%	100	88.6	100	
Emission Duration	min	0.08	0.17	0.24	
Spray Rate	mg/min	3500	17941	24854	
AVO in Finished product type	%	0.9	0.05	0.1	
RESULTS SUMMARY : exposure to AVO by inhalation					
Zone 1 Peak Concentration	mg/m^3	1.9736	0.8088	3.127	
Zone 2 Peak Concentration	mg/m^3	0.2274	0.1219	0.583	
Peak Exposure Rate	mg/min	0.0178	0.0073	0.028	
Total Cumulative Exposure	mg/d	0.0420	0.0449	0.122	0.209
Respiratory tract and SED by INHALATION	µg/kg/d	**0.700**	**0.749**	**2.04**	**3.49**
SED by DERMAL[1]	µg/kg/d	**17.9**	**2.90**	**4.82**	**25.6**
TOTAL SED	µg/kg/d	**18.5**	**3.27**	**6.61**	**29.1**

SCCS comment

As described above, the SCCS has recalculated the inhalation exposure estimates with a more conservative 2-Box-Model and adjusted parameters as discussed above. The resulting inhalation exposure estimates are 0.983, 1.28 and 2.65 µg/kg bw/d for fine fragrance spray, deodorant and hairspray, respectively. The SCCS has further applied a conservative worst-case for calculating the dermal exposure from deodorant spray, which according to Ref. 45 can be up to 23.5% deposited on the skin (instead of 11.4% reported in Table 9). The resulting dermal exposure has been estimated at 6.4 µg/kg bw /d.

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 11 (Notes of Guidance (SCCS/1647/22)).

To calculate the MOS, the deterministic aggregated systemic exposure dose for consumers was compared to the $NOAEL_{sys}$ of 58.33 mg/kg bw derived in SCCS/1559/2918
The exposure estimates presented in the new submission only refer to selected sprayable products. However, the exposure from sprayable products needs to be aggregated with other dermally applied products. For the dermally applied products, the SCCS considers the values used in SCCS/1559/2018. The aggregation was done using either the sprayable or the non-sprayable version of a product category, whichever resulted in higher exposure estimates. The comparison of sprayable and non-sprayable products is presented in Table 10. The product form selected for aggregation is marked in bold.

Table 10 : SED calculation

Categories of products	Concentration AVO (%) (%)	SED dermal (µg/kg bw/d)	SED inhalation (µg/kg bw/d)	SED dermal+inhal (µg/kg bw/d)
Fine fragrances non-spray	**0.9**	**21**		**21.0**
Fine fragrances spray	0.9	17.9	0.983	18.9
Deodorants non-spray	0.05	6.3		
Deodorants spray	**0.05**	**6.4**	**1.28**	**7.7**
Hair styling non-spray	0.1	3.3		3.3
Hair styling spray	**0.1**	**4.82**	**2.65**	**7.5**

The product forms Fragrance non-spray, deodorant spray and hair styling spray are used for the calculation of aggregate SED (by products category and route) and MOS (Table 11 below).

Table 11 . Margin of Exposure calculation

Categories of products	Concentration of Vetiver Oil % (w/w)	SED (µg/kg bw/d)	$NOAEL_{sys}$ (µg/kg bw/day)	MoS
fragrance non-spray	0.9	21.0	58,330	2778
deodorant sprays	0.05	7.7	58,330	7595
hairsprays	0.1	7.5	58,330	7806
Make-up products	0.05	4.7	58,330	12411
Face cream	0.1	12.8	58,330	4557
Hand Cream	0.1	18	58,330	3241
Body lotion	0.1	65.2	58,330	895
Bath cleansing*	0.2	9	58,330	6481
Aggregated SED for consumer		145.9	58,330	400

*soaps, shower gels, rinse-off conditioners, shampoo

The resulting MOS for each product types 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, considered AVO as a single entity on which to assess the toxicity.

General toxicological evaluation

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

Skin sensitisation

Based on the animal studies, AVO can be regarded as a moderate skin sensitiser. AVO did not induce skin sensitisation in human RIPT study. In the public literature there are no reports on sensitisation from AVO in humans.

Considering the results of the HRIPT study and the fact that AVO has been used for years in cosmetics without evidence of sensitising potential, it is unlikely that AVO would cause contact allergy in humans.

Inhalation toxicity

No data have been provided on inhalation toxicity of AVO.

Mutagenicity / genotoxicity

AVO added with 1% Alpha-tocopherol (TP) was tested in 4 GLP-compliant bacterial gene mutation studies with negative results. Additionally, AVO without Alpha-tocopherol was tested in one GLP-compliant study also with negative result. AVO added with 1% Alpha-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) In light of the data provided concerning inhalation toxicity, does the SCCS consider Acetylated Vetiver Oil (AVO) safe when used in sprayable cosmetic products with intended maximum concentrations (IMCs) of 0.9% (w/w) in fragrance pump sprays, 0.05% (w/w) in deodorant sprays and 0.1% (w/w) in hairsprays and body lotion sprays?

Having considered the data provided concerning inhalation toxicity and aggregate exposure, the SCCS considers Acetylated Vetiver Oil (AVO) (with 1% alpha-tocopherol) safe when used at the intended maximum concentrations (IMCs) of 0.9% (w/w) in fragrance pump sprays, 0.05% (w/w) in deodorant sprays and 0.1% (w/w) in hairsprays and body lotion sprays. The findings of an *in vitro* study using Mucilair™ also support this conclusion.

(2) Does the SCCS have any further scientific concerns regarding the use of Acetylated Vetiver Oil (AVO) in cosmetic products?

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5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

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

8. LIST OF ABBREVIATIONS

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

And the following additional Abbreviation:

AVO: Acetylated Vetiver Oil