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Scientific Committee on Consumer Safety

SCCS

OPINION ON

vetiveryl acetate (fragrance ingredient)

The SCCS adopted this opinion at its 8th plenary meeting

on 16 December 2014

About the Scientific Committees

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

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

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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1. BACKGROUND

Vetiveryl acetate (CAS n. 62563-80-8/ 84082-84-8/ 117-98-6) has a long history of widespread use as a woody note in perfumery.

During the 18th Plenary meeting of 25 September 2001, the Scientific Committee on Cosmetic Products and Non-Food Products intended for the Consumer (SCCNFP) adopted an opinion of an initial list of perfumery materials to be included in Annex III - List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down – to Directive 76/768/EEC (doc. n° SCCNFP/0392/00 final). Subsequently, the list was taken over in the Annexes to Regulation (EC) No 1223/2009.

The SCCNFP adopted its first update of the "Initial List of Perfumery Materials which must not form part of Cosmetic Products except subject to the restrictions and conditions laid down" during the 26th plenary meeting of 9 December 2003 (doc. N° SCCNFP/0770/03).

The ingredient vetiveryl acetate was not included in the lists mentioned above but the SCCNFP (doc. N° SCCNFP/0770/03) aimed to discuss additional substances for possible inclusion at a later date.

That is why the Submission I for vetiveryl acetate was submitted in 2005 by The European Flavour & Fragrance Association.

The Scientific Committee on Consumer Products (SCCP) adopted at its 7th plenary meeting the 28 of March 2006 the opinion (SCCP/0984/06) 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.

In June 2013 the Commission has received an update dossier by the International Fragrance Association (IFRA) on the safety assessment of vetiveryl acetate. This submission is intended to demonstrate the safety of the ingredient when used as fragrance ingredient in cosmetic leave-on and rinse-off type products. IFRA recommends a safe concentration limit for vetiveryl acetate when it is used in the specific categories of cosmetic products as developed by the International Fragrance Association (IFRA).

Limits in the finished product:			
Category 1 (Lip products)	0.07%	Category 6 (Mouthwash, toothpaste)	1.67%
Category 2 (Deodorants/antiperspirants)	0.08%	Category 8 (Make up remover, nail care)	2.00%
Category 3 (Hydro-alcoholic products for shaved skin)	0.35%	Category 9 (Shampoo, rinse-off conditioner, bar soap)	5.00%
Category 4 (Hydro-alcoholic products for unshaved skin)	1.04%		
Category 5 (Women facial cream, facial make up, hand cream)	0.55%		

2. TERMS OF REFERENCE

1. On the basis of currently available information, does the SCCS consider vetiveryl acetate 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?

2. Does the SCCS have any further scientific concerns with regard to the use of vetiveryl acetate 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 is the commonly used name to refer to a complex mixture obtained either by a) the acetylation of vetiver oil and subsequent purification or, b) the acetylation of the alcohols ("vetiverol") obtained from vetiver oil of different origin.

(Reference: 15)

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

3.1.1.1 Primary name and/or INCI name

Vetiveryl acetate
Chemical name: Not applicable (Mixture of ca. 100 substances, see 3.1.4)
INCI name: Not applicable (Mixture of ca.100 substances, see 3.1.4)

3.1.1.2 Chemical names

Synonyms for vetiveryl acetate described in the IFRA Standard (44th Amendment) are:
6-Azulenol, 1,2,3,3a,4,5,6,8a-octahydro-4,8-dimethyl-2-(methylethylidene)-acetate
6-Azulenol, 1,2,3,3a,4,5,6,8a-octahydro-4,8-dimethyl-2-(1-methylethylidene)-, acetate
2-Isopropylidene-4,8-dimethyl-1,2,3,3a,4,5,6,8a-octahydroazulen-6-yl acetate

SCCS comment
The chemical names given above relate to the main constituent of vetiveryl acetate (about 15%). Vetiveryl acetate is a complex mixture of ca. 100 substances and it cannot be identified as a single chemical substance (see 3.1.4.).

3.1.1.3 Trade names and abbreviations

Vetiveryl acetate
Vetiver acetate
Vetivert acetate
Vetyvenyl acetate
Vetiverol acetate
Vetyveryl acetate
Vetiveria zizanioides, ext., acetylated

(Reference: 89)

SCCS comment
Reference not submitted.

Vetiveria zizanioides, ext., acetylated
Acetyver
Vetiveryl acetate 112 Extra Acetivenol

(Reference: 94)

SCCS comment
Reference is only a statement from consortium.

3.1.1.4 CAS / EC number

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

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

1,2,3,3a,4,5,6,8a-octahydro-2-isopropylidene-4,8-dimethylazulen-6-yl acetate
CAS: 117-98-6
EINECS: 204-225-7

(Reference: 13)

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 description (see 3.1.4), vetiveryl acetate is a mixture of ca. 100 substances.
The rational for reporting up to five CAS and/or EC No. of vetiveryl acetate is not given.

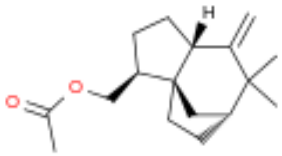
According to the Reference AR1, vetiveryl acetate has CAS No. 68917-34-0
According to the Reference AR2, vetiveryl acetate has CAS No. 62563-80-8

Reference not submitted.

3.1.1.5 Structural formula

Not applicable (Mixture of ca. 100 substances, see 3.1.4)

SCCS comment
According to the Reference AR2, the main component of vetiveryl acetate is khushimyl acetate (CAS No. 61474-33-7) with following chemical structure:



3.1.1.6 Empirical formula

Not applicable (Mixture of ca.100 substances, see 3.1.4)

According to IFRA standard (44th Amendment), vetiveryl acetate has empirical formula $C_{17}H_{26}O_2$

SCCS comment
Empirical formula of a mixture is not possible.

3.1.2 Physical form

Liquid

(Reference: 1)

SCCS comment
Reference not submitted.

3.1.3 Molecular weight

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

3.1.4 Purity, composition and substance codes

Vetiveryl acetate is a complex mixture with ca. 100 constituents present at >0.01% as identified via polar and apolar GC. The components may be broadly described as follows:

Component	Average value (%)
Sum of terpenes/sesquiterpenes	9
Sum of ketones	19
Sum of acetates	63

The main identified components, present at greater than 1%, are as follows:

Component	Average value (%)	CAS No.
Khusimyl acetate	15.45	61474-33-7
Isovalencenyl acetate	14.11	?
α Vetivone + Vetiselinenol acetate	5.88	15764-04-2 +?
Nootkatene derivative (acetate)	4.01	?
β Vetivone	3.97	18444-79-6
Nootkatone derivative (acetate)	3.10	?
α Vetivone	2.62	15764-04-2

Khusimol ester	2.21	?
β Vetivenene (eremophila-1,7(11)-diene)	1.91	?
Isonootkatol acetate	1.82	?
Ziza-6(13)-en-3-one + Eudesmadienone	1.82	?
Isokhusenyle acetate	1.72	?

(Reference: 94)

SCCS comment
Reference not submitted.

SCCS comments

- Vetiver oils of different origin (India, Indonesia, Haiti, Brazil etc) may have a different composition. The quality of commercial vetiver oils 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 that may vary with producer. Therefore the vetiveryl acetate prepared from different vetiver oils may have a different composition. Concentration ranges of various constituents of vetiveryl acetate should be provided.
- Ca. 100 constituents with a concentration of >0.01% are present in vetiveryl acetate, 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 vetiveryl acetate is composed of the unknown ca. 88 constituents.
- No documentation was provided for characterisation and quantification of the substances present in vetiveryl acetate.
- 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 vetiveryl acetate 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 vetiveryl acetate 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 vetiveryl acetate.

3.1.5 Impurities / accompanying contaminants

None identified.

(Reference: 94)

SCCS comment
Information about the residues of the reagents used for acetylation of vetiver oil/vetiverol alcohol in vetiveryl acetate was not provided.

3.1.6 Solubility

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

3.1.7 Partition coefficient (Log P_{ow})

Not submitted.

3.1.8 Additional physical and chemical specifications

Melting point: Not determined
Boiling point: Not determined
Flash point:
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /
pH: /
UV Vis spectrum (….. nm):

3.1.9 Homogeneity and Stability

Shelf life: >2 years

(Reference: 2)

SCCS comment
No data was submitted concerning stability of vetiveryl acetate and its test solutions.

General SCCS comments to physico-chemical characterisation

- Rationale for up to five chemical names and their CAS and/or EC No. reported for vetiveryl acetate is not described.
- Only partial and insufficient information on composition of vetiveryl acetate is reported (see 3.1.4).
- The composition of vetiveryl acetate may differ from batch to batch depending upon the origin of vetiver oil/vetiverol and the method of acetylation. The composition of the batches of vetiveryl acetate used in various studies is not provided except that the ester content (varying from 46 to 99%) has been provided for some batches (see Annex).
- No data was submitted concerning stability of vetiveryl acetate and its test solutions.

3.2 Function and uses

Vetiveryl acetate, as used, is a mixture of products resulting from acetylation of crude vetiver oil. Vetiveryl acetate is used as a fragrance in perfumes and in cosmetics.

Maximum use concentration of vetiveryl acetate in various types of cosmetic products is described in the following table (provided by the applicant).

Limits in the finished product:			
Category 1 (Lip products)	0.07%	Category 6 (Mouthwash, toothpaste)	1.67%

Category 2 (Deodorants/antiperspirants)	0.08%	Category 8 (Make up remover, nail care)	2.00%
Category 3 (Hydro-alcoholic products for shaved skin)	0.35%	Category 9 (Shampoo, rinse-off conditioner, bar soap)	5.00%
Category 4 (Hydro-alcoholic products for unshaved skin)	1.04%		
Category 5 (Women facial cream, facial make up, hand cream)	0.55%		

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Guideline: OECD TG 423 (1996), Directive 96/54 EEC B.1 tris (1996)
Species/strain: rat / Wistar
Group size: 3 males, 3 females,
Test substance: vetiveryl acetate (ester content: 65.9%)
Batch: 9000317035
Purity: /
Vehicle: polyethylene glycol (PEG 300)
Dose level: 2000 mg/kg bw
Administration: oral (gavage), 14 days observation period
GLP: yes
Study period: May to June 1999

The test substance (batch: 9000317035, described as a yellow liquid, ester content: 65.9%) was administered by oral gavage to 3 male and 3 female Wistar rats at a dose level of 2,000 mg/kg bw. The test material was dissolved in polyethylene glycol 300 (PEG 300) and administered at a dose volume of 10 ml/kg bw. The animals were observed for mortality/viability and changes in appearance and behaviour four times during test day 1 and once daily during days 2-15. Body weights were recorded on test day 1 (pre-administration) and once daily during days 2-15. After the 14-day observation period had been completed, animals were sacrificed and gross pathological examination was conducted.

Results:
There were no deaths, clinical signs, macroscopic findings or affected body weight gain in any of the dosed animals.

Conclusion:
The acute oral LD50 value was >2000 mg/kg bw for male and female Wistar rats.

(Reference: 70)

SCCS comment
Regarding the composition of the test substance it is mentioned in the original study report "*not specified; excluded from the Statement of Compliance*", i.e. the composition of the test substance is unknown to the SCCS.

Guideline:	/
Species/strain:	rat / Wistar
Group size:	10 males
Test substance:	labelled 'Compound No. 71-90'
Batch:	/
Purity:	/
Vehicle:	/
Dose level:	5000 mg/kg bw
Administration:	oral, 14 days observation period
GLP:	/
Study period:	/ (Original study report dated May 5, 1972)

In an older study the test substance (labelled 'Compound No. 71-90', described as a brown liquid, no information on the ester content) was administered (as a concentrate) by oral administration to 10 male Wistar rats at a dose level of 5000 mg/kg bw. The rats were fasted for at least 16 hours prior to administration of the substance. Observations for mortality were made at 1 and 6 hours after dosing and daily thereafter for 14 days. After the observation period had been completed, animals were sacrificed and gross pathological examination was conducted on all survivors.

Results:
The rats showed lethargy and diarrhoea. Two rats died within the first day after application. No findings from necropsy were reported.

Conclusion:
The acute oral LD50 value was considered to be >5000 mg/kg bw in male Wistar rats.

(Reference: 16)

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

A range-finding study in mice has been submitted. The test material (named vetyvenyl acetate, described as a clear yellow liquid, no information on the ester content) was administered undiluted by oral intubation to fasted mice (unspecified sex) at dose levels of 2.0 (2 animals), 5.0 (6 animals) and 10.0 (2 animals) ml/kg bw and were observed for up to one week. At termination, the surviving animals were killed and all animals were subjected to an autopsy.
Clinical signs of stress initiated between the first two hours after application in all animals. Mice dosed at 2.0 recovered within 18 hours, and mice dosed at 5.0 ml/kg bw all recovered within 18 to 72 hours. Both mice at the highest dosage group became ataxic after 18 hours, showed laboured breathing after 24 hours and died after 42 and 48 hours, respectively. The autopsy of the animals that died revealed bleaching of the stomach and small intestines, as well as pale/patchy liver with bleaching where in contact with the stomach, and pale kidneys. All surviving animals gained weight during the observation period and appeared normal at the autopsy. The approximate acute LD50 value was between 5.0 and 10.0 ml/kg bw.

(Reference: 48)

SCCS comment
The composition of the test substance is unknown to the SCCS.

3.3.1.2 Acute dermal toxicity

Guideline:	/
Species/strain:	rabbit / New Zealand white
Group size:	10 animals
Test substance:	labelled 'RIFM # 71-90'
Batch:	/
Purity:	/
Vehicle:	/
Dose level:	5000 mg/kg bw
Administration:	dermal, 24 hours, occluded, 7 days observation period
GLP:	/
Study period:	/ (Original study report dated May 1, 1972)

In an older study the acute dermal toxicity was investigated by occlusive application of the neat test substance (labelled 'RIFM # 71-90', described as a brown liquid, no information on the ester content) by a single 24-hour occluded patch in 10 New Zealand White rabbits. Following the exposure, the bindings were removed and observations were made for mortality and toxic effects over a seven-day period. Gross necropsies were performed on all animals at the termination of the study.

Results:
There were no deaths during the course of the study. Evidence of toxicity from percutaneous absorption of the test substance was not found. Signs of skin irritation were noted during the course of the observation period in the form of slight to moderate erythema and oedema. Both signs declined during the post treatment observation period but were still present for slight erythema in one animal at termination on day 7, while oedema was not observed from day 5 onwards. No abnormalities were noted by gross pathology.

Conclusion:
The acute dermal LD50 value was >5000 mg/kg bw in New Zealand White rabbits.

(Reference: 16)

SCCS comment
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.

3.3.1.3 Acute inhalation toxicity

No data were submitted.

SCCS overall comment on acute toxicity
Based on the submitted studies the acute toxicity of vetiveryl acetate cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate on the market is reported (see 3.1.4) and as the composition of the test substances used in the submitted acute toxicity studies is unknown to the SCCS.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline: OECD TG 404 (1992), Directive 92/69 EEC, B.4
Species/strain: rabbit / New Zealand white
Group size: 3 animals (1 male, 2 female)
Test substance: Vetiveryl acetate (ester content: 65.9%)
Batch: 9000317035
Purity: /
Vehicle: /
Dose level: neat substance
Dose volume: 0.5 ml
Observation: readings at 1, 24, 48, 72 hours, observation period 7 days
GLP: yes
Study period: June 1999

The skin irritation potential of vetiveryl acetate (batch: 9000317035, described as a yellow viscous liquid, ester content: 65.9%) was investigated in 3 New Zealand White rabbits (one male, two females). The left flank was clipped and 0.5 ml of the neat substance was applied once to a 6 cm^2 area. The semi-occlusive dressings remained in place for four hours, and then skin findings were evaluated at 1, 24, 48, and 72 hours. Skin reactions were scored according to the numerical system listed in Commission Directive 92/69/EEC.

Results:
No clinical signs of systemic toxicity were observed in the animals during the test and observation period, and no mortality occurred. The dermal exposure caused very slight erythema (grade 1) in one animal at 1-hour and 24-hours after treatment, which reversed by 48 hours.

Conclusion:
Under the conditions of the study, neat vetiveryl acetate was considered to be "not irritating" to the skin of rabbits.

(Reference: 71)

SCCS comment
Under the conditions of this study, the test substance is mildly irritating to rabbit skin.
The composition of the test substance is unknown to the SCCS.

Guideline: /
Species/strain: rabbit / New Zealand white
Group size: 8 animals
Test substance: Vetyvenyl acetate (PPL sample, ester content: 46%)
Batch: /
Purity: /
Vehicle: /
Dose level: neat substance
Dose volume: 0.5 ml
Observation readings immediately after treatment and at 24, 48 and 72 h after patch removal
GLP: /
Study period: January 1982

The skin irritation potential of vetyvenyl acetate (PPL sample, described as a pale green clear liquid, ester content: 46%) was investigated in 8 New Zealand White rabbits. The test substance (0.5 ml, neat) was applied to the clipped dorsum of rabbits under a semi-occlusive patch. The semi-occlusive dressings remained in place for four hours, and then skin findings were evaluated immediately after treatment, and at 24, 48, and 72 hours. Skin reactions were scored for erythema, oedema, cracking and scaling and any other feature using an 8-point anchored ordinate scale ranging from "a" (very slight) to "h" (severe). At the end of the test the reaction grades were converted to numerical scores which were used to calculate the total irritation score per site, and a mean irritation score per site per day for each treatment group.

Results:
Vetyvenyl acetate (PPL sample) showed mainly moderate effects 24 hours after treatment. By 72 hours the test substance showed mainly slight/moderate effects.

Conclusion:
Neat vetyvenyl acetate was slightly to moderately irritating to the skin of rabbits.

(Reference: 49)

SCCS comment
The study could not be fully evaluated by the SCCS as the submitted original study report is poorly legible.
The composition of the test substance is unknown to the SCCS.

SCCS overall comment on skin irritation
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' (described as a brown liquid, no information on the ester content), see section 3.3.1.2.
Based on the submitted studies the skin irritation potential of vetiveryl acetate cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate on the market is reported (see 3.1.4) and as the composition of the test substances used in the submitted skin irritation studies is unknown to the SCCS.

3.3.2.2 Mucous membrane irritation / Eye irritation

Guideline:	OECD TG 405 (1987), Directive 92/69 EEC B.5
Species/strain:	rabbit / New Zealand white
Group size:	3 animals (1 male, 2 females)
Test substance:	vetiveryl acetate (ester content: 65.9%)
Batch:	9000317035
Purity:	/
Vehicle:	/
Dose level:	neat substance
Dose volume:	0.1 ml
Route:	instillation in the conjunctival sac of the eye
Observation:	readings at 1, 24, 48 and 72 hours, observation period 3 days
GLP:	yes
Study period:	June 1999

The eye irritation potential of vetiveryl acetate (batch: 9000317035, described as a yellow, viscous liquid, ester content: 65.9%) was investigated by instillation of 0.1 ml of the neat test substance into the conjunctival sac of one eye of each animal, the untreated eye served

as control. Three New Zealand White rabbits (one male, two females) were used. The eyes were not rinsed. Both eyes were examined at 1, 24, 48 and 72 hours after instillation. The effects on the cornea, iris and conjunctivae (reddening, swelling, ulceration) were scored after 1, 24, 48 and 72 hours according to the numerical system listed in Commission Directive 92/69/EEC.

Results:
The single instillation caused slight reddening (grade 1) of the conjunctivae as well as moderate watery discharge in all animals within one hour after the treatment. By 24 hours the signs had been reversed. Staining and corrosion of the cornea, sclera, and conjunctivae was not observed.

Conclusion:
Under the conditions of the study, neat vetiveryl acetate was considered to be "not irritating" to the rabbit eye.

(Reference: 72)

SCCS comment
Under the conditions of this study, the test substance is mildly irritating to the rabbit eye.
The composition of the test substance is unknown to the SCCS.

Guideline:	OECD TG 405 (1987), EEC B.5 (1997)
Species/strain:	rabbit / New Zealand white
Group size:	4 females
Test substance:	vetiveryl acetate extra (CAS No. 117-98-6, ester content: 85.7%)
Batch:	20070028
Purity:	/
Vehicle:	/
Dose level:	neat substance
Dose volume:	0.1 ml
Route	instillation in the conjunctival sac of the eye
Observation:	readings at 1, 24, 48 and 72 hours, observation period 14 days
GLP:	yes
Study period:	Oct – Nov 2000

The potential irritant effect of vetiveryl acetate extra (batch: 200070028, described as a colourless to slight yellowish liquid, ester content: 85.7%) was investigated by instillation of 0.1 ml of the neat test substance into the conjunctival sac of one eye of each animal, the untreated eye served as control. Four female New Zealand White rabbits were used. The eyes were examined and the grade of ocular reaction was recorded one hour and 24 hours later. After the first 24 hour reading Fluorescein was instilled. After rinsing with isotonic sodium chloride solution the eyes were examined again to detect possible corneal damage. The eyes were also examined 48 and 72 hours, as well as 7 and 14 days after the treatment.

Results:
Conjunctival redness (grade 1 and 2) and chemosis (grade 1 and 2) were observed in all animals and persisted in one animal (grade 1) until day 7 after the treatment. No corneal damage was observed and no effects on the iris were noted except in one animal (grade 1) at the 1-hour reading. The mean score for conjunctival redness was 1.2 and the mean score for chemosis was 1.3.

Conclusion:
According to the EEC commission 93/21EEC of May 4, 1993, the test substance shall not be classified as eye irritating.

(Reference: 74)

SCCS comment
Under the conditions of this study, the test substance is irritating to the rabbit eye.
The composition of the test substance is unknown to the SCCS.

Guideline: /
Species/strain: rabbit / New Zealand white
Group size: 5 animals (2 males and 1 female in group A; 1 male in group B and C)
Test substance: Vetyvenyl acetate (PPL 81/15, ester content: 46%)
Batch: /
Purity: /
Vehicle: Tween 80
Dose levels: neat substance (group C), dilutions of 50% (group B) and 10% (group A)
Dose volume: 0.1 ml
Route: instillation in the conjunctival sac of the eye
Observation: readings at 24 and thereafter daily until day 12
GLP: /
Study period: January to February 1982

In a so-called 'limited screen test', vetyvenyl acetate (PPL 81/15, described as a colourless clear liquid, ester content: 46%) was investigated in New Zealand White rabbits (1 male and 1 female in each group) for its eye irritation potential by instillation of 0.1 ml of the neat test substance (1 male rabbit, group C) or diluted in Tween 80 at 50% (1 male rabbit, group C) or at 10% (2 males and 1 female, group A) into the conjunctival sac of one eye of each animal, the untreated eye served as control. Eyes were examined 24 hours after treatment and thereafter at daily intervals and graded for corneal, conjunctival and iridial damage.

Results:
The neat test substance (group C) caused a slight corneal lesion (grade 1), which healed by day 3.
The 50% dilution (in Tween 80, group B) caused a persistent moderate corneal opacity with slight swelling (grade 1), which persisted for up to 12 days (last reading). Moderate conjunctivitis and discharge (grade 2 and 1) were noted; no conjunctival effects were observed on day 12. Iridial damage (grade 3 and 1) was noted at day 2 and 3. The animal also developed slight peripheral pannus.
The 10% dilution (in Tween 80, group A) caused slight corneal lesions in all three animals (grade 1), which healed on or before by day 5. All animals experienced slight conjunctivitis (grade 1) and two had a slight discharge (grade 1), which disappeared on or before day 5.

Conclusion:
The 10% and undiluted preparations were only slightly irritant. The 50% preparation was a moderate irritant and caused peripheral pannus.

(Reference: 59)

SCCS comment
Under the conditions of this study, the 50% dilution is moderately irritating to the rabbit eye, whereas the neat test substance and the 10% dilution are mildly irritating to the rabbit eye.
The composition of the test substance is unknown to the SCCS.

Guideline: /

Species/strain: rabbit / New Zealand white
Group size: 4 animals (1 male and 1 female per group)
Test substance: Vetyvenyl acetate (PPL 81/15, ester content: 46%)
Batch: /
Purity: /
Vehicle: Tween 80
Dose levels: neat substance, dilution of 50%
Dose volume: 0.01 ml
Route: instillation in the conjunctival sac of the eye
Observation: readings at 24 and thereafter daily until effects disappeared
GLP: /
Study period: January to February 1982

In a so-called 'small volume test', vetyvenyl acetate (PPL 81/15, described as a colourless clear liquid, ester content: 46%) was investigated in two New Zealand White rabbits (1 male and 1 female in each group) for its eye irritation potential by instillation of 0.01 ml of the neat test substance or diluted at 50% in Tween 80 into the conjunctival sac of one eye of each animal, the untreated eye served as control. Eyes were examined 24 hours after treatment and thereafter at daily intervals and graded for corneal, conjunctival and iridial damage.

Results:
The neat test substance had no corneal effects; very slight conjunctivitis (grade 1) was present only initially at 15 minutes after instillation.
The 50% dilution (in Tween 80) caused slight corneal lesions in both rabbits (grade 1), as well as slight conjunctivitis and discharge (grade 1); no effects were observed on day four.

Conclusion:
The 50% preparation was only slight irritant. The 100% preparation was non-irritant.

(Reference: 58)

SCCS comment
Under the conditions of this study, the 50% dilution is mildly irritating to the rabbit eye, whereas the neat test substance is not irritating to the rabbit eye.
The composition of the test substance is unknown to the SCCS.

SCCS overall comment on eye irritation
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 vetiveryl acetate cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate on the market is reported (see 3.1.4) and as the composition of the test substances used in the submitted eye irritation studies is unknown to the SCCS.

3.3.3 Skin sensitisation

Vetiveryl acetate has been investigated for its sensitising potential in several adjuvant and non-adjuvant tests in experimental animals. Eight guinea pig studies were evaluated in the SCCP opinion adopted 28th March 2006 (ref. 91) and based on these studies it was concluded that vetiveryl acetate is a weak allergen. The SCCP noted that the submitted information was old, with studies being largely performed during the 1970s, and that the batches of vetiveryl acetetate tested were of unknown purity. Therefore, data on sensitisation conforming to modern standards and guidelines were required.

The Applicant has submitted Local Lymph Node Assays (LLNA) in which four different qualities of vetiveryl acetate have been tested for skin sensitising potential. Only these four studies have been evaluated in this opinion.

Guideline:	OECD TG 429
Species/strain:	mouse/CBA/J
Group size:	Test substance A, B, C 30 females (5 animals per group) D 45 females (5 animals per group)
Test substance:	A: Acetyver 230451 B: Acet vetivenyl E 112 Extra C: Vetiveryl acetate EX Haiti Clos Ha D: ROB HB
Batch:	A: 0000203938 B: 9000669418 C: B D: 1590849
Purity:	/
Control:	negative: vehicle (diethyl phthalate/ethanol in a 3:1 ratio) positive: d) hexylcinnamaldehyde (HCA), 5, 15, 35%
Dose:	0, 1, 2.5, 5, 10, 25%
GLP:	yes
Date:	2007 (all four LLNA)

The sensitising potential of four different qualities of vetiveryl acetate was tested in the murine local lymph node assay. The test substance A was named 'Acetyver 230451' (batch: 0000203938, described as a light yellow liquid, no information on the ester content), test substance B was named 'Acet vetivenyl E 112 Extra' (batch: 9000669418, described as a yellow liquid, no information on the ester content), test substance C was named vetiveryl acetate EX Haiti Clos Ha (batch: B, described as a light yellow liquid, no information on the ester content), and test substance D was named ROB HB (batch: d) 1590849, described as a yellow liquid, no information on the ester content).
Groups of 5 female CBA/J mice received once daily topical applications on the dorsal surface of both ears with 1%, 2.5%, 5%, 10% or 25% (w/v) of vetiveryl acetate in 3:1 diethyl phthalate:ethanol for three consecutive days. The negative control groups were treated with the vehicle alone. A positive control, hexylcinnamaldehyde (HCA), was included in the LLNA with test substance D and was used at the positive control for the three other LLNA run concurrently with this study.
On day 6, the mice were intravenously injected in the tail vein with 20 microCurie of radiolabelled ^{3}H-thymidine in sterile saline. The mice were killed by CO_2 asphyxiation 5 hours later, and the draining auricular lymph nodes were removed. At removal, the number of nodes collected per animal was recorded and the nodes were examined for size/appearance and the data recorded. A single cell suspension was prepared by pooling the lymph nodes from each group. After washing twice with phosphate buffered saline, cells were precipitated overnight at 2-8°C with 5% trichloroacetic acid. Incorporation of ^{3}H-thymidine was measured in a β-scintillation counter. Increases in ^{3}H-thymidine incorporation relative to the vehicle-treated control groups were then derived and recorded as stimulation indices (SI) for each group. An SI of 3 or more was considered a positive response. The EC_3 value (theoretical concentration resulting in an SI of 3) was estimated in each LLNA. The EC_3 value was then taken as a measure of relative potency and used to compare the sensitisation potential between each quality.

Results:
Three mice were found dead during the study with test substance B and one mouse was found dead in the study with test substance D; no gross lesions were found upon necropsy and the cause of dead was unknown. All other mice appeared normal throughout the study.

There was no mortality in the studies with test substance A and C and all animals appeared normal throughout the study.
No erythema or oedema was noted in any of the mice at any time during the study, except for very slight erythema on day 2 in one mouse treated with 35% HCA and on day 3 in all 5 mice treated with 35% HCA. There were no statistically significant differences in mean body weights at day 1 and day 6 or in mean body weight changes. The lymph nodes from all mice treated with the test substance at 25% and with HCA at 35% were enlarged, but otherwise appeared normal.
Stimulation indices and EC_3 values are presented in the table below.

Test substance	Concentration (%)	DPM/lymph node	Stimulation index	EC3
A	1:3 DEP/Ethanol control	812	-	9.3% (2317 µg/cm^2)
	1.0	1358	1.67	
	2.5	1543	1.90	
	5.0	2053	2.53	
	10.0	2703	3.33	
	25.0	8196	10.09	
B	1:3 DEP/Ethanol control	1302	-	10.9% (2722 µg/cm^2)
	1.0	2072	1.59	
	2.5	1151	0.88	
	5.0	2606	2.00	
	10.0	2835	2.18	
	25.0	20747	15.93	
C	1:3 DEP/Ethanol control	1481	-	13.3% (3325 µg/cm^2)
	1.0	1702	1.15	
	2.5	2209	1.49	
	5.0	3390	2.29	
	10.0	3310	2.23	
	25.0	8493	5.73	
D	1:3 DEP/Ethanol control	1578	-	13.1% (3277 µg/cm^2)
	1.0	1346	0.85	
	2.5	1469	0.93	
	5.0	2391	1.52	
	10.0	1767	1.12	
	25.0	16064	10.18	
Positive control	HCA 5	1878	1.19	
	HCA 15	2774	1.76	
	HCA 35	9944	6.30	

Conclusion:
It was demonstrated that under the conditions investigated, all four qualities of vetiveryl acetate exhibited a potential to induce dermal sensitisation in this murine local lymph node assay. Based on the potency classifications recommended by ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) in Technical RIFM# 87, these materials are considered weak to moderate dermal sensitisers. With regards to the similar EC_3 values,

there were no significant differences between the sensitisation potentials of the four qualities.

(Reference: 79)
(Reference: 80)
(Reference: 81)
(Reference: 86)

SCCS comment
All four qualities of vetiveryl acetate tested in the local lymph node assay have been shown to be moderate skin sensitisers.

SCCS overall comment on skin sensitisation
Based on the submitted studies the skin sensitisation potential of vetiveryl acetate cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate on the market is reported (see 3.1.4) and as the composition of the test substances used in the submitted LLNA studies is unknown to the SCCS.

3.3.4 Dermal / percutaneous absorption

No data have been submitted.
100% dermal absorption of vetiveryl acetate could be used for MOS calculation.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (28 days) oral toxicity

Guideline:	OECD TG 407 (2008), Commission Directive 96/54/EEC B.7
Species/strain:	Rat/Sprague-Dawley (Crl:CD® (SD) IGS BR strain)
Group size:	5 animals per sex
Test substance:	Vetiveryl acetate
Batch:	VE00085543
Purity:	/
Vehicle:	corn oil
Dose levels:	0, 125, 300, 1000 mg/kg bw/day
Dose volume:	4 ml/kg bw
Route:	oral
Administration:	gavage
GLP:	yes
Study period:	April 2010 – October 2011

Prior to the main study, a 7-day range finding study was conducted to determine the dose levels for the definitive 28-day repeated dose toxicity study. The test substance was administered by gavage in corn oil to Sprague-Dawley rats (3/sex/dose) for seven consecutive days at dose levels of 0, 250, 500 and 1000 mg/kg bw/day. As no adverse effects were observed, dose levels of 0, 125, 300 and 1000 mg/kg bw/day were selected.

The subacute toxicity of vetiveryl acetate (batch: VE00085543, described as a yellow liquid, no information on the ester content) was examined in male and female Sprague-Dawley rats after repeated oral administration for 28 consecutive days. Dose levels of vetiveryl acetate of 125, 300, 1000 mg/kg bw/day were administered orally by gavage to three groups each of five male and five female Sprague-Dawley rats for twenty-eight consecutive days. A control group of five males and five females was dosed with the vehicle alone (corn

oil). Two recovery groups, each of five males and five females, were treated with the high dose (1000 mg/kg bw/day) or the vehicle alone for twenty-eight consecutive days and then maintained without treatment for a further fourteen day period. Clinical signs, functional observations (behavioural assessments, functional performance tests, sensory reactivity), body weight/body weight gain, and food and water consumption were monitored during the study. Haematology and blood chemistry investigations were performed on all non-recovery group animals at the end of the treatment period (day 28) and for all recovery group animals at the end of the treatment-free period (day 42). Urinalysis was performed on all non-recovery group animals during week 4 and on all recovery group animals during week 6. At termination, the animals were sacrificed and necropsy was performed. Blood samples were taken for thyroid hormone assessment, but were discarded as no treatment-related effects on the pituitary-thyroid axis were identified. A vaginal smear was taken from all female animals and the stage of oestrus was recorded. The weights of adrenals, brain, epididymides, heart, kidneys, pituitary, prostate and seminal vesicles (with coagulating glands and fluids), liver, ovaries, spleen, testes, thymus, thyroid/parathyroid and uterus with cervix were measured. A comprehensive histopathological examination was done in these organs and in several additional organs.

Results:
No mortality occurred. Clinical observations were confined to increased salivation in animals in both sexes at 350 and 1000 mg/kg bw/day (from day 10 and day 4 onwards, respectively). Water consumption was increased at 1000 mg/kg bw/day during the final two weeks of treatment. There were no treatment-related effects on functional observations, body weight or body weight gain, food consumption, haematology, urinalysis, or stage of oestrus at termination. Blood chemistry evaluations showed statistically significantly increased cholesterol ($p<0.05$), total protein ($p<0.05$) and alanine aminotransferase ($p<.01$) in females treated with 1000 mg/kg bw/day; these effects were not observed in any males or the females treated with 125 or 350 mg/kg bw/day. Increased cholesterol was observed in recovery females treated with 1000 mg/kg bw/day. The changes in these blood chemical parameters were considered by the study report authors to be an adaptive response to the test item in the absence of any histopathological changes and therefore not considered to be an adverse effect.
No macroscopic abnormalities were detected at necropsy.
Statistically significantly increased liver weights (both absolute and relative) were observed in both sexes for all treatment non-recovery groups; this was considered by the study report authors to be an adaptive response to the test item in the absence of any histopathological changes and therefore not considered to be an adverse effect.
Males from all treatment groups had significantly increased kidney weights (both absolute and relative), which extended to the recovery males. Kidney histopathology observations included increased severity of tubular dilation/basophilia in males from all treated groups; increase in tubular basophilia indicated, according to the study report authors, regeneration attempt of tubular epithelium. Hyaline droplets were found in males from all treatment groups with increased severity. Additional Mallory's Heidenhain staining was performed on male kidneys confirming the alpha-2-microglobulin nature of the findings. Granular casts were also observed in 1000 mg/kg bw/day males. These changes did not regress in the recovery males, which also showed mononuclear cell foci and interstitial fibrosis in the kidneys.

Conclusion:
The subacute administration of vetiveryl acetate to male and female Sprague-Dawley rats on 7 consecutive days per week for 28 days at dose levels of 0, 125, 300 and 1000 mg/kg bw/day led to no toxicologically relevant effects in females. Only adaptive and/or effects on slightly disturbed organ function, predominantly the liver, were noted in females. Thus, the 'No Observed Adverse Effect Level' (NOAEL) for female rats can be considered to be 1000 mg/kg bw/day. The kidney effects detected in males from all treatment groups were considered to represent an adverse effect and therefore, a NOAEL for male rats was not established. However, the kidney effects can be considered as signs of the alpha-2-

microglobulin nephropathy, which is known as a species specific disturbance of the male rat and thus, not indicative of a hazard to human health. As the rat specific alpha-2-microglobulin nephropathy has no concurrent impairment in humans, the NOAEL of 1000 mg/kg bw/day will be used a default value for the human health risk assessment.

(Reference: 84)

SCCS comment

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 points at that the mechanism behind the kidney effects could be related to the accumulation of alpha-2-microglobulin in the male rat kidney. This mechanism is specific for the male rat and therefore, unlikely to occur in humans who do not synthetise a protein equivalent to alpha-2-microglobulin. Kidney damage induced in male rats via alpha-2-microglobulin accumulation has been observed with a variety of hydrocarbons derived from petroleum but also from natural sources such as limonene, a monoterpene, which share properties with some of the numerous sesquiterpenes in vetiveryl acetate.

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 were also increased in males at 1000 mg/kg bw/day, although not being significantly different compared with the control group. Relative liver weights were 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 liver, as histopathological changes in the liver cannot be expected to be observed in this study because of the short study 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 vetiveryl acetate, based on the submitted study, a NOAEL for repeated dose toxicity cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate on the market is reported (see 3.1.4) and as the composition of the test substance used in the submitted 28-day study is unknown to the SCCS.

3.3.5.2 Sub-chronic (90 days) toxicity (oral)

No data submitted.

3.3.5.3 Chronic (> 12 months) toxicity

No data submitted.

3.3.6 Mutagenicity / Genotoxicity

Vetiveryl acetate was tested for mutagenicity/genotoxicity in several studies in vitro according to current test guidelines under GLP conditions. There were 5 Bacterial Reverse Mutation Tests (Ames), Mammalian cell chromosomal aberration test using CHO and human peripheral blood and Mammalian cell *hprt* gene mutation test on Mouse lymphoma L5178Y cells.

Because of Initial batch 90001735 (purity 65,9% ester component) showed increased mutant frequency in strain TA100 study was terminated. It was realized that addition of alpha-tocopherol was capable of abolishing the mutagenic activity of vetiveryl acetate. Thus a new preparation of the test material containing alpha-tocopherol was, therefore, subjected to a complete Ames test (Study No373MOO). Then, vetiveryl acetate containing alpha-tocopherol (1%) was also subject of the genotoxicity testing for mammalian gene mutation and chromosomal aberration assays.

Test	Material	Guideline	GLP	Reference
Bacterial Reverse Mutation Test (Ames) 2003	Vetiveryl acetate (18 month, batch: 9000429043) Yellow liquid	OECD 471	Yes	RIFM 2003a, RIFM# 43187 Ref. 76
Bacterial Reverse Mutation Test (Ames) 2003	Vetiveryl acetate (24 month, batch: 9000429043, Yellow liquid	OECD 471	Yes	RIFM 2003b, RIFM# 43188, Ref 77
Bacterial Reverse Mutation Test (Ames) 2003	Vetiveryl acetate (24 month, batch: 112 extra, batch: 9000428765)	OECD 471	Yes	RIFM 2003c, RIFM# 43189, Ref 78
Bacterial Reverse Mutation Test (Ames)	Vetiveryl acetate (extra, batch: 20070028, content: 85.7%) Colourless to pale yellowish liquid	OECD 471	Yes	RIFM 2001, RIFM# 58892, ref 75
Bacterial Reverse Mutation Test (Ames)	Vetiveryl acetate (batch: 9000360016, ester: 65.0% plus 1% alpha-tocopherol) CAS No. 68917-34-0 / 84082-84-8	OECD 471	Yes	RIFM 2000a, RIFM# 43186 Ref 73
Mammalian cell chromosomal aberration test (CHO), 2010	Vetiveryl acetate (batch: VE00034228, purity: 99% plus 1% alpha-tocopherol) CAS No. 117-98-6	OECD 473	Yes	RIFM 2010a, RIFM# 59006
Mammalian cell chromosomal aberration test (HPBL), 2011	Vetiveryl acetate (batch: VE00034228, purity: 99% plus 1% alpha-tocopherol) CAS No. 117-98-6	OECD 473	Yes	RIFM 2011b, RIFM# 62942
Mammalian cell gene mutation test (MLA), 2013	Vetiveryl acetate (batch: VE00231600, purity: 99% plus 1% alpha-tocopherol), CAS No. 117-98-6, Yellow liquid (certificate), Clear colourless liquid, (Covance)	OECD 476	Yes	RIFM 2013, RIFM# 65094 Ref

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial reverse mutation tests (Ames)

Guideline:	OECD 471,
Test system:	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537
Replicates:	Triplicate plates
Test substance:	Vetiveryl acetate (stable 18 months)
Batch:	9000429043
Purity:	/
Concentrations:	Experiment I: Standard plate incorporation test (SPT) ±S9-mix: 33, 100, 333, 1,000, 2,500, 5,000 µg/plate Experiment II: Pre-incubation test (PIT) 33, 100, 333, 1,000, 2,500 µg/plate (TA98, TA100, TA102, TA1535, TA1537, -S9-mix) 10, 33, 100, 333, 1,000, 2,500 µg/plate (TA100, TA102 +S9-mix)
Treatment:	Experiment I Standard plate incorporation test Experiment II Pre-incubation test with 60 min of pre-incubation.
Vehicle:	DMSO
GLP:	in compliance
Study period:	6.2.-4.3.2003

Vetiveryl acetate was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S9-mix prepared from phenobarbital/ß-naphthoflavone induced male Wistar rat liver) according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) method. Concentrations were based on results of pre-experiment with strains TA98 and TA100. Toxicity was measured as a reduction of normal spontaneous revertant or a clearing bacterial background lawn. The experimental conditions in the pre-experiment were identical to those of experiment I and this pre-experiment is reported as part of the main experiment I. *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to the test substance (dissolved in DMSO) at concentrations ranging from 33 – 5,000 µg/plate. The negative and positive controls were in accordance with the OECD guideline.

Results
No precipitation occurred at any concentration. Bacteriotoxic effects were noted in strains TA1537 (at 5,000 µg/plate), TA100 and TA102 (≥1,000 µg/plate) in experiment I. In experiment II, bacteriotoxicity was observed in strains TA 1535 ((≥2,500 µg/plate), TA1537 (≥1,000 µg/plate), TA100 (≥1,000 µg/plate) and TA102 (≥2,500 µg/plate) all with metabolic activation. The number of revertant colonies did not differ between plates containing the test substance and those containing the negative controls either with or without metabolic activation.

Conclusion
Under conditions used vetiveryl acetate was not mutagenic in the gene mutation test.

(Reference: 76)

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 of vetiveryl acetate showed antibacterial properties, results have limited value. The applicant did not explain what vetiveryl acetate stab means. SCCS suggests 'stab' means stabilized with alpha-tocopherol. Consequently this test has no value in the evaluation of vetiveryl acetate mutagenicity.

Guideline:	OECD 471
Test system:	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537
Replicates:	Triplicate plates, 2 experiments

Test substance:	Vetiveryl acetate stab. (24 month)
Batch:	9000429043
Purity:	/
Concentrations:	Experiment I: Standard plate incorporation test (SPT), ±S9-mix: 33, 100, 333, 1,000, 2,500, 5,000 µg/plate Experiment II: Pre-incubation test (PIT), ±S9-mix: 33, 100, 333, 1,000, 2,500, 5,000 µg/plate
Treatment:	Experiment I Pre-incubation test with 60 min of pre-incubation. Experiment II Standard plate incorporation test
Vehicle:	DMSO
GLP:	In compliance
Study period:	10.-20.6.2003

Vetiveryl acetate was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S9-mix prepared from phenobarbital/ß-naphthoflavone induced male Wistar rat liver) according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) method. Concentrations were based on results of pre experiment with strains TA98 and TA100. Toxicity was measured as reduction of normal spontaneous revertant or a clearing bacterial background lawn. The experimental conditions in the pre experiment were identical to dose of experiment us this pre-experiment is reported in main experiment. I and the *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to the test substance (dissolved in DMSO) at concentrations ranging from 33 – 5,000 µg/plate. The negative and positive controls were in accordance of OECD guideline

Results
No bacteriotoxicity and no precipitation occurred at any concentration. Slight toxic effect occurred as reduction of number of revertants was observed in experiment with S9-mix exclusively. All treated plates showed normal background growth up to highest concentration with and without S9-mix in all strains used. The number of revertant colonies did not differ between plates containing the test substance and those containing the negative controls either with or without metabolic activation.

Conclusion
Under conditions used Vetiveryl acetate was not mutagenic in the gene mutation test.

(Reference: 77)

SCCS comment
Raw data of with batch 9000429043 (stab) show that there was decrease in number of revertants in samples with S9-mix in several *Salmonella typhimurium* strains. No purity data were provided. The applicant did not explain what vetiveryl acetate stab means. SCCS suggests 'stab' means stabilized with alpha-tocopherol. Consequently this test has no value in the evaluation of vetiveryl acetate mutagenicity.

Guideline:	OECD 471
Test system:	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537
Replicates:	Triplicate plates
Test substance:	Vetiveryl acetate 112 extra stab. (24 month)
Batch:	9000428765
Purity:	/
Concentrations:	Experiment I: Standard plate incorporation test (SPT), ±S9-mix: 33, 100, 333, 1,000, 2,500, 5,000 µg/plate

	Experiment II: Pre-incubation test (PIT), ±S9-mix: 33, 100, 333, 1,000, 2,500, 5,000 µg/plate
Treatment:	Experiment I Pre-incubation test with 60 min of pre-incubation. Experiment II Standard plate incorporation test
Vehicle:	DMSO
GLP:	In compliance
Study period:	11.-20.6.2003

Vetiveryl acetate was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S9-mix prepared from phenobarbital/ß-naphthoflavone induced male Wistar rat liver) according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) method. Concentrations were based on results of pre-experiment with strains TA98 and TA100. Toxicity was measured as reduction of normal spontaneous revertant or a clearing bacterial background lawn. The experimental conditions in the pre-experiment were identical to dose of experiment thus this pre-experiment is reported in main experiment. *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to the test substance (dissolved in DMSO) at concentrations ranging from 33 – 5,000 µg/plate. The negative and positive controls were in accordance with the OECD guideline.

Results
No bacteriotoxicity and no precipitation occurred at any concentration. Slight toxic effect occurred as a reduction of number of revertants was observed in some strains without and with S9-mix. All treated plates showed normal background growth up to highest concentration with and without S9-mix in all strains used. The number of revertant colonies did not differ between plates containing the test substance and those containing the negative controls either with or without metabolic activation.

Conclusion
Under conditions used, vetiveryl acetate was not mutagenic in the gene mutation test.

(Reference: 78)

SCCS comment
Raw data with 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. No purity data were provided. The applicant did not explain what vetiveryl acetate extra stab means. SCCS suggests 'stab' means stabilized with alpha-tocopherol. Consequently this test has no value in the evaluation of vetiveryl acetate mutagenicity.

Guideline:	OECD 471,
Test system:	*Salmonella typhimurium* strains TA97a, TA 98, TA100, TA102, TA1535,
Replicates:	Triplicate plates
Test substance:	Vetiveryl acetate extra
Batch:	20070028:
Purity:	85.7%
Concentrations:	Experiment I: TA100 +S9-mix: 0.005, 0.016, 0.05, 0.18 and 0.5 µg/plate; TA97a ±S9-mix, TA 1535 ± S9-mix TA98 –S9-mix 0.016, 0.05, 0.16, µg/plate; TA98+S9-mix, TA102± S9-mix: 0.05, 0.16, 0.5, 1.6 and 5 µg/plate Experiment II: TA97a+S9-mix; TA100±S9-mix, TA1535+S9-mix 0.005, 0.016, 0.05, 0.18 and 0.5 µg/plate; TA102+S9-mix, TA1535-S9-mix: 0.016, 0.05, 0.16, µg/plate;

	TA97a –S9, TA 98±S9-mix, TA102-S9-mix: 0.05, 0.16, 0.5, 1.6 and 5 µg/plate
Treatment:	Direct plate incorporation test 18 h exposure
Vehicle:	DMSO
GLP:	In compliance
Study period:	20.10.2000-17.1.2001

Vetiveryl acetate was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S9-mix prepared from phenobarbital/ß-naphthoflavone induced male Wistar rat liver) according to the plate incorporation test with 18h exposure.
The *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535 were exposed to the test substance (dissolved in DMSO) at concentrations ranging from 0.005 – 5 µg/plate with and without metabolic activation (S9-mix prepared from phenobarbital/ß-naphthoflavone induced male Wistar rat liver). Toxicity was evaluated on the bases of background lawn. The negative and positive controls were in accordance with the OECD guideline.

Results
Vetiveryl acetate did not induce gene mutations by base pair changes or frame shifts in the genome of the bacterial strains used either in the presence or absence of S9-mix and was shown to be non-mutagenic in this bacterial gene mutation test.

Conclusion
Under conditions used, vetiveryl acetate was not mutagenic in the gene mutation test.

(Reference: 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 vetiveryl acetate mutagenicity.

Guideline:	OECD 471,
Test system:	*Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535,
Replicates:	Triplicate plates and 2 experiments
Test substance:	Vetiveryl acetate extra,
Batch:	9000360016 +1% of alpha-tocopherol
Purity:	65%
Concentrations:	Experiment I and II: 20, 63.2, 200, 632 and 2000 µg/plate± S9-mix
Treatment:	Experiment I: Direct plate incorporation test, Experiment II 30 min pre-incubation assay,
Vehicle:	DMSO
GLP:	In compliance
Study period:	03.8.2000-29.9.2000

Vetiveryl acetate was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S9-mix prepared from phenobarbital/5,6 benzoflavon induced male Wistar rat liver) according to the plate incorporation and pre-incubation tests. Concentrations were based on growth Vogel-Bonner minimal agar plates in strain TA100 using the plate incorporation test.
The *Salmonella typhimurium* strains TA97, TA98, TA100, TA102 and TA1535 were exposed to the test substance (dissolved in DMSO) at concentrations ranging from 20-2000 µg/plate with and without metabolic activation S9-mix. Toxicity was evaluated on the basis of

background lawn. The negative and positive controls were in accordance with the OECD guideline

Results
No overt precipitation was apparent two days after incubation. The number of revertant colonies did not increase for any of the five strains after treatment with the test material and thus, vetiveryl acetate was not mutagenic under the study conditions.

Conclusion
Under conditions used, vetiveryl acetate was not mutagenic in the gene mutation test.

(Reference: 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 vetiveryl acetate mutagenicity.

***In vitro* mammalian cell gene mutation test**

Guideline/Method:	OECD 476, UKEMS Guidelines
Test system:	Mouse lymphoma L5178Y cells
Replicates:	Duplicate cultures, two independent experiments
Test substance:	Vetiveryl acetate plus 1% alpha-ocopherol
Batch:	VE00231600
Purity:	99%
Concentrations:	Experiment I (exp. I) -S9-mix: 0*, 15*, 30*, 40*, 50, 60, 70, 75, 80, 85, 90, 100 µg/mL +S9-mix: 0*, 40*, 80*, 120*, 140*, 160*, 180, 190, 200, 225, 250 µg/ml Experiment II (exp. II) -S9-mix: 0*, 10*, 20*, 25*, 30*, 35*, 40*, 42.5*, 45*, 47.5*, 50, 60, 80 µg/ml +S9-mix: 0*, 40*, 80*, 120*, 130*, 140, 150, 160, 170, 180, 200, 250 µg/ml (* selected for analysis)
Exposure period:	3 hours
Expression period:	7 days
Vehicle:	DMSO
Positive Controls:	-S9-mix: 4-nitroquinoline (NQO), 0.15 and 0.20 µg/ml +S9-mix: Benzo[a]pyrene (B[a]P), 2.0 and 3.0 µg/ml
Negative control:	DMSO
GLP:	in compliance
Study period:	7.12.-4.3.2013

Vetiveryl acetate (VE00231600) was tested for its potential to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus (6-thioguanine [6TG] resistance) in mouse lymphoma cells using a fluctuation protocol. The test article was dissolved in DMSO and was tested in the presence and absence of metabolic activation (S9-mix prepared from Aroclor 1254 induced male Sprague-Dawley rat liver) incubated for 3 hours, followed by a 7-day expression period. The selection of the concentrations was based on a cytotoxicity range-finder experiment followed by two independent experiments in duplicate cultures. In the cytotoxicity range-finder experiment, six concentrations were tested in the absence and presence of S9-mix, ranging from 23.97 to 767 µg/ml. In Experiment 1 eleven concentrations, ranging from 15 to 100 µg/ml, were tested in the absence of S-9 and ten concentrations, ranging from 40 to 250 µg/ml, were tested in the presence of S-9. In Experiment 2 twelve concentrations, ranging from 10 to 80 µg/ml, were

tested in the absence of S9-mix and eleven concentrations, ranging from 40 to 250 µg/ml, were tested in the presence of S9-mix.
The results were analysed for plating efficiency (PE), percentage of relative survival (RS) and mutant frequency (MF). Positive control treatments with NQO (0.15 and 0.20 µg/ml) for the non-activation set and B[a]P (2.0 and 3.0 µg/ml) requiring activation were included in each mutation experiment. A negative (solvent) control (DMSO) was also included in the test.

Results:
Precipitation was observed at ≥30 µg/ml ±S-9 mix in experiment I and at ≥50 µg/ml +S-9 mix in experiment II. In the range-finder experiment, complete toxicity was observed at 95.88 µg/ml and above in the absence of S-9 and at 383.5 µg/ml and above in the presence of S-9. The highest concentrations to give >10% RS were 47.94 µg/ml in the absence of S-9 and 191.8 µg/ml in the presence of S-9, which gave 146% and 11% RS, respectively. In both independent experiments, no statistically significant increases in mutant frequency were observed following treatment with vetiveryl acetate at any concentration tested in the absence and presence of S-9 and there were no significant linear trends. Although only three test article concentrations were analysed in the absence of S-9 in experiment 1 due to steep concentration related cytotoxicity, a suitable concentration (47.5 µg/ml, giving 16% RS) was identified in experiment 2, therefore the whole study was considered suitably robust and acceptable. The sensitivity of the test system was shown since the positive controls, NQO in the –S9-mix assays and B[a]P in the +S9-mix assay, showed significant increases in the mutant frequency.

Conclusion:
Under the conditions of the assay, vetiveryl acetate was considered non mutagenic in the gene mutations at the *hprt* locus of L5178Y mouse lymphoma cells.

(Reference: 91)

SCCS comment
The purity of 99% of vetiveryl acetate mixture was assumed by the study report authors because the sample of the test substance contained 1% alpha-tocopherol. The composition of vetiveryl acetate 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.
Vetiveryl acetate was tested with 1% alpha-tocopherol alpha and there is no justification for that. SCCS objects to testing vetiveryl acetate with alpha-tocopherol. Consequently this test has no value in the evaluation of vetiveryl acetate mutagenicity.

In vitro mammalian cell chromosomal aberration tests

Guideline/Method:	OECD 473
Test system:	Chinese hamster ovary cells (CHO-WBL)
Replicates:	Duplicate cultures
Test substance:	Vetiveryl acetate plus 1% alpha-tocopherol
Batch:	VE00034228
Purity:	99%
Concentrations:	Experiment I (exp. I) 4 + 20 h: -S9-mix: 0*, 2.5, 5, 10*, 20*, 30*, 40 µg/ml 4 + 20 h: +S9-mix: 0*, 10, 20, 30*, 40*, 60*, 80 µg/ml Experiment II (exp. II) 24 h: -S9-mix: 0*, 5*, 10*, 20*, 25, 30, 35, 40 µg/ml

	4 + 20 h: +S9-mix: 0*, 10*, 20*, 40*, 50*, 60, 70 µg/ml (* selected for metaphase analysis)
Vehicle:	DMSO
GLP:	in compliance
Study period:	2.-19.11.2009

Vetiveryl acetate (VE00034228) was tested for its potential to induce structural chromosome aberrations in Chinese hamster ovary (CHO) cells *in vitro*. The test article was dissolved in DMSO and was tested in the presence and absence of metabolic activation (S9-mix prepared from phenobarbital and ß-naphthoflavone induced male Sprague-Dawley rat liver) incubated for 4 hours, followed by a 20 hour recovery time. The concentration range without metabolic activation was 2.5 - 40 µg/ml. The concentration range with metabolic activation was 10 - 80 µg/ml. In a second experiment, the cells were incubated 24 hours without metabolic activation at 5 - 35 µg/ml. An additional set of cell cultures underwent a 4-hour exposure with S9-mix activation prior to a 20-hour recovery at 10 - 70 µg/ml. In both experiments, the CHO cells were treated with demecolcine to arrest mitosis. A total of 1000 cell nuclei were counted and the number of metaphases were recorded and expressed as mitotic index. One hundred metaphases from each culture were analysed for structural and numerical aberrations. Mitomycin C (0.2/0.05 µg/ml) for the non-activation set and Cyclophosphamide (5/8 µg/ml) requiring activation served as positive control substances. A negative (solvent) control (DMSO) was also included in the test.

Results:
Precipitation was observed visually at ≥30 µg/ml ±S9-mix in experiment I and at ≥50 µg/ml +S-9 mix in experiment II. In the –S9-mix and +S9-mix assays, a concentration-dependent increase in cell growth inhibition was observed at the higher test concentrations. In experiment I, a small but statistically significant increase in the frequencies of cells with aberrations was observed, in both the absence and presence of metabolic activation,. However, these were either only marginally outside the historical control data or did not show a concentration dependency, except in one of the duplicates noted or at above concentrations, which also led to precipitation. No corresponding effect was noted in experiment II. Consequently, the increases were considered as incidental and not of any toxicological significance. The sensitivity of the test system was shown since the positive controls, MMC in the assays, and CP in the +S9-mix assay showed significant structural chromosomal aberrations.

Conclusion:
Under the experimental conditions used, vetiveryl acetate did not induce any significant increase in the frequency of cells with chromosomal aberrations and thus, showed no clastogenic effect in Chinese hamster ovary cells.

(Reference: 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. Vetiveryl acetate was tested with 1% alpha-tocopherol and there is no justification for that. However, SCCS considers this test as positive.

Mammalian cell chromosomal aberration test in peripheral blood lymphocytes

Guideline/Method:	OECD 473,
Test system:	Human peripheral blood lymphocytes
Replicates:	Duplicate cultures
Test substance:	Vetiveryl acetate plus 1% alpha-tocopherol

Batch:	VE00085543 (purity: 99%)
Concentrations:	Experiment I (exp. I) 4 + 20 h: -S9-mix: 0*, 10, 20*, 30*, 35, 40*, 45*, 50, 60 µg/ml 4 + 20 h: +S9-mix: 0*, 40*, 60*, 80*, 100*, 110, 120, 130, 140 µg/ml Experiment II (exp. II) 24 h: -S9-mix: 0*, 10, 20*, 40*, 60, 80*, 100*, 110, 120 µg/ml 4 + 20 h: +S9-mix: 0*, 20*, 40*, 60*, 80*, 100, 110, 120, 140 µg/ml (* selected for metaphase analysis)
Treatment:	Experiment I 4 + 20 h Experiment II 24h without S9-mix and 4+20h with S9-mix
Vehicle:	DMSO
GLP:	in compliance
Study period:	13.6.-28.7.2011

Vetiveryl acetate (VE00085543) was tested for its potential to induce structural chromosome aberrations in Human peripheral blood lymphocytes *in vitro*. The test article was dissolved in DMSO and was tested in the presence and absence of metabolic activation (S9-mix prepared from phenobarbital and ß-naphthoflavone induced male Sprague-Dawley rat liver) incubated for 4 hours, followed by a 20-hour recovery time. The concentration range without metabolic activation was 10 - 60 µg/ml. The concentration range with metabolic activation was 40 - 140 µg/ml. In a second experiment, the cells were incubated 24 hours without metabolic activation at 10 - 120 µg/ml. An additional set of cell cultures underwent a 4-hour exposure with S9-mix activation prior to a 20-hour recovery at 20 - 140 µg/ml. In both experiments, the cells were treated with demecolcine to arrest mitosis. A total of 2000 cell nuclei were counted and the number of metaphases were recorded and expressed as mitotic index. One hundred metaphases from each culture were analysed for structural and numerical aberrations. Mitomycin C (0.4/0.2 µg/ml) for the non-activation set and Cyclophosphamide (4.5 µg/ml) requiring activation served as positive control substances. A negative (solvent) control (DMSO) was also included in the test.

Results:
Precipitation was not observed at any concentration. In the –S9-mix and +S9-mix assays, a concentration-dependent increase in cell growth inhibition was observed at the higher test concentrations. In both experiments, the test substance did not induce any statistically significant or biologically relevant increases in the frequency of cells with aberrations in the absence and presence of metabolic activation. The sensitivity of the test system was shown since the positive controls, MMC in the –S9-mix assays, and CP in the +S9-mix assay showed significant structural chromosomal aberrations.

Conclusion:
Under the conditions of the assay, vetiveryl acetate did not induce an increase in the number of cells with chromosomal aberrations and thus, showed no clastogenic potential in human peripheral blood lymphocytes.

(Reference: 85)

SCCS Comment
Vetiveryl acetate 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 of vetiveryl acetate mutagenicity.

SCCS overall comment on mutagenicity / genotoxicity

Overall, the genotoxicity of vetiveryl acetate was exclusively investigated in a gene mutation test in bacteria. This study was not finished. In the study report No's 293M99, 361M99, 373MOO (batch: 9000360016) it is stated: "*Vetiveryl Acetate has been evaluated for genotoxic activity using the Salmonella/mammalian microsome (Ames) test. Initially a batch (9000317035) with a degree of purity (ester component) of 65.9% was subjected to a range finder assay with strain TA100 (Study No. 293M99, GLP study). Since the batch was found to cause an increase of the mutation frequency starting at a dose of 500 µg/plate, the experimentation with this batch was terminated. A series of further preparations were investigated in strain TA100 (Study No. 361M99; non-GLP study) to assess possible impurity or degradation related effects. It was realized 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 vetiveryl acetate 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 vetiveryl acetate containing 1% alpha-tocopherol have no value in the evaluation of the genotoxic potential of vetiveryl acetate alone. Therefore, on the basis of the results from the study mentioned above vetiveryl acetate has to be considered genotoxic.

Vetiveryl acetate containing 1% alpha-tocopherol was tested for mutagenicity/genotoxicity for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Exposure to vetiveryl acetate 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. Vetiveryl acetate 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 of vetiveryl acetate cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate on the market is reported (see 3.1.4) and as the composition of the test substances used in the submitted mutagenic/genotoxic studies is unknown to the SCCS.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

No data submitted.

3.3.7 Carcinogenicity

No data submitted.

3.3.8 Reproductive toxicity

No data submitted.

3.3.9 Toxicokinetics

No data submitted.

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity *in vitro*

Neutral Red Uptake Phototoxicity Assay

Guideline:	OECD 432 (adaptation)
Test system:	BALB/c 3T3 mouse fibroblasts (passage number < 100)
Replicates:	Duplicate
Test substance:	Vetiveryl acetate
Batch:	/
Purity:	/
Concentrations:	+ UV-A: eight concentrations ranging from 9.07 to 556 µg/ml - UV-A: eight concentrations ranging from 16.3 to 1000 µg/ml
Vehicle:	Hanks' Balanced Salt Solution (HBSS)
Positive control:	Chlorpromazine (+ UV-A: 0.156 – 9.53 µg/ml; -UV-A: 1.63 – 100 µg/ml)
Negative control:	Blanks and HBSS
Source of light:	Dermalight SOL 3 solar simulator, equipped with a UVA H1 filter (320 - 400 nm),
Intensity of irradiation:	UV-A: 1.7 ± 0.1 mW/cm² (total dose: 5 J/cm²)
GLP:	Yes
Study period:	May 2010

Vetiveryl acetate (batch: no information, described as a clear yellow semi-viscous liquid, no information on the ester content) was tested for its phototoxic and cytotoxic potential in the Neutral Red Uptake (NRU) Phototoxicity Assay in BALB/c 3T3 mouse fibroblasts. The assay used is an adaptation of the procedures described in the OECD 432.
Prior to the main study, a solubility test was performed to determine the most appropriate solvent for preparing the test substance dosing dilutions and a dose range finding assay was performed to establish appropriate testing concentrations. Based on the results, the test substance was dissolved in Hanks' Balanced Salt Solution (HBSS), and eight concentrations ranging from 9.07 to 556 µg/ml with UV-A irradiation and ranging from 16.3 to 1000 µg/ml without UV-A irradiation were examined in the definitive assay.
Two independent trials (trial 1 and 2) were performed in the main study. Hereto, 10^4 cells per well were grown in 96-well plates for 24 hours. Subsequently, the test solution, negative control and positive control were applied to the bioassay plates and were incubated at standard culture conditions for about 1 hour. Thereafter, the plates designated for the photo exposure were exposed (with the lid on) to 1.7 ± 0.1 mW/cm² UVA light by a Dermalight SOL 3 solar simulator, equipped with a UVA H1 filter (320 - 400 nm) for 50 ± 2 minutes (+UVA) at room temperature resulting in an irradiation dose of 5 J/cm². Duplicate plates designated for the cytotoxicity assay were kept in the dark for 50 ± 2 minutes (-UVA). Following exposure, the test substance dilutions were decanted from the plates, the cells were washed, supplemented with growth medium and then additionally incubated for 24 ± 1 hours. Thereafter, 100 µL of filtered neutral red solution (33 µg/ml) were added to all test wells and the plates were returned to the incubator for approximately 3 hours. After incubation, the neutral red containing medium was decanted, the wells rinsed and blotted to remove the excess HBSS, and 100 µL of neutral red solvent were added to the bioassay wells followed by incubation for an minimum of 20 minutes. Finally, the absorbance at 550 nm (OD_{550}) was measured with a Molecular Devices Vmax plate reader. The relative survival was obtained by comparing the amount of neutral red taken up by test article treated groups to the neutral red taken up by the vehicle treated group on the same plate. An IC_{50}, the mean photo effect (MPE) and photo-irritancy factor (PIF) were calculated by means of PHOTOTOX software provided by ZEBET, Berlin, Germany, for both the UV-A irradiated or non-irradiated testing solutions.

Results
Precipitation was observed in the stock dilution for the test substance at all concentrations in both definitive trials. In the dose range finding assay, trial 1 and 2, the test substance caused a left shift in the concentration-response curve (i.e., induced greater toxic effects) in the presence of UV-A irradiation, when compared to the test substance in the absence of UV-A exposure. This shift was reflected in the PIF and MPE values and was considered as indicative of a phototoxic potential. The sensitivity of the study was confirmed as the positive control chlorpromazine led to the expected MPE shift indicative for phototoxicity and the solvent controls exposed to UV-A met the acceptance criterion for this assay.

Conclusion
The test substance was predicted to have a photo-toxic potential in the NRU phototoxicity assay in Balb/c 3T3 mouse fibroblasts *in vitro* (i.e., PIF = 5.0 and MPE = 0.150).

(Reference: 90)

SCCS comment
No information was provided on the composition of the test substance.
A UV/vis absorption spectrum of the test item should be present, even before biological testing.
Because of precipitation problems, the exact concentrations of vetiveryl acetate to which the cells have been exposed could be lower than the nominal concentrations and thus the measured phototoxic potential might be underestimated. On the other hand, precipitation may have also created false positive results under these circumstances. Therefore, the results obtained are not reliable.

Phototoxicity Assay *in vitro*: Human skin model test

Guideline: /
Test system: (EpiDerm™human skin model
Replicates: Duplicate
Test substance: Vetiveryl acetate (112 Extra)
Batch: VE00196943
Purity: /
Concentrations: 0.1, 0.316, 1.0, 3.16, 10% (v/v, with/without light irradiation)
Vehicle: Sesame oil
Positive control: /
Negative control: Sesame oil
Source of light: Sunlight simulator (Dr. Hönle SOL 500 solar simulator in combination with the solar standard filter H1 to keep UV-B irradiation as low as possible, spectrum: >320 nm)
Intensity of irradiation: UV-A: 6 J/cm² (= 1.6 - 1.8 mW/cm²)
GLP: Yes
Study period: August 2012

Vetiveryl acetate 112 Extra (batch: VE00196943, no information on description, no information on the ester content) was tested for its phototoxic potential using a three dimensional human epidermis model (EpiDerm™); procedures according to MatTek Corporation 1997, Phototoxicity Protocol for use with EpiDerm™ Model (EPI-200). The test substance was dissolved in sesame oil and concentrations of 0.1, 0.316, 1.0, 3.16, 10% (v/v) were examined. The test solutions were applied onto filter pads which were then applied onto the skin equivalents. Sesame oil was used as negative control. Each concentration, including negative control, was tested at a volume of 20 µl per tissue in duplicates. One test group of skin equivalents treated with the test substance concentrations and the negative control was irradiated with artificial sunlight for 60 minutes at 1.8 mW/cm^2 UV-A corresponding to an irradiation dose of 6 J/cm^2 UV-A. The other test

group of skin equivalents treated with the test substance concentrations and the negative control were kept in the dark for 60 minutes. Tissues were then rinsed with PBS to remove test material, transferred to new 6 well plates with fresh medium and incubated overnight. The next day, the assay medium was replaced by MTT-medium and tissues were incubated for 3 hours with MTT. Tissues were then rinsed with PBS, and the formazan salt was extracted with isopropanol. Optical density (OD) was determined at 540/570 nm in a plate spectrophotometer and cell viability was calculated for each tissue as % of the corresponding vehicle control either irradiated or un-irradiated.

Results
No cytotoxic effects were observed after treatment of the skin equivalents with the test substance at any concentration, neither in the presence nor in the absence of irradiation with artificial sunlight. The validity of the study was confirmed as the mean OD of the negative control for irradiated tissues (OD = 1.653) and non-irradiated tissues (OD = 1.766) was ≥0.8.

Conclusion
Vetiveryl acetate was shown to exhibit no phototoxic potential on reconstructed human skin (EpiDerm™) *in vitro.*

(Reference: 88)

SCCS comment
No information was provided on the composition of the test substance.
A UV/vis absorption spectrum of the test item should be present, even before biological testing.
The SCCS noted that the submitted report is a draft. As no positive control group was included in the study, the results cannot be used by SCCS to assess the phototoxic potential of vetiveryl acetate.

SCCS overall comment on phototoxicity *in vitro*
A UV/vis absorption spectrum of the test item should be present.
Because of precipitation, the first study RIFM 2012d, RIFM# 63844 using the NRU phototoxicity assay with Balb/c 3T3 mouse fibroblasts cannot be used to assess the phototoxicity of vetiveryl acetate. Likewise, the second follow-up study RIFM 2012b, RIFM# 63835 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 vetiveryl acetate cannot be evaluated.
In addition, only partial and insufficient information on the composition of vetiveryl acetate 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.

3.3.10.2 Phototoxicity / photoirritation *in vivo*

An explorative screening for the potential to induce skin irritation and staining or a photo-toxic response was performed with undiluted Vetiveria zizanioides, ext., acetylated. The test compound was applied to hairless mice (hrhr) and Handord strain mini pigs by pipetting it onto a 5.0 cm2 area of skin. Subsequently a 1.0 cm circle was exposed to light at the centre of the application area for a dose of two minutes. The light source used (Berger, WG3 filter) produced a spectrum of 295 nm to 410 nm to simulate the summer sunlight of 40 degree north latitude. The light source was filtered through to produce an identical spectrum in such a way that only longwave ultraviolet light was utilised. Each group was compared to a negative (vehicle) and a positive (0.1% 8MOP) control. Other light sources used were the sun, the Osram XBF xenon lamp (filtered through glass) and fluorescent black light lamps.

Vetiveria zizanioides, ext., acetylated revealed an absorption peak of <300nm. Neither irritant nor photo-toxic skin reactions were observed.

(Reference: 20)

SCCS comment
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'.

SCCS overall comment on phototoxicity / photoirritation
Based on the submitted data, the phototoxic/photoirritation potential of vetiveryl acetate cannot be evaluated.
In addition, only partial and insufficient information on the composition of vetiveryl acetate on the market is reported (see 3.1.4) and the composition of the test substances used in the submitted studies is also unknown to the SCCS.

3.3.11 Human data

Vetiveryl acetate has been investigated for its sensitising potential in several maximisation tests in humans. These studies were included in the SCCP opinion adopted 28th March 2006 (ref. 91), but were considered to be unethical. The SCCP noted that the submitted information was old, with studies mainly being performed during the 1970s, and that the batches of vetiveryl acetetate tested were of unknown purity. Therefore, data on sensitisation conforming to modern standards and guidelines were required.

The Applicant has submitted a human repeated insult patch test (HRIPT) and a phototoxicity test on human volunteers. Only these two studies have been evaluated in this opinion.

Photoirritation and photosensitization

Study Design:	
Date of report:	2008
Guideline:	Repeated insult patch test according to approved study protocol and standard operating procedures
Species:	Human
Group size:	120 enrolled volunteers (40 males, 79 females)/112 completed (37 males, 75 females)
Test substance:	Vetiveryl acetate (H373-1, H373-2, H373-3)
Batch:	no data
Route:	Semi-occlusive epicutaneous application
Scoring system:	Modified scoring scale of the ICDRG (International Contact Dermatitis Research Group)
GLP:	Yes
Published:	No

A repeated insult patch test (RIPT) according to an approved study protocol was performed with three qualities of vetiveryl acetate (H373-1, H373-2, H373-3) on a panel of 120 volunteers (40 males, 79 female volunteers under GLP conditions). During the induction phase, approximately 0.3 ml of the test material was applied on a Webril/adhesive patch to the dry-wiped skin on the left side of the back of each volunteer. The patch was semi-occlusively covered and remained on the skin for 24 hours. Thereafter, the patches were

removed and the skin was scored. The patch removal was followed by a rest period of 24 hours on workdays or 48 hours on the weekends. A series of 9 induction patches was completed over a period of 3 weeks. The last induction patching was followed by a rest period of two weeks with no application. After the rest period, a Webril/adhesive patch was applied with 0.3 ml of the test material and fixed semi-occlusively on the virgin, right side of the back of each volunteer for 24 hours. After removal, the application sites were scored at about 24, 48, 72 and 96 hours post-patching. The complete test was conducted under the supervision of a Board-Certified Dermatologist, which also participated in scoring the volunteers.

Results
A total of 112 volunteers (37 males and 75 females, age range: 18 - 70) completed the study and 8 discontinued but not due to the test material reaction.
Vetiveryl acetate (H373-1): During the induction period, five volunteers exhibited low level, transient (±) reactions. At challenge, two other exhibited low level (±) reactions.
Vetiveryl acetate (H373-2): During the induction period, three volunteers exhibited low level, transient (±/1) reactions. At challenge, two other exhibited low level (±) reactions.
Vetiveryl acetate (H373-3): During the induction period, three volunteers exhibited low level, transient (±) reactions. At challenge, no reactions were exhibited.

Conclusion
There was no indication for an irritative or sensitising potential of the three qualities of vetiveryl acetate under the conditions of the RIPT study in male and female human volunteers. An explorative screening for the potential to induce skin irritation was performed with vetiveryl acetate at 5%, 15% and 20% in white Vaseline or lanolin in 42, 29 or 44 human volunteers, respectively. Each test material was applied to the backs by occlusive patches for 48 h. After removal and rest for 23 h, the backs were irradiated by UV. The skin reactions were scored at 48 h, 72 and 96 h. No skin irritation was recorded (Reference: RIFM 1975, RIFM# 15092). The sensitising potential of Vetiveryl acetate was comprehensively investigated in numerous Human maximization tests. In all of these studies, the irritating potential to determine the minimal irritating dose (MED) was investigated in pre-tests as a rule.

(Reference: 82)

SCCS comment
No information was provided on the composition of the test substances.
The experimental detail is deficient in that the concentrations of the applied vetiveryl acetate are not stated in the report (RIFM 2008d/RIFM 54473). The tabulated data from RIFM 2008d (RIFM 54473) show +/- reactions on challenge in 3 subjects out of 112 tested. The report RIFM 2008d (RIFM 54473) 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.

Phototoxicity

Guideline/Method:	Modified phototoxicity test according to approved study protocol and standard operating procedures
Species:	Human
Group size:	28 enrolled volunteers/27 completed (13 males/14 females, age range: 18 - 64)
Test substance:	Vetiveryl acetate
Batch:	No data
Concentrations:	0, 2.5, 7.5, 25%
Vehicle:	Diethyl phthalate:ethanol (3:1)
Controls:	a) saline

	b) vehicle
Route:	Occlusive epicutaneous application
Scoring system:	Modified scoring scale of the International Contact Dermatitis Research (Group)
Irradiation:	Source of light: Harrison Research Laboratory custom made light sources using four Philips F40BL fluorescent tubes (peak: 369 nm; half-power bandwidth: 16 nm (362 - 379 nm)
Intensity of irradiation:	UV-A: 4.6±0.2 mW/cm2 UV-B: 1.4 ±0.2 mW/cm2 (based on minimal erythema dose (MED)
Testing schedule:	Day 1: Duplicate patches occlusively applied for 24 h Day 2: 24 h post-patching removal of patches, irradiation with UV-A and UV-B, scoring prior to and after irradiation, non-irradiated sites protected from light and scored after patch removal Day 3/4: about 48/72 h post patching scoring of irradiated and non- irradiated sites
GLP:	Yes
Published:	No

Vetiveryl acetate was tested for its phototoxic potential in a modified photo-toxicity test on human volunteers as a result of a single application and UV-B and UV-A irradiation to induce a phototoxic response in humans, according to approved study protocol and standard operating procedures. Twenty-eight were enrolled and 27 volunteers completed the study (13 males/14 females, age range: 18 - 64). The material was tested as 2.5%, 7.5% and 25% preparation in diethyl phthalate:ethanol (3:1) and the vehicle and saline were used as control application. For UV-A irradiation a total dose of 4.6±0.2 mW/cm2 in a wavelength range between 320 nm and 400 nm and for UV-B irradiation a wavelength range of 280 nm to 320 nm and UVB irradiation of 1.4 ±0.2 mW/cm2 was used. The light sources consisted of four Philips F40BL fluorescent tubes. Prior to the application the minimal erythemal dose (MED) was determined. On study day 1 duplicate patches were occlusively applied for 24 h and on day 2 the patches were removed and the skin was irradiated with UV-A and UV-B. The skin sites were scored prior to and after irradiation. The non-irradiated sites were protected from light and were also scored after patch removal. On day three and four (about 48/72 h post patching) the irradiated and non-irradiated sites were scored again.

Results

25% vetiveryl acetate: Two volunteers exhibited low-level (±/1) reactions on both the irradiated and the non-irradiated contact sites. Nineteen other volunteers exhibited low-level (±/1) reactions on the irradiated contact site only. One subject exhibited a slight tanning response on the irradiated contact site. 7.5% vetiveryl acetate: Twenty-three volunteers exhibited low-level (±/1) reactions on the irradiated Test Material contact site only. Two volunteers exhibited slight tanning responses on the irradiated Test Material contact site. 2.5% vetiveryl acetate: One subject exhibited low-level (±) reactions on both the irradiated and the non-irradiated contact sites. Twenty-three other volunteers exhibited low-level (±/1) reactions on the irradiated contact site only. Three volunteers exhibited slight tanning responses on the irradiated contact site. Saline control: Twenty-five volunteers exhibited low-level (±/1) reactions on the irradiated contact site only. Two volunteers exhibited a slight tanning response on the irradiated contact site. Vehicle control: One volunteer exhibited low-level (±) reactions on both the irradiated and the non-irradiated contact sites. All other volunteers, 26 in total, exhibited low-level (±/1) reactions on the irradiated contact site only. Two volunteers exhibited slight tanning responses on the irradiated contact site.

Conclusion

No serious adverse events related to the test material preparations occurred during this test. Low levels effects seen at all concentrations were also noted in the saline and vehicle control groups. Vetiveryl acetate did not induce a phototoxic response on the skin of human

volunteers under the conditions of this modified phototoxicity test. An explorative screening for the potential to induce a photo-toxic response was performed with vetiveryl acetate at 5%, 15% and 20% in white Vaseline or lanolin in 42, 29 and 44 human volunteers, respectively. Each test material was applied to the backs by occlusive patches for 48 h. After removal and rest for 23 h, the backs were irradiated by UV. The skin reactions were scored at 48 h (prior to irradiation), 72 and 96 h. No phototoxic skin response was recorded.

(Reference: 87)

SCCS comment

No information was provided on the composition of the test substance.
The available test results do not indicate a phototoxic potential.

SCCS overall comment on the human studies

Based on the submitted human data, the photoirritation / photosensitisation and phototoxic potential of vetiveryl acetate cannot be evaluated.
In addition, only partial and insufficient information on the composition of vetiveryl acetate on the market is reported (see 3.1.4) and the composition of the test substances used in the submitted studies is also unknown to the SCCS.

3.3.12 Special investigations

No data submitted.

3.3.13 Exposure

The following table has been provided by the applicant.

Product type	% fragrance mixture in product	% VA in fragrance mixture	% VA in product	% penetration	Daily Amount product (g/day)	Applicat. per day	RF	Rel. daily exposure product (mg/kg bw/d)	SED (µg/kg/ day)
Shower gel	1,2	2,8	0,0336	100	18,67	1,43	0,01	2,79	0,938
Hand wash soap	1,5	0,13	0,00195	100	20	10	0,01	3,33	0,065
Shampoo	0,5	1,16	0,0058	100	10,46	1	0,01	1,51	0,088
Hair conditioner	0,5	1,16	0,0058	100	3,92	0,28	0,01	0,60	0,035
Hair styling products	0,5	2,58	0,0129	100	4	1,14	0,1	5,74	0,740
Body lotion	0,4	2,26	0,00904	100	7,82	2,28	1	123,20	11,137
Face cream	0,3	2,26	0,00678	100	1,54	2,14	1	24,14	1,637
Hand cream	0,4	2,26	0,00904	100	2,16	2	1	32,70	2,956
Liquid foundation	1	2,2	0,022	100	0,51	1	1	7,90	1,738
Make-up remover	1	2,2	0,022	100	5	1	0,1	8,33	1,833
Eye shadow	1	2,2	0,022	100	0,02	2	1	0,33	0,073
Mascara	1	2,2	0,022	100	0,025	2	1	0,42	0,092
Eyeliner	1	2,2	0,022	100	0,005	2	1	0,08	0,018
Lipstick, lip salve	1	2,2	0,022	100	0,057	2	1	0,90	0,198
Deodorant non spray	1	0,82	0,0082	100	1,5	2	1	22,08	1,811
Deodorant aerosol spray (EtOH)	8	4,52	0,3616	100	1,43	2	1	20,63	74,598
Deodorant aerosol spray (not EtOH)	15	4,52	0,678	100	0,69	2	1	10,00	67,800
Sun care cosmetics	2	0,000014	0,00000028	100	36	2	1	600,00	0,002
						Total exposure			**165,759**
VA = Vetiveryl acetate, RF = Retention factor									

3.3.14 Safety evaluation (including calculation of the MoS)

Not applicable.

3.3.15 Discussion

Vetiveryl acetate is used as a fragrance ingredient in perfumes and in cosmetics.

In the previous opinion on vetiveryl acetate, the SCCP was of the opinion that the information submitted was inadequate to assess the safe use of the substance. Before further consideration, information such as characterisation of the test substance and clarification on purity and impurities was required (SCCP/0984/06).

Physico-chemical properties

Vetiveryl acetate is a mixture of products resulting from acetylation of crude vetiver oil derived from grass vetiveria zizaniodes, or acetylation of alcohols extracted from vetiver oil. The vetiver oils originating from various countries (India, Indonesia, Haiti, Brazil etc.) may have different compositions, and thus the vetiveryl acetate prepared from different vetiver oils will also have different compositions. The quality of commercial vetiver oils 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 that may vary with producer. IFRA Standard (44th amendment) recommends 3 different methods for acetylation of vetiver oils. It is possible that different methods of acetylation of vetiver oils may result in different composition of vetiveryl acetate. Finally, the composition of vetiveryl acetate prepared from an alcohol extract of vetiver oil (vetiverol) will be different from that prepared by the acetylation of vetiver oil.

The documentation of chemical characterisation and quantification of vetiveryl acetate is not provided. The information provided on the composition of vetiveryl acetate is insufficient (see 3.1.4). Not all of the chemically characterised substances of vetiveryl acetate are identified by their CAS/EC No. More than 40% of the vetiveryl acetate is composed of ca. 88 unknown constituents. Moreover, the concentration ranges of the chemically characterised constituents, which may reflect different vetiveryl acetate preparations, have not been provided. Identifying vetiveryl acetate by one chemical substance (CAS No. 117-98-6, 1,2,3,3a,4,5,6,8a-octahydro-2-isopropylidene-4,8-dimethylazulen-6-yl acetate) is not correct. Rationale for up to five chemical names and their CAS and/or EC No. reported for vetiveryl acetate is not described. No data was submitted concerning the stability of vetiveryl acetate and its test solutions.

Adequate information regarding chemical characterisation and quantification of constituents of a typical vetiveryl acetate (including the concentration range of the constituents) is required.

In the absence of knowledge of the composition of the test substance used in the submitted studies, the relevance of test results is questionable.

General toxicity

The test substances used in the acute toxicity studies are of low acute toxicity with LD50 values after oral administration above 2000 mg/kg bw and above 5000 mg/kg bw following dermal application.

In the 28-day study in rats, a NOAEL of 300 mg/kg bw/day is considered for the test substance used, based on increased cholesterol and increased relative liver weights (magnitude above 50%) at the highest dose level (1000 mg/kg bw/day). A 90-day study was not submitted.

Based on the submitted studies, the acute and repeated dose toxicity of vetiveryl acetate cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate 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.

No data on reproductive toxicity were submitted.

Irritation/sensitization

The test substance was mildly irritating to rabbit skin under the conditions of an OECD TG 404 study. In another study, the test substance was slightly to moderately irritating to the skin of rabbits. In addition, signs of skin irritation were also observed in the acute dermal toxicity study.

Under the conditions of two OECD TG 405 studies, the test substances were either mildly irritating or irritating to rabbit eye. In a third study, the neat test substance and the 10% dilution were mildly irritating to the rabbit eye whereas the 50% dilution was moderately irritating. In yet another study, the neat test substance was not irritating to the rabbit eye, whereas the 50% dilution was mildly irritating.

All four qualities of the test substance tested in the local lymph node assay have been shown to be moderate skin sensitisers.
Based on the submitted studies, the skin and eye irritation potential, as well as the skin sensitisation potential of vetiveryl acetate, cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate on the market is reported and as the composition of the test substances used in the submitted irritation and sensitisation studies is unknown to the SCCS.

Phototoxicity
Based on the submitted data, the photosensitising / photoirritation and phototoxic potential of the test substances cannot be evaluated.
In addition, only partial and insufficient information on the composition of vetiveryl acetate on the market was reported and the composition of the test substances used in the submitted studies is also unknown to the SCCS.

Dermal absorption
No data were submitted. However, the applicant has proposed to use 100% dermal absorption of vetiveryl acetate.

Mutagenicity/Genotoxicity
Vetiveryl acetate was tested for mutagenicity/genotoxicity *in vitro* according to current test guidelines under GLP conditions in following studies: several Bacterial Reverse Mutation Tests (Ames), Mammalian cell chromosomal aberration test using CHO and human peripheral blood and Mammalian cell *hprt* gene mutation test on mouse lymphoma L5178Y cells.

In the first Ames test initial batch 90001735 (purity 65.9% ester component) showed increased mutant frequency in bacterial strain TA100. It was found that the addition of alpha-tocopherol was capable of abolishing the mutagenic activity of vetiveryl acetate. Thus a new preparation of the test material containing alpha-tocopherol (1%) was subjected to a complete Ames test (Study No373MOO) and to all other genotoxicity tests. The SCCS considers this test modification inappropriate because of the known anti-mutagenic properties of alpha-tocopherol.

Further studies performed with vetiveryl acetate containing alpha-tocopherol (1%) showed no genotoxic/mutagenic potential in several bacterial gene mutation assays with *Salmonella typhimurium* in the presence or absence of metabolic activation. However, vetiveryl acetate showed antibacterial properties, thus bacterial Ames is not recommended for testing this substance.

Both mammalian gene mutation and chromosomal aberration tests in human lymphocytes were performed with 1% of alpha-tocopherol and were negative. However, only short 3h exposure was used in mammalian gene mutation test at the *hprt* locus that might be too short for assessing mutagenic potential. The need for longer treatment time (without metabolic activation) is also considered in the revision of the OECD TG.

Results from *in vitro* mammalian cell chromosomal aberration test in Chinese hamster ovary cells are also not sufficient as precipitation occurred in both experiments with and without metabolic activation. Additionally, results from one experiment gave a weak but positive response, even when the test was performed in the presence of alpha-tocopherol.

Genotoxicity data are inadequate to exclude the genotoxic/mutagenic effect of vetiveryl acetate. There was no justification for the use of alpha-tocopherol. There is no

documentation showing that the test substance(s) used in the mutagenicity tests are representative for vetiverlyl acetate from different origin and compositions.
Based on the submitted studies, the mutagenic/genotoxic potential of vetiveryl acetate cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate 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.
New data on the genotoxicity of vetiveryl acetate need to be provided without inclusion of alpha-tocopherol.

Carcinogenicity
No data were submitted.

4. CONCLUSION

The main concern with the safety assessment of the fragrance 'vetiveryl acetate' is that it is a mixture of many different constituents and that the composition of the fragrance will vary considerably depending on the origin of the grass *Vetiveria zizaniodes* from which the crude vetiver oil is derived, as well as the different manufacturing processes of the fragrance from the vetiver oil.

In the absence of knowledge of the composition of the test substances used in the submitted studies, the relevance of test results is questionable.

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

In the previous opinion on vetiveryl acetate, the SCCP was of the opinion that the information submitted was inadequate to assess the safe use of the substance. Before further consideration, information such as characterisation of the test substance and clarification on purity and impurities was required (SCCP/0984/06).

Based on the newly submitted studies, the safety of vetiveryl acetate on the market cannot be evaluated as only partial and insufficient information on the composition of vetiveryl acetate on the market has been reported and as no information has been provided on the composition of the test substances used in the submitted toxicological studies.

Adequate information regarding chemical characterisation and quantification of constituents of 'vetiveryl acetate' on the market, including the concentration range of the constituents, is required. Furthermore, documentation and justification is required to allow judgement on whether the test substances used in the submitted toxicological studies can be considered representative for what is considered as 'vetiveryl acetate' on the market.

2. Does the SCCS have any further scientific concerns with regard to the use of vetiveryl acetate as fragrance ingredient in cosmetic leave-on and rinse-off type products?

Based on the available data, there is evidence that vetiveryl acetate has skin and eye irritation potential and is a moderate skin sensitiser. Concern was raised in the previous opinion (SCCP/0984/06) that vetiveryl acetate may also have a phototoxic potential; however, based on the submitted data, the photosensitising / photoirritation and phototoxic potential of the test substances cannot be evaluated.

Genotoxicity data are inadequate to exclude the genotoxic/mutagenic effects of vetiveryl acetate which were observed in an Ames test. There was no justification for the use of alpha-tocopherol. New data on genotoxicity of vetiveryl acetate without inclusion of alpha-tocopherol need to be provided.

On the basis of the inadequate data provided, a reliable safety assessment cannot be performed whether vetiveryl acetate on the market is safe for use in cosmetics at the concentration limits proposed by the IFRA. However, due to the major concern of genotoxicity the SCCS considers vetiveryl acetate unsafe as a cosmetic ingredient.

5. MINORITY OPINION

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ANNEX

Vetiveryl acetate: Overview of test substances used in the toxicological studies

Study	Test substance	Batch number	Ester content %	Description	Reference
Acute oral tox rat	Vetiveryl acetate	9000317035	65.9	Yellow liquid	Ref: 70 RIFM 1999, RIFM# 35747
Acute oral tox rat	Compound No. 71-90	-	-	Brown liquid	Ref: 16 RIFM 1972, RIFM# 2536
Acute oral tox mouse	Vetyvenyl acetate	-	-	Clear yellow liquid	Ref: 48 RIFM 1981, RIFM# 49324
Acute dermal tox	RIFM # 71-90	-	-	Brown liquid	Ref: 16 RIFM 1972 RIFM#2536
Skin irritation	Vetiveryl acetate	9000317035	65.9	Yellow, viscous liquid	Ref: 71 RIFM 1999a, RIFM# 35070
Skin irritation	Vetyvenyl acetate PPL sample	-	46	Pale green clear liquid	Ref: 49 RIFM 1982a, RIFM# 49323
Eye irritation	Vetiveryl acetate	9000317035	65.9	Yellow, viscous liquid	Ref: 72 RIFM 1999b, RIFM# 35069
Eye irritation	Vetiveryl acetate extra CAS No. 117-98-6	20070028	85.7	Colourless to slight yellowish liquid	Ref: 74 RIFM 2000b, RIFM# 58891
Eye irritation	Vetyvenyl acetate PPL 81/15	-	46	Colourless clear liquid	Ref: 59 RIFM 1982k, RIFM# 49326

Eye irritation	Vetyvenyl acetate PPL 81/15	-	46	Colourless clear liquid	Ref: 58 RIFM 1982j, RIFM# 49325
Skin sensitisation LLNA a)	Acetyver 230451	0000203938	-	Light yellow liquid	Ref.: 79 RIFM 2008a, RIFM# 55336
Skin sensitisation LLNA b)	Acet vetivenyl E 112 Extra	9000669418	-	Yellow liquid	Ref.: 80 RIFM 2008b, RIFM# 55337
Skin sensitisation LLNA c)	Vetiveryl acetate ex Haiti Clos Ha	B	-	Light yellow liquid	Ref.: 81 RIFM 2008c, RIFM# 55338
Skin sensitisation LLNA d)	ROB HB	d) 1590849	-	Yellow liquid	Ref.: 86 RIFM 2011c, RIFM# 55339
28-day oral gavage rat	Vetiveryl acetate CAS No. 117-98-6	VE00085543	- [99 + 1% alpha-tocopherol]	Yellow liquid	Ref.: 84 RIFM 2011a, RIFM# 62943
Ames test	Vetiveryl acetate Stab. (18 months)	9000429043	-	Yellow liquid	Ref: 76 RIFM 2003a, RIFM# 43187
Ames test	Vetiveryl acetate Stab. (24 months)	9000429043	-	Yellow liquid	Ref: 77 RIFM 2003b, RIFM# 43188
Ames test	Vetiveryl acetate 112 Extra Stab. (24 months)	9000428765	-	Yellow to brownish liquid	Ref: 78 RIFM 2003c, RIFM# 43189

Ames test	Vetiveryl acetate extra CAS No. 117-98-6	20070028	85.7	Colourless to pale yellowish liquid	Ref: 75 RIFM 2001, RIFM# 58892
Ames test	Vetiveryl acetate CAS No. 68917-34-0 / 84082-84-8	9000360016	65 + 1% alpha-tocopherol	Yellow, viscous liquid	Ref: 73 RIFM 2000a, RIFM# 43186
Mouse lymphoma test	Vetiveryl acetate CAS No. 117-98-6	VE00231600	- (99%) + 1% alpha-tocopherol	Yellow liquid (certificate) Clear colourless liquid (Covance)	Ref.: xx RIFM 2013, RIFM# 65094
CA in CHO in vitro	Vetiveryl acetate CAS No. 117-98-6	VE00034228	- + 1% alpha-tocopherol	Yellow liquid	Ref.: 83 RIFM 2010a, RIFM# 59006
CA in human peripheral lymphocytes in vitro	Vetiveryl acetate CAS No. 117-98-6	VE00085543	99 + 1% alpha-tocopherol	Yellow liquid	Ref.: 85 RIFM 2011b, RIFM# 62942
NRU phototox in vitro	Vetiveryl acetate	-	-	Clear yellow semi-viscous liquid	Ref.: 90 RIFM 2012d, RIFM# 63844
Phototoxicity Assay in vitro: Human skin model test	Vetiveryl acetate 112 extra	VE00196943	-	-	Ref.: 88 RIFM 2012b, RIFM# 63835

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