EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

Oak moss / Tree moss

(sensitisation only)

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCCP adopted this opinion at its 15th plenary of 15 April 2008

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 Products (SCCP), 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 Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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Opinion to be cited as: SCCP (Scientific Committee on Consumer Products), Opinion on oak moss / tree moss (sensitisation only), 15 April 2008

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1. BACKGROUND

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at its 14th plenary meeting of 24 October 2000 an opinion (SCCNFP/0421/00) concerning Oakmoss/Treemoss, that "… *oakmoss/treemoss extracts, present in cosmetic products, have a well-recognised potential to cause allergic reactions in the consumer as fragrance ingredients…*"

Based on the submission by EFFA[1] of a study "Local nymph Node Assay (LLNA)-Sensitisation dossier on Atranol and Chloroatranol", the Scientific Committee on Consumer Products (SCCP) adopted at its 2nd plenary meeting of 7 December 2004 an opinion (SCCP/0847/04) on Atranol and Chloroatranol present in natural extracts (e.g. Oakmoss and Treemoss extract) with the conclusion:

"Because chloroatranol and atranol are components of a botanical extract, oakmoss absolute, it has been impossible to trace exposure.
Chloroatranol was shown to cause elicitation of reactions by repeated open exposure at the ppm level (0.0005%) and at the ppb level on patch testing (50% elicit at 0.000015%).
As chloroatranol and atranol are such potent allergens (and chloroatranol particularly so), they should not be present in cosmetic products."

Oak-/treemoss extracts are regulated in Annex III, entries 91 and 92 respectively, for labelling purposes when present in concentrations above 10 ppm for leave-on products and 100 ppm for rinse-off products.

In December 2005, EFFA submitted submission II on Oakmoss only. Submission III from December 2006 is a sensitisation dossier on oakmoss/treemoss, treated to remove selectively atranol and chloroatranol.

According to the current IFRA[2] standards Oakmoss extracts (e.g. absolute, resinoid, concrete, etc) are obtained from *Evernia prunastri,* and Treemoss extracts (e.g. absolute, resinoid, concrete, etc) are obtained from *Usnea* and *Pseudevernia furfuracea*. Therefore, qualities marketed as cedar moss are also covered.

2. TERMS OF REFERENCE

1. *Does the SCCP consider oakmoss/treemoss extracts safe for consumers when used in cosmetic products in a total concentration up to 0.1% as currently recommended by IFRA, taken into account the scientific data provided?*

2. *Does the SCCP recommend any further restrictions with regard to the use of oakmoss/treemoss extract in cosmetic products?*

[1] EFFA – European Flavour & Fragrance Association
[2] IFRA – International Fragrance Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Oakmoss absolute (*Evernia spp.*)

Treemoss absolute (*Pseudevernia furfuracea*)

The lichen *Evernia furfuracea* found on Pine trees is also collected from cedar trees and extracts from lichens obtained from this tree are called Cedar moss extracts. Cedar moss is included based on this relationship.

3.1.1.2. Chemical names

Oakmoss
Evernia prunastri (oakmoss) extract; *Evernia prunastri*, ext.; Evernia absolute; oakmoss resinoid (*Evernia spp*.); oakmoss concrete (so-called); Evernia resinoid; *Evernia prunastri* (oakmoss) extract.

Treemoss
Pseudevernia furfuracea extract; Treemoss concrete (*Pseudevernia furfuracea*); Treemoss resinoid (*Pseudevernia furfuracea*)

3.1.1.3. Trade names and abbreviations

/

3.1.1.4. CAS / EINECS number

CAS: 9000-50-4 Oakmoss
68648-41-9 Treemoss
EINECS: 289-861-3 (registered as CAS No. 90028-68-5) Evernia prunastri, ext.
283-658-3 (registered as CAS No. 84696-53-7), Usnea barbata, ext
289-860-8 (registered as CAS No. 90028-67-4) Evernia furfuracea, ext.

3.1.1.5. Structural formula

Not applicable

3.1.1.6. Empirical formula

Not applicable

3.1.2. Physical form

Oakmoss absolute is a dark green, semi-solid to solid mass or a dark brownish-green liquid. Oakmoss resinoid is an almost black-green or brownish-green waxy mass.

Treemoss absolute is a dark green, semi-solid to solid mass or a dark brownish-green liquid.

3.1.3.	Molecular weight

Not applicable

3.1.4.	Purity, composition and substance codes

/

3.1.5.	Impurities / accompanying contaminants

/

3.1.6.	Solubility

/

3.1.7.	Partition coefficient (Log P_{ow})

Log P_{ow}: /

3.1.8.	Additional physical and chemical specifications

Melting point:	/
Boiling point:	/
Flash point:	/
Vapour pressure:	(calculated): <0.01 mm Hg at 20°C (Oakmoss) (calculated): 0.01 mm Hg at 20°C (Treemoss)
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV Absorbance:	λ_{max} below 300 nm

3.2. Function and uses

Characterization: oakmoss is the lichen, *Evernia Prunastri* which grows primarily on oak trees. Extracts are produced from the botanical material and include concretes (produced by extraction with hydrocarbon solvents), absolutes (produced by alcohol extraction of the concrete) and resinoids (produced by hot alcohol extraction).

The IFRA Standard on Treemoss extracts (e.g. absolute, resinoid, concrete, etc.), Usnea and *Pseudevernia furfuracea** (* The Standard therefore also covers qualities marketed as cedar moss), states that the material should not be used such that the level in consumer products exceeds 0.1%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

Not applicable

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Not applicable

3.3.2.2. Mucous membrane irritation

Not applicable

3.3.3. Skin sensitisation

OAKMOSS, LLNA

Guideline:	OECD 429
Group:	28 female CBA/J mice 5 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 1 group of 4 for positive control
Substance:	Mousse Chêne DM HYP
Purity:	/
Atranol/chloroatranol content:	< 50 ppm each (undocumented in report)
Batch:	119459
Vehicle:	Dimethyl formamide (DMF)
Dilutions:	2.5, 5, 10, 25 and 50%
Controls:	negative – DMF Positive – 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP:	in compliance
Date:	29 June – 13 July 2004

For the main study, oakmoss absolute, treated to lower the level of atranol and chloroatranol, was tested at five concentrations: 50%, 25%, 10%, 5% and 2.5% in DMF.
Groups of female CBA/J mice (n=5) were dosed topically on the dorsum of both ears with 25 µl of test material, the same volume of vehicle alone acted as a control. Dosing occurred daily for three consecutive days. Ear thickness measurements were recorded during the assay and used as an indicator of local irritation. The animals "rested" for two days and on the sixth day after the first application, all mice were injected intravenously by the tail vein with 250 µl of sterile saline containing 20 µCi of radiolabelled methyl thymidine (3HTdR). Five hours later, the mice were killed and the draining auricular lymph nodes were excised and a single cell suspension was prepared from the lymph nodes of each test group. Suspensions of the lymph node cells were prepared by mechanical disaggregation. The cell suspensions were washed with 15 ml of 0.9% saline and centrifuged. The pellets obtained were re-suspended, the cellularity and viability of the cells was determined by trypan blue exclusion. The suspensions were then centrifuged and the pellets were precipitated overnight at 4°C with 5% w/v trichloroacetic acid (TCA). The samples were then pelleted by centrifugation for a last time and precipitated with 5% TCA. The cells were re-suspended in 1 ml of 5% TCA and transferred to scintillation vials containing 3 ml of scintillation fluid. The incorporation of 3HTdR was measured by β-scintillation counting and expressed as mean disintegrations per minute (dpm) per lymph node for each experimental group.

For each concentration of test material, a stimulation index (SI) relative to the concurrent vehicle-treated control was calculated. The SI values were calculated by dividing the mean dpm at a given dose level by the mean dpm of the vehicle control group. (A material is considered a sensitizer if at least one concentration is observed to result in an SI value of 3 or more.) An EC3 value, or estimated concentration of test material required to elicit an SI of 3 or more, was derived from the dose-response data by linear interpolation.

No signs of toxicity were observed over the course of the study. Dryness of the skin was noted on day 6 in 1/4 and 2/4 animals in the 25% and 50% dose groups, respectively. Slight to moderate increases in ear thickness were noted in the 50%, 25% and 10% dose groups.

		Stimulation Index
DMF		-
2.5%	Mousse Chêne DM HYP	1.03
5%	Mousse Chêne DM HYP	0.54
10%	Mousse Chêne DM HYP	0.79
25%	Mousse Chêne DM HYP	0.99
50%	Mousse Chêne DM HYP	3.42
25%	α-hexylcinnamaldehyde	2.14

A positive lymphoproliferative response was recorded at the highest concentration tested – 50%. However, as this concentration was observed to be irritating and no evidence of a dose-response relationship was observed, the study authors considered the response at this dose to be due to an irritant effect and thus not to be considered for the calculation of an EC3 value. They concluded that the sample was not likely to be a sensitizer under the conditions of the test.

Ref.: 3, subm. III

Comment
The positive control, 25% α-hexylcinnamaldehyde, did not reach the Stimulation index benchmark of 3.

Guideline: OECD 429
Group: 20 female CBA/J mice
3 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 1 group of 4 for positive control
Substance: CHENE IFRA INCO 20
Purity: /
Batch: FG2.231.2
Solvent: acetone/olive oil (4/1, v/v):
Atranol content: < 50 ppm (undocumented in report)
Chloroatranol content: < 20 ppm (undocumented in report)
Dilutions: 25, 50 and 100%
Controls: negative – acetone/olive oil
Positive – 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP: in compliance
Date: 21 December 2004 – 3 January 2005

CHENE IFRA INCO 20 was found to be soluble in 4:1 acetone:olive oil (AOO) and to be non-irritating up to 100% (which was selected as the highest concentration for the main study).

CHENE IFRA INCO 20 was tested at three concentrations – 25%, 50% and 100% in AOO. No significant irritation, as measured by ear thickness, was observed in any of the dose groups. A positive response was observed at a concentration of 50%.

		Stimulation Index
vehicle		-
25%	CHENE IFRA INCO 20	2.43
50%	CHENE IFRA INCO 20	3.35
100%	CHENE IFRA INCO 20	2.74
25%	α-hexylcinnamaldehyde	11.37

The EC3 value was calculated to be 40.5% (10.125 µg/cm²).

Ref.: 4, subm. III

Guideline: OECD 429
Group: 28 female CBA/J mice
5 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 1 group of 4 for positive control
Substance: OAKMOSS ABSOLUTE F0724
Purity: /
Batch: FG2-294-1
Atranol/chloroatranol content: < 2 ppm each (undocumented in report)
Solvent: acetone/olive oil (4/1 v/v)
Dilutions: 1, 2.5, 5, 10 or 25%,
Controls: negative – acetone/olive oil
Positive – 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP: in compliance
Date: 22 – 27 November 2006

The assay was conducted as described above. A preliminary study was conducted to determine the solubility of the test sample in the recommended vehicles for the LLNA and to assess the potential for irritation for the purpose of dose selection. The test material was found to be soluble in 4:1 acetone:olive oil (AOO) and to be non-irritating up to 25% (which was selected as the highest concentration for the main study).

In the main study, OAKMOSS ABSOLUTE F0724 was tested at five concentrations – 1.0%, 2.5%, 5.0%, 25.0% and 100% in AOO. No significant irritation, as measured by ear thickness, was observed in any of the dose groups.

		Stimulation Index
vehicle		-
1.0 %	OAKMOSS ABSOLUTE F0724	1.10
2.5 %	OAKMOSS ABSOLUTE F0724	1.06
5 %	OAKMOSS ABSOLUTE F0724	1.66
10 %	OAKMOSS ABSOLUTE F0724	2.26
25 %	OAKMOSS ABSOLUTE F0724	3.48
25%	α-hexylcinnamaldehyde	12.80

A positive response was observed at a concentration 25%. The EC3 value was calculated to be 19% (4750 µg/cm²).

Ref.: 5, subm. III

Guideline: OECD 429
Group: 28 female CBA/J mice
5 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 1 group of 4 for positive control
Substance: Absolue Mousse de Chêne SCPP (221129)
Purity: /
Batch: 1469610

Atranol/chloroatranol content: < 50 ppm each (undocumented in report)
Solvent: acetone/olive oil (4/1 v/v)
Dilutions: 0.5, 1, 2.5, 5 or 10%,
Controls: negative – acetone/olive oil
Positive – 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP: in compliance
Date: 22 – 27 June 2006

The sensitization potential of a sample of Absolue Mousse de Chêne SCPP (221129) was assessed in the Local Lymph Node Assay (LLNA). The assay was conducted as described above. A preliminary study was conducted to determine the solubility of the test sample in the recommended vehicles for the LLNA and to assess the potential for irritation for the purpose of dose selection. The test material was found to be soluble in 4:1 acetone:olive oil (AOO) and to be slightly irritating at concentrations greater than 25%. Therefore, the highest concentration selected for the main study was 10%.

In the main study, Absolue Mousse de Chêne SCPP (221129) was tested at five concentrations – 0.5%, 1.0%, 2.5%, 5.0% and 10.0% in AOO. No significant irritation, as measured by ear thickness, was observed in any of the dose groups.

		Stimulation Index
vehicle		-
0.5 %	Absolue Mousse de Chêne SCPP (221129)	1.08
1.0 %	Absolue Mousse de Chêne SCPP (221129)	0.91
2.5 %	Absolue Mousse de Chêne SCPP (221129)	1.06
5 %	Absolue Mousse de Chêne SCPP (221129)	3.65
10 %	Absolue Mousse de Chêne SCPP (221129)	19.19
25%	α-hexylcinnamaldehyde	12.80

A positive response was observed at a concentration of 5.0%. The EC3 value was calculated to be 4.4% (1100 µg/cm²).

Ref.: 6, subm. III

Guideline: OECD 429
Group: 35 female CBA/J mice
7 groups of 5 female mice for test substance
Substance: Arginine treated Oakmoss 05-203-02
Purity: /
Batch: X3243
Atranol/chloroatranol content: < 10 ppm each (undocumented in report)
Solvent: propylene glycol
Dilutions: 2.5, 5, 10, 25 and 50%,
Controls: negative – solvent
positive – 35% α-hexylcinnamaldehyde
GLP: in compliance
Date: 7 – 13 April 2005

A LLNA was conducted using the above material. The initial study protocol indicated the use of DEP/ethanol (75/25) as the solvent but as the study material was found to be insoluble in this vehicle, propylene glycol was used.

Arginine treated Oakmoss 05-203-02 was tested at five concentrations – 2.5, 5, 10, 25 and 50%, in propylene glycol. The SI for the dilutions were 0.94, 0.89, 1.67, 1.52 and 2.51 respectively. As no SI was > 3, the test substance was considered non-sensitising. The positive control had an SI of 26.99.

Ref.: 8, subm. II

Guideline: OECD 429
Group: 35 female CBA/J mice
7 groups of 5 female mice for test substance
Substance: 05-203-01(untreated oakmoss)
Purity: /
Batch: X3243
Atranol content: 4.5% (undocumented in report)
Chloroatranol content: 2.6% (undocumented in report)
Solvent: diethyl phthalate/ethanol 3:1
Dilutions: 2.5, 5, 10, 25 and 50%,
Controls: negative – solvent
positive – 35% α-hexylcinnamaldehyde
GLP: in compliance apart from test substance "not characterised according to GLP" and not tested for purity or stability.
Date: 6 – 12 April 2005

A LLNA was conducted using the above material.

Dilution of 05-203-01 %	**Stimulation Index (SI)**
2.5	0.95
5.0	1.41
10	2.36
25	8.35
50	16.61
35% α-hexylcinnamaldehyde	7.93

The EC3 of the test substance 05-203-01 was calculated to be 11.6.

Ref.: 7, subm. II

Guideline: OECD 429
Group: 35 female CBA/J mice
7 groups of 5 female mice for test substance
Substance: 05-203-03 (untreated)
Purity: /
Batch: E9876
Atranol content: 1.44% (undocumented in report)
Chloroatranol content: 0.83% (undocumented in report)
Solvent: diethyl phthalate/ethanol 3:1
Dilutions: 2.5, 5, 10, 25 and 50%,
Controls: negative – solvent
positive – 35% α-hexylcinnamaldehyde
GLP: in compliance apart from test substance "not characterised according to GLP" and not tested for purity or stability.
Date: 5 – 12 April 2005

A LLNA was conducted using the above material.

Dilution of 05-203-03 %	Stimulation Index (SI)
2.5	0.79
5.0	1.75
10	3.80
25	12.32
50	20.45
35% α -hexylcinnamaldehyde	5.83

The EC3 of the test substance 05-203-03 was calculated to be 8.05.

Ref.: 5, subm. II

Comment
The chemical identity of the test material is not described in the laboratory report.

Guideline:	OECD 429
Group:	35 female CBA/J mice 7 groups of 5 female mice for test substance
Substance:	05-203-04 (JPL treated)
Purity:	/
Batch:	E9876
Atranol/chloroatranol content:	< 10 ppm each (undocumented in report)
Solvent:	diethyl phthalate/ethanol 3:1
Dilutions:	2.5, 5, 10, 25 and 50%,
Controls:	negative – solvent positive – 35% α-hexylcinnamaldehyde
GLP:	in compliance apart from test substance "not characterised according to GLP" and not tested for purity or stability.
Date:	5 – 12 April 2005

A LLNA was conducted using the above material.

Dilution of 05-203-04 %	Stimulation Index (SI)
2.5	0.95
5.0	1.01
10	1.10
25	1.93
50	5.73
35% α-hexylcinnamaldehyde	3.81

The EC3 of the test substance 05-203-04 was calculated to be 32.04.

Ref.: 6, subm. II

Comment
The chemical identity of the test material is not described in the laboratory report.

Guideline:	OECD 429
Group:	female CBA/Ca mice 5 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 3 groups of 4 for positive control
Substance:	oakmoss (LLNA-OM-A to OM-E)
Purity:	/
Batch:	/
Atranol/chloroatranol content:	< 50 ppm each (undocumented in report, detection limit)
Solvent:	1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP)
Dilutions:	2.5%, 5.0%, 10.0%, 25.0% and 50.0%
Controls:	negative – 1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP) Positive – 5, 10, 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP:	in compliance apart from stability and achieved concentration of test substance
Date:	27 April – 3 May 2005

A LLNA was conducted using the above material.

Dilution of LLNA OM-A to OM-E %	Stimulation Index (SI)
2.5	2.2
5.0	1.4
10	1.7
25	5.5
50	2.9
25% α-hexylcinnamaldehyde	<25

The anomalous result at 50% was considered to be possibly due to evaporation.
The EC3 of the test substance LLNA-OM-A to OM-E was calculated to be 15.1% (equivalent to 3775 µg/cm^2) indicating that the test substance is a sensitiser.

Ref.: 4, subm. II

Comment
A 'sample note' states "Oakmoss absolute processed for the selective elimination of atranol and chloroatranol. Atranol not detected. Chloroatranol not detected. Analysis by HPLC. The lower limit of detection was reported to be 50 ppm or less."

Guideline:	OECD 429
Group:	female CBA/Ca mice 5 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 3 groups of 4 for positive control
Substance:	oakmoss (LLNA-OM-1 to OM-5)
Purity:	/
Batch:	/
Atranol content:	0.11% (undocumented in report)
Chloroatranol content:	1.26% (undocumented in report)
Solvent:	1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP)
Dilutions:	2.5%, 5.0%, 10.0%, 25.0% and 50.0%
Controls:	negative – 1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP) Positive – 5, 10, 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP:	in compliance apart from stability and achieved concentration of test substance
Date:	27 April – 3 May 2005

A LLNA was conducted using the above material.

Dilution of LLNA OM-1 to OM-5 %	Stimulation Index (SI)
2.5	1.6
5.0	2.8
10	4.4
25	7.0
50	12.8
25% α-hexylcinnamaldehyde	< 25

The EC3 of the test substance LLNA-OM-1 to OM-5 was calculated to be 5.6% (equivalent to 1400 µg/cm^2) indicating that the test substance is a sensitiser.

Ref.: 3, subm. II

Comment
A 'sample note' states "Samples with typical levels of atranol and chloroatranol."

Guideline: OECD 429
Group: 25 female CBA/J mice
5 groups of 5 female mice for test substance and controls
Substance: Oakmoss absolute Ref A (04-223-09)
Purity: /
Batch: F8406
Atranol content: 3.38% (undocumented in report)
Chloroatranol content: 1.81% (undocumented in report)
Solvent: diethyl phthalate/ethanol 3:1
Dilutions: 7.5, 15 and 30%,
Controls: negative – solvent
positive – 35% α-hexylcinnamaldehyde
GLP: in compliance apart from test substance "not characterised according to GLP" and not tested for purity or stability
Date: 28 July – 3 August 2004

A LLNA was conducted using the above material.

Dilution of 04-223-09%	Stimulation Index (SI)
7.5	1.92
15.0	2.29
30	5.36
35% α-hexylcinnamaldehyde	4.08

The EC3 of the test substance 04-223-09 was calculated to be 18.47.

Ref.: 1, subm. II

Guideline: OECD 429
Group: 25 female CBA/J mice
5 groups of 5 female mice for test substance and controls
Substance: Oakmoss absolute without aldehydes (04-223-10)
Purity: /
Batch: OMLMR1
Atranol content: < 75 ppm (undocumented in report)
Chloroatranol content: < 25 ppm (undocumented in report)
Solvent: diethyl phthalate/ethanol 3:1
Dilutions: 7.5, 15 and 30%,
Controls: negative – solvent
positive – 35% α-hexylcinnamaldehyde
GLP: in compliance apart from test substance "not characterised according to GLP" and not tested for purity or stability
Date: 28 July – 3 August 2004

A LLNA was conducted using the above material.

Dilution of 04-223-10%	Stimulation Index (SI)
7.5	1.11
15.0	1.40
30	1.65
35% α-hexylcinnamaldehyde	5.14

No EC3 of the test substance 04-223-10 could be calculated. The substance was not considered a skin sensitiser.

Ref.: 2, subm. II

TREEMOSS

Guideline: OECD 429
Group: 28 female CBA/J mice
5 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 1 group of 4 for positive control
Substance: Absolue Mousse d'arbre IFRA
Purity: /
Batch: 42-3-44
Atranol content: < 50 ppm (undocumented in report)
Chloroatranol content: < 50 ppm (undocumented in report)
Solvent: acetone/olive oil (4/1 v/v)
Dilutions: 2.5%, 5.0%, 10.0%, 25.0% and 50.0%
Controls: negative – acetone/olive oil
Positive – 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP: in compliance
Date: 20 June – 3 July 2006

The sensitization potential of Absolue Mousse d'arbre IFRA was assessed in the Local Lymph Node Assay (LLNA). The assay was conducted as described above.
A preliminary study was conducted to determine the solubility of the test sample in the recommended vehicles for the LLNA and to assess the potential for irritation for the purpose of dose selection. The test material was found to be soluble in 4:1 acetone:olive oil (AOO) and not excessively irritating at the maximum concentration of 50%. Therefore, the highest concentration selected for the main study was 50%.
In the main study, Absolue Mousse d'arbre IFRA was tested at five concentrations – 2.5%, 5.0%, 10.0%, 25.0% and 50.0% in AOO. No signs of toxicity were observed over the course of the study. Erythema was noted on day 6 in 1/4 and 4/4 animals in the 25.0% and 50.0% dose groups, respectively. Slight increases in ear thickness were noted in the 25.0% and 50.0% dose groups.

		Stimulation Index
vehicle		-
2.5 %	Absolue Mousse d'arbre IFRA	1.59
5.0 %	Absolue Mousse d'arbre IFRA	2.21
10 %	Absolue Mousse d'arbre IFRA	1.84
25 %	Absolue Mousse d'arbre IFRA	3.04
50 %	Absolue Mousse d'arbre IFRA	8.19
25%	α-hexylcinnamaldehyde	12.62

A positive lymphoproliferative response was recorded at the two highest concentration tested – 25.0% and 50.0%. However, as these concentrations were observed to be irritating and no evidence of a dose-response relationship was observed, the response at these doses were considered to be due to an irritant effect and thus not considered for calculation of an EC3 value. It was concluded that an EC3 was not calculable under the conditions of the test.

Ref.: 7, subm. III

Guideline: OECD 429
Group: female CBA/Ca mice
5 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 3 groups of 4 for positive control
Substance: LLNA-TM-2 to TM-6
Purity: /
Batch: /
Atranol content: 0.86% (undocumented in report)

Chloroatranol content:	1.14% (undocumented in report)
Solvent:	1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP)
Dilutions:	2.5%, 5.0%, 10.0%, 25.0% and 50.0%
Controls:	negative – 1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP) Positive – 5, 10, 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP:	in compliance
Date:	18 - 24 May 2005

The sensitization potential of a sample of treemoss absolute (LLNA-TM-2 to TM-6) was assessed in a series of Local Lymph Node Assays (LLNA) at concentrations of 2.5%, 5.0%, 10.0%, 25.0% and 50.0% in 1:3 EtOH:DEP. There was no analysis certificate with the study report but was stated in the submission to contain typical levels of atranol and chloroatranol.
Groups of female CBA/Ca mice (n=4) were dosed topically on the dorsum of both ears with 25 µl of test material, the same volume of vehicle alone acted as a control. Dosing occurred daily for three consecutive days. The animals "rested" for two days and on the sixth day after the first application, all mice were injected intravenously by the tail vein with 250 µl of phosphate buffered saline containing 20 µCi of radiolabelled methyl thymidine (3HTdR). Five hours later, the mice were killed and the draining auricular lymph nodes were excised and pooled for each experimental group. Suspensions of the lymph node cells were prepared by mechanical disaggregation through 200-mesh stainless steel gauze. The cell suspensions were washed twice with phosphate buffered saline (PBS) and precipitated overnight at 4°C with 5% w/v trichloroacetic acid (TCA). The samples were then pelleted by centrifugation. The cells were re-suspended in 1 ml of TCA and transferred to scintillation vials containing 10 ml of scintillation fluid. The incorporation of 3HTdR was measured by β-scintillation counting and expressed as disintegrations per minute (dpm) per lymph node for each experimental group. For each concentration of test material, a stimulation index (SI) relative to the concurrent vehicle-treated control was calculated. The SI values were calculated by dividing the dpm at a given dose level by the dpm of the vehicle control group.

		Stimulation Index
vehicle		-
2.5 %	LLNA-TM-2 to TM-6	2.7
5.0 %	LLNA-TM-2 to TM-6	2.8
10 %	LLNA-TM-2 to TM-6	3.1
25 %	LLNA-TM-2 to TM-6	7.8
50 %	LLNA-TM-2 to TM-6	18.8
25%	α-hexylcinnamaldehyde	6.6

An EC3 value, or estimated concentration of test material required to elicit an SI of 3 or more, was derived from the dose-response data by linear interpolation. A positive lymphoproliferative response was recorded at the three highest concentration tested – 10.0%, 25.0% and 50.0%. The calculated EC3 value was 8.3% (2075 µg/cm²).

Ref.: 1, subm. III

Comment
TM may refer to treemoss but this is not stated in report.

Guideline:	OECD 429
Group:	female CBA/Ca mice 5 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 3 groups of 4 for positive control
Substance:	LLNA-TM-B to TM-F
Purity:	/
Batch:	/

Atranol content: 0.96% (undocumented in report)
Chloroatranol content: not stated0.82% (undocumented in report)
Solvent: 1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP)
Dilutions: 2.5%, 5.0%, 10.0%, 25.0% and 50.0%
Controls: negative – 1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP)
Positive – 5, 10, 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP: in compliance
Date: 18 - 24 May 2005

The sensitization potential of a sample of treemoss absolute (LLNA-TM-B to TM-F) was assessed in a series of Local Lymph Node Assays (LLNA) at concentrations of 2.5%, 5.0%, 10.0%, 25.0% and 50.0% in 1:3 EtOH:DEP. There was no analysis certificate with the study report but was stated in the submission to contain typical levels of atranol and chloroatranol.

		Stimulation Index
vehicle		-
2.5 %	LLNA-TM-B to TM-F	1.7
5.0 %	LLNA-TM-B to TM-F	2.6
10 %	LLNA-TM-B to TM-F	3.1
25 %	LLNA-TM-B to TM-F	4.2
50 %	LLNA-TM-B to TM-F	11.8
25%	α-hexylcinnamaldehyde	6.6

A positive lymphoproliferative response was recorded at the three highest concentration tested – 10.0%, 25.0% and 50.0%. The calculated EC3 value was 9.0% (2250 µg/cm²).
Ref.: 2, subm. III

Comment
TM may refer to treemoss but this is not stated in report.

CEDAR MOSS

Guideline: OECD 429
Group: 28 female CBA/J mice
5 groups of 4 mice for test substance, 1 group of 4 for vehicle control, 1 group of 4 for positive control
Substance: Mousse de Cedre incolore
Purity: /
Batch: 1526344
Atranol content: not stated
Chloroatranol content: not stated
Solvent: acetone/olive oil (4/1 v/v)
Dilutions: 5, 10, 25, 50 and 100%
Controls: negative – acetone/olive oil
Positive – 25% α-hexylcinnamaldehyde in acetone/olive oil (4/1 v/v)
GLP: in compliance
Date: 11 – 16 August 2005

A preliminary study was conducted to determine the solubility of the test sample in the recommended vehicles for the LLNA and to assess the potential for irritation for the purpose of dose selection. The test material was found to be soluble in 4:1 acetone:olive oil (AOO) and not excessively irritating at the maximum concentration of 100%. Therefore, the highest concentration selected for the main study was 100%. In the main study, Mousse de

Cedre incolore was tested at five concentrations – 5.0%, 10.0%, 25.0%, 50.0% and 100.0% in AOO. No signs of toxicity or irritation were observed over the course of the study.

		Stimulation Index
vehicle		-
5 %	Mousse de Cedre incolore	1.36
10 %	Mousse de Cedre incolore	1.89
25 %	Mousse de Cedre incolore	4.36
50 %	Mousse de Cedre incolore	12.35
100 %	Mousse de Cedre incolore	10.26
25%	α-hexylcinnamaldehyde	9.55

A positive lymphoproliferative response was recorded at concentrations equal to and greater than 25%. The EC3 value was calculated to be 16.74% (4185 µg/cm²).

Ref.: 8, subm. III

Human Studies; Marzulli-Maibach Human Maximization

Guideline: /
Group: 100 subjects; 85 male, 15 female; age 18-60years
Substance: Absolue Mousse de Chêne Ram Lot 1
Purity: Chromatogram shows no peak for atranol, chloroatranol (detection limit not described)
Batch: 091404_03sample#3
Solvent: DEP/ethanol (75/25)
Dilution: 3%
Dose: 30 µL in Finn chamber (0.024 µL/mm^2 of active)
GCP: in compliance
Date: 2004

The substance was applied on days 1, 3, 5, 8, 10, 12, 15, 17 and 19 in the induction period. After a 2 week rest, there was a challenge on day 36 with readings after 1 day of occlusion and then on days 2 and 3.

The only reaction observed was a "very slight reaction" in 1 subject on day 37 but this does not appear on the results tables.

Under the conditions of the study, the authors considered that Absolue Mousse de Chene Ram Lot 1 was non-sensitising. The NOAEL was 2420 µg/cm^2.

Ref.: 9, subm. II

3.3.4. Dermal / percutaneous absorption

Not applicable

3.3.5. Repeated dose toxicity

Not applicable

3.3.6. Mutagenicity / Genotoxicity

Not applicable

3.3.7. Carcinogenicity

Not applicable

3.3.8. Reproductive toxicity

Not applicable

3.3.9. Toxicokinetics

Not applicable

3.3.10. Photo-induced toxicity

Not applicable

3.3.11. Human data

See 3.3.3. Sensitisation

3.3.12. Special investigations

Not applicable

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

3.3.14. Discussion

Table 1: overview of the data submitted on oakmoss

Test substance	Atranol/chloroatranol level	LLNA, EC3	µg/cm²	Positive control, SI	Ref.
Oakmoss, treated	< 75 ppm (atranol) < 25 ppm (chloroatranol)	/ (SI <3 up to 30%)	/	35% α-hexylcinnamaldehyde, 5.14	2, II
Oakmoss, treated	< 50 ppm each	15.1%	3775	25% α-hexylcinnamaldehyde, < 25	4, II
Oakmoss, treated	< 10 ppm each	32.04%	8010	35% α-hexylcinnamaldehyde, 3.81	6, II
Oakmoss, treated	< 50 ppm each	/	/	25% α-hexylcinnamaldehyde, 2.14 (did not reach the SI benchmark of 3)	3, III
Oakmoss, treated	< 50 ppm (atranol) < 20 ppm (chloroatranol)	40.5%	10 125	25% α-hexylcinnamaldehyde, 11.37	4, III
Oakmoss, treated	< 2 ppm each	19%	4750	25% α-hexylcinnamaldehyde, 12.80	5, III
Oakmoss, treated	< 50 ppm each	4.4%	1100	25% α-hexylcinnamaldehyde, 12.80	6, III
Oakmoss, treated	< 10 ppm each	/	/	35% α-	8, II

Test substance	Atranol/chloroatranol level	LLNA, EC3	µg/cm²	Positive control, SI	Ref.
		(SI <3 up to 50%)		hexylcinnamaldehyde, 26.99	
Oakmoss, untreated	3.38% atranol 1.81% chloroatranol	18.47%	4617	35% α-hexylcinnamaldehyde, 4.08	1, II
Oakmoss, untreated	0.11% atranol 1.26% chloroatranol	5.6%	1400	25% α-hexylcinnamaldehyde, < 25	3, II
Oakmoss, untreated	1.44% atranol 0.83% chloroatranol	8.05%	2012	35% α-hexylcinnamaldehyde, 5.83	5, II
Oakmoss, untreated	4.5% atranol 2.6% chloroatranol	11.6%	2900	35% α-hexylcinnamaldehyde, 7.93	7, II

Table 2: overview of the data submitted on treemoss

Test substance	Atranol/chloroatranol level	LLNA, EC3	µg/cm²	Positive control, SI	Ref.
Treemoss	0.86% atranol 1.14% chloroatranol	8.3	2075	25% α-hexylcinnamaldehyde, 6.6	1, III
Treemoss	0.96% atranol 0.82% chloroatranol	9.0	2250	25% α-hexylcinnamaldehyde, 6.6	2, III
Treemoss, treated	< 50 ppm each	/ (positive results attributed to irritation)	/	25% α-hexylcinnamaldehyde, 12.62	7, III

The main identified allergens in oakmoss are chloroatranol and atranol. The 'typical' levels of these chemicals have been reduced to levels described variously as < 2ppm to < 75ppm each. Details of the methods of allergen reduction are not described in the provided documents but at least two methods appear to have been used. Adequate details of the analytical procedures used to determine levels of atranol and chloroatranol have not been provided. Demonstration of limits of detection has not been provided.

For untreated oakmoss, EC3 values of between 5.6% and 18.47% were found; for oakmoss treated to reduce the levels of chloroatranol and atranol, EC3 values of between 4.4% and 40.5% were found. These large variations may, in part, be due to laboratory differences. Although higher EC3 values were observed in some experiments with treated extracts, a clear correlation between atranol/chloroatranol content and results of LLNA tests did not become apparent.

In recognition of the fact that contact allergy to oakmoss/treemoss is important, product ingredient labelling is required. Such labelling, as a secondary measure to prevent disease, is helpful only to that group of the European population who have a recognised contact allergy to oakmoss/treemoss (following diagnostic clinical patch testing). Labelling is not helpful to the group who have unrecognised contact allergy.
The submitted data on oakmoss suggests that reduction of the main identified allergens (chloroatranol and atranol) to levels of < 2 ppm each is possible in commercial quantities. Opinion SCCP/0847/04 *stated* that *"As chloroatranol and atranol are such potent allergens (and chloroatranol particularly so), they should not be present in cosmetic products."* A level of 10 ppm of individual allergens in oakmoss present at 0.1% in a cosmetic product equates to 0.000001% (0.01 ppm or 10 ppb) of the individual allergens. Such reduction of the identified allergens is likely to reduce the sensitising potential of oakmoss and the elicitation of allergic reactions in consumers previously sensitised. However, any reduction in elicitation will need to be demonstrated by appropriate clinical testing of subjects previously sensitised.

Insufficient data is available to comment on treemoss and cedar moss but reduction in the levels of chloroatranol and atranol would reduce any sensitising potential of the products due to the presence of these two allergens.

4. CONCLUSION

1. *Does the SCCP consider oakmoss/treemoss extracts safe for consumers when used in cosmetic products in a total concentration up to 0.1% as currently recommended by IFRA, taken into account the scientific data provided?*

Oakmoss/treemoss contain atranol and chloroatranol. In the opinion of the SCCP of 7 December 2004 (SCCP/0847/04) it was stated that these allergens should not be present in cosmetic products. Therefore, untreated oakmoss/treemoss is not safe for the consumer. In treated preparations of oakmoss, it is possible to reduce the levels of atranol and chloroatranol to less than 2 ppm. Although it might be anticipated that reduction of these allergens could reduce elicitation on the skin of previously sensitised individuals. This will need to be demonstrated by appropriate clinical studies. Until such studies are available, it is not possible to estimate if oakmoss, containing atranol and chloroatranol to 2 ppm each, when present up to 0.1% in finished cosmetic products is safe for the consumer.

Little data is available for treemoss and cedar moss. However, if they are treated to reduce the levels of atranol and chloroatranol to less than 2 ppm as is possible for oakmoss, than, if present at 0.1% in finished cosmetic products, the comments above for oakmoss apply. Similar, appropriate clinical studies as above are required.

The primary prevention of the induction of contact allergy can be achieved by eliminating consumer exposure or reducing exposure to a level at which induction is improbable.

2. *Does the SCCP recommend any further restrictions with regard to the use of oakmoss/treemoss extract in cosmetic products?*

It appears that it is possible to reduce, on a commercial scale, the levels of the main allergens (chloroatranol and atranol) in oakmoss to < 2 ppm each in the 'neat' product. The levels of these allergens, which would then be present in cosmetic products where oakmoss is used at 0.1%, would be such that the risks of induction and elicitation of allergic reactions to them would be low. However, appropriate clinical studies on individuals with characterised contact allergy to oakmoss are required to demonstrate the dose response characteristics of allergic elicitation reactions with the modified preparations.

5. MINORITY OPINION

Not applicable

6. REFERENCES

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