



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL
Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Coumarin

(sensitisation only)

Adopted by the SCCP
during the 8th plenary meeting of 20 June 2006

TABLE OF CONTENTS

1.	BACKGROUND	3
2.	TERMS OF REFERENCE	3
3.	OPINION	3
4.	CONCLUSION	15
5.	MINORITY OPINION	15
6.	REFERENCES	15
7.	ACKNOWLEDGEMENTS	17

1. BACKGROUND

The Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) adopted at its plenary session of 8 December 1999, an opinion concerning Fragrance Allergy in Consumers (SCCNFP/0017/98). Based on this opinion, Coumarin was regulated within the 7th Amendment of the Cosmetic Directive (76/768/EEC) which required a labelling in the ingredients, if the substance is present in concentration higher than 10 ppm in leave-on products and 100 ppm in rinse-off products.

Industry has asked for a re-evaluation of coumarin at a purity of > 99.99%, which is claimed of having no sensitising properties.

2. TERMS OF REFERENCE

1. *Does the SCCP consider that Coumarin with the specified purity (> 99.99%) has no sensitizing properties when used in cosmetic products?*
2. *If yes, does the SCCP propose any restrictions or conditions for its use in cosmetic products?*
3. *Do the data provided change the opinion of the SCCP concerning Fragrance Allergy in Consumers (SCCNFP/0017/98)?*

3. OPINION

Introduction

Coumarin (anhydride of o-coumaric acid) is a white, crystalline lactone, obtainable naturally from several plants, such as tonka bean, lavender, sweet clover grass, strawberries, and cinnamon, or produced synthetically from an amino acid, phenylalanine. Coumarin has a characteristic odour like that of vanilla beans. It is used for the preparation of flavours and fragrances. The coumarin nucleus (benzo-2-pyrone) is derived from cinnamic acid (phenylacrylic skeleton) in the biosynthesis. Accordingly, the hydroxy group attached to coumarin structure at 7 position is important in biosynthesis pathway. Umbelliferone (7-hydroxy coumarin), esculetin (6,7-Dihydroxycoumarin), scopoletin (7-hydroxy-6-methoxycoumarin) are the widespread coumarins in nature.

Coumarin derivatives are used as therapeutic anticoagulants and as rodenticides by causing fatal haemorrhage. Synthetic 7-hydroxy coumarins are used as UV absorbers and in the synthesis of certain drugs.

Coumarin is a widely used fragrance ingredient. It was found in 57% of 73 deodorants on the European market in a 1998 published study (Rastogi et al 1998).

Coumarin is regulated within the 7th Amendment of the Cosmetic Directive (76/768/EEC) which required labelling if present in a concentration of 10 ppm or higher in leave-on or 100 ppm in rinse-off products.

Opinion on Coumarin (sensitisation only)

There is no upper limit to the concentration of coumarin which may be present in finished cosmetic products according to Council Directive 76/768/EEC on cosmetics or in the IFRA standards

Coumarin has caused allergic reactions on 1.2 - 6.8% of patients suspected for fragrance contact allergy, (ref.: SCCNFP/0017/98 and 11).

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Coumarin

3.1.1.2. Chemical names

2H-1-Benzopyran-2-one; 1,2-Benzopyrone; 2-Oxo-1,2-benzopyran; 3-(2-hydroxyphenyl)-delta-lactone-2-Propenoic acid; cis-o-Coumaric acid lactone; 2-Oxo-2H-1-benzopyran; benzo-alpha-pyrone; o-hydroxycinnamic acid delta-lactone;; o-hydroxycinnamic acid lactone; o-hydroxycinnamic lactone;; 2H-benzopyran-2-one; Benzo-2-pyrone; Benzopyran-2-one; 3-(2-hydroxyphenyl)-2-Propenoic acid, delta-lactone

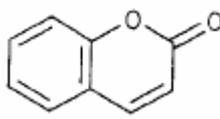
3.1.1.3. Trade names and abbreviations

Tonka bean camphor; coumarinac lactone

3.1.1.4. CAS / EINECS number

CAS: 91-64-5
EINECS: 202-086-7

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₉H₆O₂

3.1.2. Physical form

White crystals, flakes or powder

Opinion on Coumarin (sensitisation only)

3.1.3. Molecular weight

Molecular weight : 146

3.1.4. Purity, composition and substance codes
--

> 99.9% (the purity of the substance considered in this submission)

Remark

The purity of coumarin has not been determined. The stated purity represents 100% minus the percentage of specific impurities.

3.1.5. Impurities / accompanying contaminants

Potential impurities: 3,4-dihydrocoumarin
3-methylcoumarin
3-ethylcoumarin

3.1.6. Solubility

Soluble in alcohol, ether, chloroform and fixed volatile oils; slightly soluble in water.

3.1.7. Partition coefficient (Log P _{ow})

Log P_{ow}: /

3.1.8. Additional physical and chemical specifications
--

Organoleptic properties: fragrant odour similar to vanilla
Melting point: 69 °C
Boiling point: 290 °C
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /

3.2. Function and uses

Coumarin is a fragrance ingredient. It is used for the preparation of flavours and fragrances. Coumarin is used as an additive in perfumes and fragranced consumer products at concentrations ranging from 0.5% to 6.4% in fine fragrances and at less than 0.01% in detergents (ref 15).

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

Patch testing, humans

Guideline: /

Group: study 1: 100 patients referred to hospital for investigation of possible contact dermatitis (patch tested with coumarin at 1% and 10%) and 279 patients (patch tested with coumarin at 2%)

study 2: 101 patients allergic to the standard fragrance mix (patch tested at 2%)

study 3: 30 patients with a relevant positive patch test to their own perfumed products (patch tested at 2%)

Substance: coumarin

Batch: /

Purity: 99.9%

Dose: /

Control: /

Coumarin (99.9%) was dispersed in liquid petrolatum (at 45°C) at concentrations ranging from 2 to 10%. Homogeneity was checked by UV analysis against petrolatum. Control of the presence of coumarin and of the homogeneity was done at the beginning and at the end of the studies.

Three different clinical studies were performed:

Study 1

379 patients referred to the hospital for evaluation of possible contact dermatitis. The first 100 patients were patch tested to coumarin at 1 and 10%. The remaining 279 were patch tested to coumarin 2% only. There was no reaction.

Results

One patient reacted to several chemicals including coumarin at 2%. There were no other reactions to coumarin.

Study 2

101 Patients positive to the fragrance mix.

Results

One patient was positive to coumarin 2% (++)

Study 3

30 patients with the presence of a relevant positive patch test to their own perfumed product. Patients received coumarin at 2% on the back.

Results

No patient showed a positive allergic reaction to coumarin.

Ref.: Vocanson 2006

Comment

The paper is confusing in part as it is unclear as to whether some patients in study 1 were tested to 1% or 2% coumarin. Also, the authors dismiss the reaction to coumarin observed in one individual in study 1.

Animal data

The contact sensitization of 11 coumarin isomers and derivatives were examined by subcutaneous sensitizing of guinea pigs, and the structure-activity relationship and cross-reactivity were investigated. Esculetin, 4-methylesculetin and daphnetin were found to be strong sensitizers. 4-Hydroxy-coumarin was found to be a moderate sensitizer. Other coumarin isomers and derivatives were weak to mild sensitizers. The results from the abstract of this study suggest that the introduction of a second hydroxy group, especially adjacent substitution at the 6, 7, and 8 positions of the coumarin ring, may play an important role in exhibiting the contact sensitization activity. The cross-reactivity was observed between esculetin and 4-methylesculetin, esculin or isoscopoletin, and also between daphnetin and 4-methylumbelliferone or umbelliferone, although there was no cross-reactivity between esculetin and daphnetin. It is interesting to note that guinea pigs, which had a weak sensitivity to umbelliferone, showed a strong cross-reactivity to daphnetin, while those, which had a weak

sensitivity to daphnetin, showed a weak cross-reactivity to umbelliferone. It is assumed that a skin-protein conjugation at 5 or 6 positions of the coumarin ring is important to elicit the cross-reactivity of esculetin or daphnetin groups.

Ref.: Masamoto 2001(abstract only)

Comment

Study cannot be evaluated because of the lack of sufficient information.

LLNA studies

Guideline:	OECD 429
Species:	female CBA/j and BALB/c mouse strains
Group:	4 groups, 4 animals per group
Substance:	coumarin; coumarin A; coumarin B
Batch:	/
Purity:	coumarin: > 99.9% Coumarin A and B: not stated (main impurities: 6-chlorocoumarin, benzochromene, 3,4-dihydrocoumarin)
Concentration:	coumarin preparations: 10, 25 and 50% in N,N-dimethylformamide (DMF) 6-chlorocoumarin: 2.5, 5 and 10% in DMF benzochromene: 2.5, 5 and 10% in DMF 3,4-dihydrocoumarin: 2.5, 5 and 10% in DMF
Dose:	25 µl
Control:	alpha-hexylcinnamaldehyde (25% in DMF)
GLP:	not in compliance

Three different coumarin preparations were tested in the local lymph node assay.

Pure coumarin was synthesised according to a controlled Perkin process from pure salicylaldehyde. Two other coumarin samples, coumarin A and coumarin B produced from o-cresol (2-methylphenol) were purchased.

The LLNA was conducted according to the design validated by ICCVAM (Interagency coordination Committee on the Validation of Alternatives methods) and the OECD guideline. HCA was used as a positive control. All the chemicals were dissolved in N,N-dimethylformamide (DMF).

Coumarin preparations were tested at 10, 25 and 50%. 6-chlorocoumarin, 3,4-dihydrocoumarin and benzochromene were tested at 2.5, 5 and 10%.

4 groups of 4 mice were painted topically at the dorsum of both ears daily for 3 consecutive days. More groups were tested but data not given.

Five days after initial application the common procedure was used to excised the draining lymph nodes.

Results

99.9% coumarin did not induce significant cell proliferation in that the stimulation index derived from the data did not reach the threshold value of 3 (2.4 at 50%). In contrast, coumarin A gave a SI of 3.2 and coumarin B a SI of 4. (see Table)

Opinion on Coumarin (sensitisation only)

Table: Assessment of the sensitizing activity of different coumarins and impurities in the mouse LLNA *

Conc.	Stimulation Index (SI)					
	99.9% coumarin	Coumarin A	Coumarin B	6-chloro-coumarin	DHC	Benzo-chromene
2.5%				2.7	2.1	1.8
5%				4.95	5.1	2.6
10%	1.9	0.9	1.9	3	7	3.4
25%	1.8	2.05	3.7			
50%	2.4	3.2	3.5			
HCA 25%	6.7	14.4	7.4	15.9	15.9	10.3
Pos. LLNA experiments	0/5	2/4	1/4	2/4	2/2	1/1

*The authors state that “only representative experiments for each chemical are shown”. Only the SI is shown here. Vehicle: DMF

Conclusion

Under the conditions of this murine local lymph node assay (LLNA), the test substances Coumarin A, Coumarin B, 6-chlorocoumarin, 3,4-dihydrocoumarin (DHC) and benzochromene induced delayed contact hypersensitivity (Stimulation Index ≥ 3). “Pure Coumarin” did not induce delayed contact hypersensitivity (Stimulation Index < 3).

Ref.: Vocanson 2006

Comment

Only a selection of results (“representative experiments”) is shown in the reference. It is stated, but no data shown, that LN cell proliferation induced by the different coumarin preparations and impurities was obtained in the two strains of mice.

The SI obtained from repeated testing with the positive control (HCA) had wide variability (from 6.7 to 15.9 displayed).

The purity of coumarin has not been determined. The stated purity represents 100% minus the percentage of specific impurities.

Guideline:	EEC 96/54/EC Part B, Method B.6
Species/strain:	CBA/J mice
Groups:	5 groups of 4 female mice
Substance:	Rhodiascent TM Coumarine supplied by Rhodia Services - RSP
Batch:	“labelling 0013101”
Purity:	not stated
Dose:	25 μ l of 5%, 10% and 25% (w/v) Rhodiascent TM Coumarine
Vehicle:	acetone/olive oil (4/1, v/v)
Negative control:	acetone/olive oil (4/1, v/v)
Positive control:	α -hexylcinnamaldehyde (HCA) at 25% in acetone/olive oil

GLP: in compliance

Skin sensitization was evaluated in a murine Local Lymph Node Assay (LLNA). Each animal received a daily topical application of 25 µL on the dorsal surface of each ear for 3 consecutive days. A negative control group received the vehicle (acetone/olive oil (4/1)). Five days after the first topical application, mice were injected intravenously through the tail vein with [³H]methylthymidine (³HTdR) in 0.9% NaCl. After 5 hours, draining auricular lymph nodes were removed and pooled for each group. Single cell suspensions of lymph node cells were prepared. The proliferative response was measured by incorporation of [³H]TdR. The values obtained were used to calculate Stimulation Indices (SI). A positive control group received a reference substance, α-hexylcinnamaldehyde (HCA).

Results

The SI was 2.72 at 5% concentration, 2.92 at 10%, and 2.31 at 25%. The sensitivity of the test system was shown by the positive control, HCA at 25%, for which the SI was 6.38.

Conclusion

Under the conditions of this murine Local Lymph Node Assay, Rhodiascent TM Coumarine did not induce delayed contact hypersensitivity (SI <3).

Ref.: CIT 2001

Guideline:	OECD draft 429 (2000)
Species/strain:	CBA/J mice
Group size:	4 female mice
Substances:	Coumarine Rhodiascent TM Coumarine - Chine 0013090/01 Ex PRC Coumarine – Chine Tianjin freeword 6-Chloro-Coumarine All supplied by Rhodia Services
Purity:	not stated
Dose:	25 µl (see tables for concentrations)
Vehicle	N,N-Dimethylformamide (DMF)
Negative control:	DMF
Positive control:	α-hexyl cinnamaldehyde (HCA) at 25% in DMF
GLP:	not in compliance

Skin sensitization was evaluated in a murine Local Lymph Node Assay (LLNA). The vehicle and concentrations were chosen, based on results from a pilot study. 3 sets of experiments were performed with each of the 4 substances at different concentration respectively and the negative and positive control (tables 1-4). Each animal received a daily topical application of 25 µL on the dorsal surface of each ear for 3 consecutive days. Five days after the first topical application, mice were injected intravenously through the tail vein with [³H]methylthymidine (3HTdR) in 0.9% NaCl. After 5 hours, draining auricular lymph nodes were removed and pooled for each group. Single cell suspensions of lymph node cells were prepared. The proliferative response was measured by incorporation of (3HTdR). The values obtained were used to calculate Stimulation Indices (SI).

Opinion on Coumarin (sensitisation only)

Results

Table 1: Results from the LLNA with Coumarine Rhodiascent TM in DMF

Concentration of substance or HCA	Stimulation Index (SI)*		
	Experiment 1	Experiment 2	Experiment 3
10%	1.3	1.6	1.3
25%	0.8	2.3	1.8
50%	0.6	3.1	2.4
HCA 25%	5.8	2.8	6.7

*Poor printing quality of figures in the reference

Table 2: Results from the LLNA with Coumarine – Chine 0013090/01 Ex PRC in DMF

Concentration of substance or HCA	Stimulation Index (SI)*		
	Experiment 1	Experiment 2	Experiment 3
10%	2.0	1.7	0.6
25%	2.6	1.8	1.4
50%	3.0	1.6	0.3
HCA 25%	5.3	5.7	7.4

*Poor printing quality of figures in the reference.

Table 3: Results from the LLNA with Coumarine – Chine Tianjin freeword in DMF

Concentration of substance or HCA	Stimulation Index (SI)*		
	Experiment 1	Experiment 2	Experiment 3
10%	1.1	1.9	1.2
25%	2.4	3.7	0.3
50%	1.9	4.0	1.8
HCA 25%	7.8	2.8	7.4

*Poor printing quality of figures in the reference

Table 4: Results from the LLNA with 6-Chloro-Coumarine in DMF

Concentration of substance or HCA	Stimulation Index (SI)*		
	Experiment 1	Experiment 2	Experiment 3
1%	-	0.6	-
2.5%	-	1.5	2.4
5%	3.4	0.8	1.8
10%	3.3	-	1.8
HCA 25%	2.8	6.7	7.4

*Poor printing quality of figures in the reference

Conclusion

The results indicate that each of the 4 Coumarin substances tested may be a skin sensitizer, as a Stimulation Index (SI) of ≥ 3 , with a positive dose response, was obtained for each substance in 1 of 3 experiments. The reproducibility of test results between experiments was however low.

Ref.: INSERM 2003

Comments

The purity of the test substances is not given.

The tables of results are difficult to read due to poor quality. The reporting of results contains mistakes, and the design is not clearly described, particularly concerning the number of tests performed with the negative and positive controls respectively.

All SI ≥ 3 were interpreted by the authors as due to skin irritation caused by the test substance, and thus not considered relevant.

The SI obtained from repeated testing with the positive control (HCA) and with different coumarin substances showed a wide variability. EC3 values were not reported.

Guideline:	OECD draft 429 (2000)
Species/strain:	BALB/c mice
Group size:	4 females
Substances:	Coumarine Rhodiascent TM Coumarine - Chine 0013090/01 Ex PRC Coumarine – Chine Tianjin freeword 6-Chloro-Coumarine Coumarine – Chine Tianjin freeword (CA : 145 CRL : 03 RAN 1483) Coumarine – SRD aromatics LTD – Indian no. 2 Dihydrocoumarine All supplied by Rhodia Services
Purity:	not stated
Dose:	25 μ l (see tables for concentrations)
Vehicle	N,N-Dimethylformamide (DMF)
Negative control:	DMF
Positive control:	α -hexyl cinnamaldehyde (HCA) at 25% in DMF
GLP:	not in compliance

Skin sensitization was evaluated in a murine Local Lymph Node Assay (LLNA). The vehicle and concentrations were chosen, based on results from a pilot study. A total of 3 sets of experiments were performed, including 2-4 of the 7 substances at different concentration respectively (table 1), and the negative and positive control. Each animal received a daily topical application of 25 μ L on the dorsal surface of each ear for 3 consecutive days. Five days after the first topical application, mice were injected intravenously through the tail vein with [3 H]methylthymidine (3HTdR) in 0.9% NaCl. After 5 hours, draining auricular lymph nodes were removed and pooled for each group. Single cell suspensions of lymph node cells were prepared. The proliferative response was measured by incorporation of (3HTdR). The values obtained were used to calculate Stimulation Indices (SI).

Opinion on Coumarin (sensitisation only)

Results

Table: Results from the LLNA with 7 Coumarin substances and HCA in DMF

Substance	Conc.	Stimulation Index (SI)		
		Experiment 1	Experiment 2	Experiment 3
Coumarine Rhodiascent TM	10%	0.83	-	-
- " -	25%	1.69	-	-
- " -	50%	2.88	-	-
Coumarine - Chine 0013090/01 Ex PRC	10%	-	0.93	-
- " -	25%	-	2.05	-
- " -	50%	-	3.19	-
Coumarine – Chine Tianjin freeword*	10%	-	0.67	-
- " -	25%	-	2.11	-
- " -	50%	-	2.11	-
6-Chloro-Coumarine	2.5%	-	-	2.74
- " -	5%	-	1.16	4.94
- " -	10%	-	0.49	2.98
Coumarine – Chine Tianjin freeword*	10%	-	0.8	-
- " -	25%	-	1.02	-
- " -	50%	-	1.34	-
Coumarine – SRD aromatics LTD – Indian no. 2	10%	0.54	-	-
- " -	25%	1.36	-	-
- " -	50%	0.95	-	-
Dihydrocoumarine	2.5%	-	-	2.08
- " -	5%	-	-	5.13
- " -	10%	5.66	-	7.03
- " -	25%	11.43	-	-
- " -	50%	11.68	-	-
HCA	25%	7.9	14.42	15.87

*Unclear which of the two Coumarine – Chine Tianjin freeword substances the results refer to
- = not tested

Conclusion

Under the conditions of this murine local lymph node assay (LLNA), the test substances Coumarine - Chine 0013090/01 Ex PRC, 6-Chloro-Coumarine and Dihydrocoumarine induced delayed contact hypersensitivity (Stimulation Index ≥ 3 in the LLNA).

Under the conditions of this LLNA, Coumarine Rhodiascent TM, Coumarine – Chine Tianjin freeword, and Coumarine – SRD aromatics LTD – Indian no. 2 did not induce delayed contact hypersensitivity (Stimulation Index < 3 in the LLNA).

Ref.: INSERM 2004

Opinion on Coumarin (sensitisation only)

Comments

The purity of the test substances is not given. The animal strain used (BALB/c) is not one of the preferred, according to OECD guideline 429. The rationale for this modification is not given in the submission. The SI obtained from repeated testing (3 experiments) with the positive control (HCA) showed remarkable variability (7.9 to 15.89).

A poster was included in the submission, with content however not relevant for the evaluation of Coumarin as skin sensitiser.

Ref.: Roger R et al.

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

A 44 year-old patient has been reported to be sensitized to coumarin. The patient showed negative results on patch testing to fragrance mix. The eau de toilette was chemically fractionated. Each fraction obtained was afterwards tested on the patients using a ROAT and/or a patch test. Only 1 fraction gave a positive ROAT result. This fraction was analyzed and found to contain coumarin and ethyl vanillin. Coumarin, one of the most widely used fragrance compounds that is not present in the fragrance mix, was confirmed as being the sensitizer.

Ref.: Mutterer 1999

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

Some of the information provided in the submission is confusing. There are errors in the documents relating to the patch test concentrations. The purity of the coumarin used in some of the experiments was not stated.

The coumarin used in the various experiments was 99.9%. According to the authors, this purity was not shown to be an allergen in the LLNA undertaken. However, the same purity of coumarin was used for patch testing, several cohorts of patients with or suspected to have contact allergy. Two individuals reacted on patch testing to coumarin 2% (99.9%).

The purity of the coumarin on the European market and to which the consumer is presently exposed, is unknown. Therefore, without this information on purity it is not possible to compare the data provided in the present submission with the data already published concerning the epidemiology of contact allergy to coumarin.

4. CONCLUSION

Coumarin with the purity of 99.9% has been shown to be able to elicit allergic contact reactions in individuals patch tested to a 2% dilution.

The test substance has not been identified by batch number with an associated chemical analysis.

The data submitted does not cause the SCCP to change the Opinion “Fragrance allergy in consumers doc n°SCCNFP/0017/98.

5. MINORITY OPINION

Not applicable

6. REFERENCES

1. Mutterer V, Gimenez Arnau E, Lepoittevin JP, Johansen JD, Frosch PJ, Menne T, Andersen KE, Bruze M, Rastogi SC, White IR. Identification of coumarin as the sensitizer

- in a patient sensitive to her own perfume but negative to the fragrance mix. *Contact Dermatitis* 1999; 40:196-9
2. Frosch PJ, Pirker C, Rastogi SC, Andersen KE, Bruze M, Svedman C, Goossens A, White IR, Uter W, Arnau EG, Lepoittevin JP, Menne T, Johansen JD. Patch testing with a new fragrance mix detects additional patients sensitive to perfumes and missed by the current fragrance mix. *Contact Dermatitis* 2005; 52:207-15.
 3. Paulsen E. Contact sensitization from Compositae-containing herbal remedies and cosmetics. *Contact Dermatitis* 2002; 47:189-98.
 4. Frosch PJ, Johansen JD, Menne T, Pirker C, Rastogi SC, Andersen KE, Bruze M, Goossens A, Lepoittevin JP, White IR. Further important sensitizers in patients sensitive to fragrances. *Contact Dermatitis* 2002; 47:78-85.
 5. Masamoto Y. Sensitization and crossreaction of simple coumarins. *Yakugaku Zasshi* 2001;121:97-103. Japanese
 6. Kunkeler AC, Weijland JW, Bruynzeel DP. The role of coumarin in patch testing. *Contact Dermatitis* 1998; 39:327-8.
 7. Beckley-Kartey SA, Hotchkiss SA, Capel M. Comparative in vitro skin absorption and metabolism of Coumarin (1,2-benzopyrone) in human, rat, and mouse. *Toxicol Applied Pharmacol* 1997; 145:34-42.
 8. Yourick JJ, Bronaugh RL. Percutaneous absorption and metabolism of Coumarin in human and rat skin. *J Applied Toxicology* 1997; 17:153-8.
 9. Malten KE, van Ketel WG, Nater JP, Liem DH. Reactions in selected patients to 22 fragrance materials. *Contact Dermatitis* 1984;11:1-10.
 10. Kaidbey KH, Kligman AM. Photosensitization by coumarin derivatives. *Arch Dermatol* 1981; 117:258-63.
 11. Frosch P J, Johansen J D, Menne T et al. Further important sensitizers in patients sensitive to fragrances. Reactivity to 14 frequently used chemicals. *Contact Dermatitis* 2002;47:78 – 85. 741 –743.
 12. Rastogi SC, Lepoittevin JP, Johansen JD, Frosch PJ, Menné T, Bruze M, Dreier B, Andersen KE, White IR. Fragrances and other materials in deodorants: search for potentially sensitizing molecules using combined GC-MS and structure activity relationship (SAR) analysis. *Contact Dermatitis*, 1998 Dec; 39(6):293-303.
 13. Felter SP, Vassallo JD, Carlton BD, Daston GP. A safety assessment of coumarin taking into account species-specificity of toxicokinetics. *Food and Chemical Toxicology* 2006;44:462-475.
 14. Lake B. Coumarin metabolism, toxicity, and carcinogenicity: relevance for human risk assessment. *Food and Chemical Toxicology* 1999;37:423-453.
 15. Floc'h F, Mauger F, Desmurs JR. Coumarin in plants and fruits: implications in perfumery. *Perfumer and Flavorist* 2002;27:32-37.
 16. Vocanson, Goujon, Chabeau. The skin allergenic properties of chemicals may depend on contaminants. Evidence from studies on coumarin. 2006 which journal?
- CIT/Study No. 21214 TSS/Rhodiascent TM Coumarine/Rhodia Services –RSP 13 December 2001

INSERM U503/Société Rhodia Services. Evaluation du potentiel de sensibilisation cutanée des Coumarines à l'aide du Local Lymph Node Assay murin. 10 décembre 2003

INSERM U503/Société Rhodia Services. Evaluation du potentiel de sensibilisation cutanée des Coumarines à l'aide du LLNA. 26 mai 2004

Roger R, Rebion S, Durand D, Da Silva P, de Jouffrey S, Forster R, Le Bigot JF, Lebrech H, Pallardy M. Local Lymph Node Assay (LLNA): discrimination of irritant and sensitizing potential and evaluation of sex differences. Centre International de Toxicologie (CIT) Poster – publication data not given

7. ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

Dr. C. Chambers
Prof. R. Dubakiene
Dr. R. Grimalt (rapporteur)
Dr. B. Jazwiec-Kanyion
Prof. V. Kapoulas
Prof. J. Krutmann
Prof. C. Lidén
Prof. J.-P. Marty
Dr. S. Rastogi
Prof. J. Revuz
Prof. V. Rogiers
Prof. T. Sanner (chairman, acting)
Dr. I.R. White