Evaluation of selected sensitizing fragrance substances- A LOUS follow-up project

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Preface

The List of Undesirable Substances (LOUS) was established by the Danish Environmental Protection Agency (EPA) as a guide for enterprises. It addresses chemical substances of concern, based on their hazardous properties and the volumes used in Denmark. The latest version of LOUS from 2009 includes 40 chemical substances or groups of substances (DK EPA 2010).

During the period 2012-2015, all substances listed on LOUS have been surveyed and further need for risk management measures will be evaluated. In certain cases, implementation projects have been launched to achieve the goals laid down in the strategies for each of these substances/substance groups.

The present project "Evaluation of selected sensitising fragrance substances" was initiated as a LOUS follow-up project by the Danish EPA. The objective of this study was to evaluate selected fragrance substances in relation to the classification criteria for strong sensitisers (Category 1A sensitisers) according to the CLP Regulation on classification, labelling and packaging of substances and mixtures (EC no. 1272/2008)[1].

The project was carried out from July to November 2015 at the National Food Institute, Technical University of Denmark.

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[1] REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006, with amendments M1-M9 and corrigenda C1-C2, 1 June 2015. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1272-20150601&from=en

Summary and conclusion

Fragrances are widely used in many different types of consumer products. Therefore, the general population can be exposed to fragrances from many different sources and consequently can have a substantial exposure despite the fact that fragrances most often are used in a relatively low concentration in individual consumer products.

Many fragrances have been shown to cause harmful effects to human health. Skin sensitisation (contact allergy) is identified as the critical effect for a wide range of fragrances. Many fragrances are already classified as skin sensitisers (Skin Sens Cat 1) according to the CLP Regulation (EC no. 1272/2008). The CLP criteria for classification of skin sensitisers were revised in 2011 and now provide possibility for sub-categorising skin sensitisers in two sub-categories, sub-category 1A (strong sensitisers) or 1B (other skin sensitisers). Some of the fragrances that are already classified as skin sensitisers in Category 1 may possibly fulfil the CLP criteria for classification as strong sensitisers in sub-category 1A.

A classification of a specific substance in sub-category 1A implies that classification and labelling of mixtures containing the substance is required at a lower concentration (factor 10) compared to skin sensitisers in Category 1. The more stringent labelling requirements for sub-category 1A sensitisers will apply for mixtures under the scope of the CLP regulation such as e.g., washing and cleaning products. Consequently, the labelling may increase the protection of users (workers, consumers) as it will allow sensitised individuals to take precautionary measures to prevent direct skin contact with a product containing a strong sensitiser.

The purpose of this project was to retrieve and review the available data for 42 selected fragrances already classified as skin sensitisers in Category 1 (harmonised and/or notified classification) in order to assess whether these substances fulfil the criteria for classification in sub-category 1A. The 42 fragrances (substances / natural extracts), hereafter referred to as substances, were selected by the Danish EPA based on information in a LOUS survey of selected fragrances (DK EPA, 2015) and in an SCCS opinion on fragrance allergens in cosmetic products (SCCS, 2012), as well as on other criteria set up by the Danish EPA, as described below in Chapter 2. The list of substances for evaluation as provided by the Danish EPA is presented in Appendix 2.

The project was divided in two phases:

In Phase 1, relevant information was retrieved for all the selected 42 substances as described in Chapter 3, section 3.1. The relevant information for the purpose of the screening in this project is human data (primarily patch tests in unselected and/or selected dermatitis patients) and animal data (local lymph node assay, guinea pig maximization test and the Buehler assay). Then, a preliminary evaluation of the relevant data was performed in order to identify possible sub-category 1A candidates. Therefore, a thorough evaluation of the retrieved data was not performed in Phase 1.

In Phase 2, a more detailed assessment of the data for selected sub-category 1A candidates was performed as described in Chapter 3, section 3.2. The quality of the data was assessed according to the Klimisch criteria (Klimisch et al., 1997) as described in section 3.2.1. The 'reliable' data (assigned score 1 or 2) were then assessed against the CLP classification criteria for skin sensitisation with special focus on whether classification in sub-category 1A is justified. Other data

(assigned score 4) were also included in the assessment. Not 'reliable' data (assigned score 3) were not included in the assessment. The CLP classification criteria for classification in sub-category 1A are summarised in section 3.2.2.

Based on the preliminary assessment in Phase 1, each of the 42 selected substances was given a priority 1 or 2 as described in Chapter 4. Priority 1 was given to those substances which were considered as possible sub-category 1A candidates, in total 20 substances (listed in the first table in Chapter 4). Priority 2 was given to those substances which were not considered as possible sub-category 1A candidates, in total 22 substances. The justification for the prioritisation of the substances as 1 or 2 is presented in Appendix 3.
Among the 20 identified possible sub-category 1A candidates, a further prioritisation was performed for the selection of the Phase 2 substances. The highest priority was given to the substances identified in the SCCS opinion (SCCS, 2012) as established contact allergens in humans and considered to be of special concern (marked with an 'X' in the right column of the table in Chapter 4) and/or based on the clinical experience from the National Allergy Research Centre. The 11 substances taken forward to Phase 2 are listed in the table below:

Substances	CAS RN	Substances of special concern according to SCCS (2012)
Citral	5392-40-5	X
Cinnamaldehyde	104-55-2	X
Cinnamyl alcohol	104-54-1	X
Coumarin	91-64-5	X
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
7-Hydroxycitronellal	107-75-5	X
Methyl oct-2-ynoate	111-12-6	
***Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext.**	8007-80-5 / 84649-98-9	
***Evernia prunastri*, ext.**	90028-68-5	X

The results of the detailed assessment performed for the 11 substances selected for Phase 2 are presented in Chapter 5 as a summary of the available data, a comparison with CLP Regulation criteria for classification in sub-category 1A, and a conclusion on classification for each substance. Appendices 4-14 present the full overview of the available studies/data for the substances and the evaluation of their skin sensitising potential. For all 11 substances, a classification as a skin sensitiser in sub-category 1A is justified based on the available data.

For nine of the substances, sub-category 1A is justified based on human patch test data i.e. frequencies in unselected and/or selected dermatitis patients and/or a high number of cases. For methyl oct-2-ynate, sub-category 1A is justified primarily based on non-human data (very low EC3 values in the two LLNAs and results from GPMT and Buehler tests, and supported by human evidence from HRIPT studies). For *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext., sub-category 1A is justified based on their constituents by read across to the major compounds such

as cinnamaldehyde (for Cassia bark extract, Cassia oil, Cinnamon bark extract and Cinnamon bark oil) and eugenol (for Cinnamon leaf oil).

The experiences from the assessments in this project are discussed in Chapter 6. In general, a decision whether a classification of a substance as a skin sensitiser in sub-category 1A is justified or not is based on the available human data. This classification is generally not supported by the available non-human data available for the 11 substances assessed in this project. However, as a substance can be classified in sub-category 1A on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals it seems clear that sub-category 1A is justified based on the available human data.
As illustrated by one of the 11 Phase 2 substances, methyl oct-2-ynate, sub-category 1A can also be justified primarily based on non-human data, in this case based on a very low EC3 value in the two LLNAs, and the results from GPMT and Buehler tests (and in this case, supported by human evidence from HRIPT studies).
As illustrated by another of the 11 Phase 2 substances, *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext., read across to major constituents in the fragrance could justify a classification in sub-category 1A.

For seven of the 42 selected substances, the available data were too limited for an indication as a possible sub-category 1A candidate. Thus, it cannot be excluded whether these substances would turn out to be sub-category 1A candidates should additional human studies and/or non-human data become available.

A relatively large proportion of the available studies have only been available from secondary sources, mainly attributed to unpublished data by the industry. Inclusion of these studies in this project is justified due to the purpose of the project, i.e. a screening of the available data for a preliminary assessment whether the criteria for classification in sub-category 1A according to the CLP criteria are fulfilled.
Secondary sources have a reliability score 4 “not assignable” according to the reliability criteria proposed by Klimisch et al. (1997). For a genuine assessment of studies only available from secondary sources, the original study report should be available for the assessment.

Conclusion:
For all 11 substances selected for detailed assessment in Phase 2, a classification as a skin sensitiser in sub-category 1A is justified based on the available data. Based on the assessments in this project, a decision whether a classification of a substance as a skin sensitiser in sub-category 1A is justified is generally based on the available human patch test data.
One of the elements in the criteria for classification in sub-category 1A based on human patch test data is ‘relatively low exposure’. For the 11 substances, relatively low exposure from use as fragrance in cosmetics and in other consumer products has been evaluated based on the IFRA standard limit of each substance in each of the 11 product categories for dermal sensitisation which have been established by IFRA based on quantitative risk assessment (QRA). The IFRA standard limits are still under evaluation. Revisions of the QRA approach might result in changes of the current IFRA standard limits and could thus have an impact on the classification as a skin sensitiser in sub-category 1A for the 11 substances.
A relatively large proportion of the cited studies have only been available from secondary sources, mainly attributed to unpublished data by the industry. For a genuine assessment of such studies, the original study reports should be available for the assessment. Thus, it cannot be excluded whether the outcome of the assessments performed in this project could turn out differently should the original study reports become available for the final assessment.

Sammenfatning og konklusion

Duftstoffer er meget udbredt i mange forskellige typer af forbrugerprodukter. Den generelle befolkning kan således blive udsat for duftstoffer fra mange forskellige kilder og kan derfor have en væsentlig udsættelse herfor til trods for, at duftstoffer oftest anvendes i en relativt lav koncentration i de enkelte forbrugerprodukter.

Mange duftstoffer har vist sig at kunne være skadelige for menneskers sundhed. Hudsensibilisering (kontaktallergi) er blevet identificeret som den kritiske effekt for en lang række duftstoffer. Mange duftstoffer er allerede klassificeret som hudsensibiliserende (Skin Sens Kat 1) i henhold til CLP-forordningen (EF nr. 1272/2008). CLP-kriterierne for klassificering af hudsensibiliserende stoffer blev revideret i 2011 og giver nu mulighed for at klassificere hudsensibiliserende stoffer i to subkategorier, subkategori 1A (stærkt sensibiliserende stoffer) eller 1B (andre hudsensibiliserende stoffer). Nogle af de duftstoffer, der allerede er klassificeret som hudsensibiliserende i kategori 1, kan muligvis opfylde CLP-kriterierne for klassificering som stærkt sensibiliserende i subkategori 1A.

Klassificering af et specifikt stof i subkategori 1A vil medføre, at kemiske blandinger (produkter) indeholdende stoffet skal klassificeres og mærkes ved en lavere koncentration (faktor 10 lavere) i forhold til stoffer klassificeret i kategori 1. De skærpede mærkningskrav for stærkt sensibiliserende stoffer (subkategori 1A) gælder for produkter, der er omfattet af CLP forordningens mærkningskrav som f.eks. vaske- og rengøringsmidler. Mærkningen vil således kunne øge beskyttelsen af brugerne (arbejdere, forbrugere), da mærkningen vil kunne gøre det muligt for sensibiliserede individer at tage forholdsregler for at undgå direkte hudkontakt med et produkt, der indeholder et stærkt sensibiliserende stof.

Formålet med dette projekt var at søge og vurdere de tilgængelige data for 42 udvalgte duftstoffer, som allerede er klassificeret som hudsensibiliserende i kategori 1 (harmoniseret og / eller notificeret klassificering) med henblik på at vurdere, om disse stoffer kan opfylder kriterierne for klassificering i subkategori 1A baseret på de tilgængelige data.
De 42 duftstoffer (stoffer / naturlige ekstrakter), efterfølgende benævnt stoffer, blev udvalgt af Miljøstyrelsen baseret på oplysninger i et LOUS kortlægningsprojekt af udvalgte duftstoffer (DK EPA, 2015) og i en SCCS opinion om allergene duftstoffer i kosmetiske produkter (SCCS, 2012), såvel som på andre kriterier sat af Miljøstyrelsen, som beskrevet nedenfor i kapitel 2. Listen over de udvalgte stoffer er præsenteret i Appendix 2.

Projektet var opdelt i to faser:

I fase 1 blev der indsamlet relevante oplysninger for alle de udvalgte 42 stoffer, som beskrevet i kapitel 3, afsnit 3.1. Relevante oplysninger med henblik på screeningen i dette projekt er humane data (primært lappetest i ikke-selekterede og / eller selekterede dermatitis patienter) og data fra studier i dyreforsøg (local lymph node assay, guinea pig maximization test og Buehler assay). Derefter blev der lavet en foreløbig vurdering af de relevante data med henblik på at identificere mulige subkategori 1A kandidater. Der er således i fase 1 ikke foretaget en grundig evaluering af de indsamlede data.

I fase 2 blev der foretaget en mere detaljeret vurdering af data for de udvalgte subkategori 1A kandidater, som beskrevet i kapitel 3, afsnit 3.2. Kvaliteten af data blev vurderet i henhold til

Klimisch kriterierne (Klimisch et al., 1997), som beskrevet i afsnit 3.2.1. 'Reliable' data (tildelt score 1 eller 2) blev derefter vurderet i forhold til CLP kriterierne for hudsensibilisering med særligt fokus på, hvorvidt klassificering i subkategori 1A er berettiget. 'Other data' (tildelt score 4) blev også inkluderet i vurderingen. Not 'reliable' data (tildelt score 3), blev ikke medtaget i vurderingen. CLP kriterierne for klassificering i subkategori 1A er sammenfattet i afsnit 3.2.2.

På baggrund af den foreløbige vurdering i fase 1 fik hvert enkelt af de 42 udvalgte stoffer en prioritet 1 eller 2, som beskrevet i kapitel 4. De stoffer, der blev vurderet som mulige subkategori 1A kandidater, fik en prioritet 1, i alt 20 stoffer (præsenteret i den første tabel i kapitel 4). De stoffer, der ikke blev vurderet som mulige subkategori 1A kandidater, fik en prioritet 2, i alt 22 stoffer. Begrundelsen for prioriteringen af stofferne er præsenteret i Appendix 3.

Blandt de 20 stoffer, der blev vurderet som mulige subkategori 1A kandidater, blev der foretaget en yderligere prioritering med hensyn til udvælgelsen af fase 2 stoffer. Den højeste prioritet blev givet til de stoffer, som i SCCS opinion (SCCS, 2012) er blevet udpeget som erkendte kontaktallergener hos mennesker og dermed betragtes som værende særligt bekymrende (markeret med et 'X' i højre kolonne i tabellen i kapitel 4), og/eller baseret på klinisk erfaringer fra Videncenter for Allergi. De 11 stoffer udvalgt til fase 2 er præsenteret i den efterfølgende tabel:

Stof	CAS RN	Stoffer som er særligt bekymrende (SCCS, 2012)
Citral	5392-40-5	X
Cinnamaldehyd	104-55-2	X
Cinnamyl alkohol	104-54-1	X
Coumarin	91-64-5	X
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
7-Hydroxycitronellal	107-75-5	X
Methyl oct-2-ynoat	111-12-6	
***Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext.**	8007-80-5 / 84649-98-9	
***Evernia prunastri*, ext.**	90028-68-5	X

Resultaterne af den detaljerede vurdering udført for de 11 stoffer udvalgt til fase 2 er præsenteret i kapitel 5 i form af et sammendrag af de tilgængelige data, en sammenligning med CLP kriterierne for klassificering i subkategori 1A, og en konklusion for klassificering af hvert enkelt stof. I Appendix 4-14 er samlet de fulde vurderinger for de enkelte stoffer. For alle 11 stoffer er en klassificering som hudsensibiliserende i subkategori 1A begrundet på grundlag af de tilgængelige data.

For ni af stofferne er subkategori 1A begrundet baseret på data fra humane lappetest data dvs. frekvenser i ikke-selekterede og/eller selekterede dermatitis patienter og/eller et højt antal tilfælde. For methyl oct-2-ynat er subkategori 1A primært begrundet baseret på data fra dyreforsøg (meget lave EC3 værdier i de to LLNA tests samt resultater fra GPMT og Buehler tests, og støttet af resultater HRIPT studier). For *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext., er

subkategori 1A begrundet baseret på read across til hovedindholdsstofferne såsom cinnamaldehyd (for Cassia bark ekstrakt, Cassia olie, kanel bark ekstrakt og kanel bark olie) og eugenol (for Cinnamon blade).

Erfaringerne fra vurderingerne i dette projekt er diskuteret i kapitel 6. Generelt er vurderingen om, hvorvidt en klassificering af et stof som hudsensibiliserende i subkategori 1A er begrundet eller ej, baseret på de tilgængelige humane data. Denne klassificering er generelt ikke understøttet af de tilgængelige data fra dyreforsøg for de 11 stoffer, der er vurderet i dette projekt. Da et stof kan klassificeres i subkategori 1A på grundlag af pålidelige humane data af god kvalitet og/eller data fra dyreforsøg, så er subkategori 1A berettiget på grundlag af de tilgængelige humane data.
Som illustreret med et af de 11 fase 2 stoffer, methyl oct-2-ynat, så kan klassificering i subkategori 1A også begrundes primært baseret på data fra dyreforsøg, i dette tilfælde baseret på en meget lav EC3 værdi i de to LLNA tests samt resultaterne fra GPMT og Buehler tests (og i dette tilfælde, støttet af dokumentation fra fra HRIPT studier).
Som illustreret med et andet af de 11 fase 2 stoffer, *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext., så kan klassificering i subkategori 1A begrundes baseret på read across til hovedindholdsstofferne i duftstoffet.

For syv af de 42 udvalgte stoffer blev de tilgængelige data vurderet til at være for begrænsede til en vurdering af stoffet som en mulig subkategori 1A kandidat. Det kan således ikke udelukkes, hvorvidt disse stoffer ville kunne vise sig at være subkategori 1A kandidater, hvis yderligere humane data og/eller data fra dyreforsøg bliver tilgængelige.

En forholdsvis stor del af de tilgængelige studier har kun været tilgængelige fra sekundære kilder, primært i form af upublicerede data fra industrien. Inddragelse af disse studier i dette projekt er berettiget som følge af formålet med projektet, dvs. en screening af de tilgængelige data med henblik på en indledende vurdering af, hvorvidt CLP kriterierne for klassificering i subkategori 1A er opfyldt.
Sekundære kilder har en 'reliability' score 4 "not assignable" i henhold til Klimisch kriterierne (Klimisch et al., 1997). For at kunne foretage en uvildig vurdering af studierne citeret fra sekundære kilder, så bør man have adgang til den originale studierapport.

Konklusion:
For alle 11 stoffer udvalgt til den detaljerede vurdering i fase 2 er en klassificering som hudsensibiliserende i subkategori 1A berettiget på grundlag af de tilgængelige data.
Baseret på vurderingerne i dette projekt så er vurderingen om, hvorvidt en klassificering af et stof som hudsensibiliserende i subkategori 1A er begrundet eller ej, generelt baseret på de tilgængelige humane patch test data.
Et af elementerne i kriterierne for klassificering i subkategori 1A baseret på humane patch test data er 'relativ lav eksponering'. For de 11 stoffer er relativ lav eksponering ved anvendelse som duftstof i kosmetik og andre forbrugerprodukter evalueret baseret på IFRA standard grænser for hvert enkelt stof i hver enkelt af de 11 produktkategorier for dermal sensibilisering som IFRA har opstillet på baggrund af kvantitativ risikovurdering (QRA). IFRA standard grænserne baseret på kvantitativ risikovurdering (QRA) er stadig under evaluering. Revidering af QRA tilgangen kunne således medføre ændringer i de nuværende IFRA standard grænser og kunne dermed influere på klassificeringen som hudsensibiliserende i subkategori 1A for de 11 stoffer.
En forholdsvis stor del af de citerede studier har kun været tilgængelige fra sekundære kilder, primært i form af upublicerede data fra industrien. For at kunne foretage en uvildig vurdering af studierne citeret fra sekundære kilder, så bør man have adgang til den originale studierapport. Det kan således ikke udelukkes, at vurderingerne i dette projekt kunne falde anderledes ud, hvis de originale studierapporter bliver tilgængelige for den endelige vurdering.

1. Introduction

Fragrances are widely used in many different types of consumer products. Therefore, the general population can be exposed to fragrances from many different sources and the exposure may be substantial despite the fact that fragrances most often are used in relatively low concentrations in individual consumer products.

Many fragrances have been shown to cause harmful effects to human health as well as to the environment. In relation to human health, skin sensitisation (contact allergy) is identified as the critical effect for a wide range of fragrances. Many fragrances are already classified as skin sensitisers (Skin Sens Cat 1) according to the CLP Regulation on classification, labelling and packaging of substances and mixtures (EC no. 1272/2008).

The CLP criteria for classification of skin sensitisers were revised in 2011 and now provide possibility for sub-categorising sensitisers in Category 1 in two sub-categories: Sub-category 1A or 1B. Sub-category 1A comprises sensitisers for which exposure to a low amount of the substance may cause a high frequency of occurrence of skin sensitisation in humans and/or a high potency in animals (strong sensitisers), while sub-category 1B comprises other skin sensitisers. Sub-categorisation of skin sensitisers in either category 1A or 1B should thus be done if justified by the available data. If the data do not allow for sub-categorisation, the substance should be classified in Category 1 according to the criteria specified.

Some of the fragrances that are already classified as skin sensitisers in Category 1 and other fragrances which are known to cause skin sensitisation may possibly fulfil the CLP criteria for classification in sub-category 1A. A classification of a specific substance in sub-category 1A implies that classification and labelling of mixtures containing the substance is required at a lower concentration compared to skin sensitisers in Category 1. The hazard statement H317 (May cause an allergic skin reaction) will thus be required at concentrations ≥ 0.1% and labelling with the supplemental hazard statement EUH208 ("Contains <name of sensitising substance>. May produce an allergic reaction") will be required at concentrations ≥ 0.01% for strong sensitisers (sub-category 1A) according to the CLP Regulation. These concentration limits are a factor 10 lower than the concentration limits for classification and labelling of mixtures containing sensitisers in Category 1.

The more stringent labelling requirements for strong sensitisers (sub-category 1A) will apply for mixtures under the scope of the CLP regulation such as e.g., washing and cleaning products. Consequently, the labelling may increase the protection of users (workers, consumers) as it will allow sensitised individuals to take precautionary measures to prevent direct skin contact with a product containing a strong sensitiser.

In an SCCS (EU Scientific Committee on Consumer Safety) opinion on fragrance allergens in cosmetic products it is mentioned that based on data from the fragrance industry, 80 % of the total fragrance chemical volume is used in cosmetics and 20 % in household products (SCCS, 2012). It should be noted that cosmetic products are not subject to classification and labelling under the scope of the CLP regulation (EC no. 1223/2009). However, according to the Cosmetic Regulation "*Perfume and aromatic compositions and their raw materials shall be referred to by the terms 'parfum' or 'aroma'. Moreover, the presence of substances, the mention of which is required under the column 'Other' in Annex III, shall be indicated in the list of ingredients in addition to the terms*

parfum or aroma." Annex III (entries 67-92) includes 26 fragrances which have been identified as human allergens and must be indicated in the list of ingredients when the concentration of the fragrance exceeds 0.001 % in leave-on products and 0.01 % in rinse-off products.

As part of the Danish Environmental Protection Agency's (Danish EPA) survey of the 'List of Undesirable Substances' (LOUS) a survey of selected fragrances has been carried out (DK EPA, 2015). In that survey, 15 substances with a classification (harmonised/notified) for their skin sensitising potential in Category 1 according to the CLP criteria were identified.

Furthermore, 82 substances / natural extracts were identified in the SCCS opinion on fragrance allergens in cosmetic products (SCCS, 2012) as established contact allergens in humans.

Based on the LOUS review and the SCCS (2012) opinion the Danish EPA has selected 42 substances / natural extracts (hereafter referred to as substances) for a screening of the available data regarding skin sensitisation, see Chapter 2.

The purpose of this project was to retrieve and review the available data for 42 selected substances in order to assess whether the selected substances fulfil the criteria for classification in sub-category 1A according to the CLP criteria.

The project was divided in two phases:

- Phase 1: Screening of the available data for the 42 selected substances, for a preliminary evaluation whether the data justify a classification as a skin sensitiser in sub-category 1A (strong sensitisers).
- Phase 2: A more detailed assessment of the data for those substances identified in Phase 1 as possible sub-category 1A candidates.

2. Selection of substances

The Danish EPA has selected 42 substances for a screening of the available data regarding skin sensitisation. The substances were selected based on information in the LOUS report (DK EPA, 2015) and in the SCCS opinion (SCCS, 2012) as described below, as well as on other criteria set up by the Danish EPA.

2.1 Selection of substances from the LOUS survey report

As part of the Danish EPA's survey of the 'List of Undesirable Substances' (LOUS) a survey of selected fragrances has been carried out (DK EPA, 2015). In that survey *"… it was decided to focus on harmonised classified substances registered under REACH AND included in the list of fragrance substances developed by IFRA"*, the International Fragrance Association.

In total, 44 substances with a harmonised classification (health and/or environment) were included in the IFRA list and these 44 substances (appear on a blue background in Appendix 1 of the LOUS report) were included for further assessment in the LOUS survey report. The group of the 44 substances both includes substances which are associated with a scent and substances which are used in fragrance mixtures to keep the fragrance liquid (solvent), preserve the fragrance (and therefore also the scent), adjuvants (i.e. substances that modifies the effect of other substances), and pigments which are applied in order to achieve a certain wanted colour etc.

Among the 44 fragrance substances in the LOUS survey report 15 of the substances were classified as skin sensitisers in Category 1 (either by a harmonised classification or a self-classification).

2.2 Selection of substances from the SCCS (2012) opinion

The EU Scientific Committee on Consumer Safety (SCCS) has published an opinion on fragrance allergens in cosmetic products (SCCS, 2012). In this opinion, 82 substances were identified as established contact allergens in humans, listed in Table 7-1 (54 individual fragrance chemicals) and 7-5 (28 natural extracts) in the opinion.

Seven of the 82 substances are among the 15 substances identified in the LOUS report with a notified classification for their skin sensitising potential.

2.3 The substances selected for this project

Among the 15 substances identified in the LOUS report with a classification (harmonised and/or notified) for their skin sensitising potential and the 82 substances identified in the SCCS (2012) opinion as established contact allergens in humans (a total of 90 substances), the Danish EPA has selected 42 substances (56 CAS numbers) for the screening in this project. All of the 42 substances either have a harmonised classification for skin sensitisation or have been self-classified for skin sensitisation by companies placing the substance on the market in the EU.

The 42 substances were selected based on the following criteria:

- High tonnage (> 100 tonnes/year) and large extent of agreement of sensitising properties:

The substance is registered under REACH and is classified as a skin sensitiser (either a harmonised classification or a self-classification). In case of self-classification >75% of the notifying companies have self-classified as a skin sensitiser (Skin Sens Cat 1) *or*

- Low tonnage but full agreement on sensitising properties:
  The substance is not registered under REACH but has a harmonised classification as a skin sensitiser (Skin Sens Cat 1) *or*
- Low tonnage but indication of the substance being a strong sensitiser:
  The substance is not registered under REACH but one or more companies have self-classified the substance as a strong sensitiser (Skin Sens Cat 1A) *or*
- None of the above criteria are fulfilled but the substance is identified as a 'high risk substance' in relation to sensitisation by SCCS (2012): The substance has been identified to be of special concern by SCCS (2012) due to a high number of reported positive human cases of sensitisation

The remaining 48 substances that were not selected for further evaluation in Phase 1 included substances that were:

- Low tonnage (substances not registered under REACH) and which did not fulfil any of the above criteria
- High tonnage but no or very low indication of sensitising properties (substances registered under REACH and for which none or only a few companies have notified a self-classification for skin sensitisation).

The 42 selected substances are listed in the following table:

Substances	CAS RN	Substances of special concern according to SCCS (2012)[2]
Butyl methacrylate	97-88-1	
Benzaldehyde	100-52-7	
Citral	5392-40-5	X
(R)-p-Mentha-1,8-diene (d-limonene)	5989-27-5	
(S)-p-Mentha-1,8-diene (l-limonene)	5989-54-8	
2-Methyl-4-phenylpentanol	92585-24-5	
A mixture of: trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran	131766-73-9	
Turpentine oil	8006-64-2	X
[3R-(3α,3aβ,7β,8aα)]-1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)ethan-1-one (Acetylcedrene)	32388-55-9	
(E)-Anethole (trans-anethole)	4180-23-8	
4-Methoxybenzyl alcohol (Anise alcohol)	105-13-5	
Benzyl salicylate	118-58-1	
2-(4-tert-Butylbenzyl)propionaldehyde	80-54-6	

[2] Special concern in the SCCS opinion (SCCS, 2012) is defined as due to the high number of reported cases, (>100 cases).

Substances	CAS RN	Substances of special concern according to SCCS (2012)[2]
(Butylphenyl methylproprional)		
d-p-Mentha-1(6),8-dien-2-one (Carvone) / l-p-Mentha-1(6),8-dien-2-one (Carvone) / (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one (Carvone)	99-49-0 / 6485-40-1 / 2244-16-8	
Cinnamaldehyde	104-55-2	X
Cinnamyl alcohol	104-54-1	X
Citronellol / (R)-3,7-dimethyloct-6-en-1-ol / (-)-3,7-dimethyloct-6-en-1-ol	106-22-9 / 1117-61-9 / 7540-51-4	
Coumarin	91-64-5	X
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one (delta-DAMASCONE)	57378-68-4	
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
4-(4-Hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde (Hydroxyisohexyl 3-cyclohexene carboxaldehyde, HICC)	31906-04-4 / 51414-25-6	X
7-Hydroxycitronellal	107-75-5	X
Isoeugenol	97-54-1	X
Limonene	138-86-3	X
Methyl oct-2-ynoate	111-12-6	
Pin-2(3)-ene / Pin-2(10)-ene	80-56-8 / 127-91-3	
p-Mentha-1,4(8)-diene (Terpinolene)	586-62-9	
Ylang ylang ext. / Ylang ylang oil	83863-30-3 / 8006-81-3	X
Cinnamomum cassia leaf oil / *Cinnamomum zeylanicum*, ext.	8007-80-5 / 84649-98-9	
Neroli Oil / Orange, sour, ext.	8016-38-4 / 72968-50-4	
Lemon, ext.	84929-31-7	
Orange, sweet, Valencia, ext. / Orange, sweet, ext.	97766-30-8 / 8028-48-6	
Clove leaf oil	8000-34-8	X
Evernia furfuracea, ext.	90028-67-4	X
Evernia prunastri, ext.	90028-68-5	X

Substances	CAS RN	Substances of special concern according to SCCS (2012)[2]
Jasmine, *Jasminum grandiflorum*, ext. / Jasmine, *Jasminum officinale*, ext. / Extract Jasmine (oil), Jasmine, *Jasminum grandiflorum*, ext.	84776-64-7 / 90045-94-6 / 8022-96-6	X
Oils, peppermint (*Mentha piperita*) / Peppermint, ext.	8006-90-4 / 84082-70-2	
Spearmint, ext. (*Mentha spicita*)	84696-51-5	
Balsams, Peru (*Myroxylon pereirae*)	8007-00-9	X
Sandalwood, ext. / Sandalwood oil	84787-70-2 / 8006-87-9	X

The 42 substances selected for this project include 19 of the 20 substances considered by the SCCS to be of special concern (SCCS, 2012) and marked with an 'X' in the right column in the table above. The remaining of the 20 substances considered by the SCCS (2012) to be of special concern, linalool, was not selected for this project as a harmonised classification as Skin Sens Cat 1B recently has been endorsed by the EU Risk Assessment Committee (RAC).

The full list of substances for evaluation in Phase 1 as provided by the Danish EPA is presented in Appendix 2. The list includes information of CAS Registry Number (CAS RN), substance name, harmonised classification for skin sensitisation in Category 1, notified classification for skin sensitisation in Category 1 (including sub-category 1A or 1B), approximate percentage of notified classifications including a classification for skin sensitisation in Category 1, REACH registration status, and priority according to the criteria set up by the Danish EPA.

3. Data collection and evaluation

The project was divided in two phases:

- Phase 1: Screening of the available data for the 42 selected substances with the purpose of performing a preliminary assessment whether the data justify a classification as a skin sensitiser in sub-category 1A (strong sensitisers) according to the CLP criteria, i.e. an identification of possible sub-category 1A candidates.
- Phase 2: A more detailed assessment of the data for those substances identified in Phase 1 as possible sub-category 1A candidates.

Data were collected for all the selected 42 substances as described in section 3.1 and the retrieved data were evaluated as described in section 3.2.

3.1 Data collection

Data from the SCCS (2012) opinion have been collected for 39 of the 42 selected substances. The three remaining substances (butyl methacrylate, 2-methyl-4-phenylpentanol, and the mixture of: trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran) were not included in the SCCS (2012) opinion.
The SCCS (2012) opinion was considered as the primary source of data for these 39 substances up to year 2011 for the purpose of the screening in this project as a very comprehensive literature search was performed as part of this opinion.

A supplementary literature search in the open literature has been performed covering the period from January 2009 and until October 2015 for the 39 substances addressed in the SCCS (2012) opinion in order to ensure that potentially relevant studies published after the adoption of the SCCS (2012) opinion also are taken into account. For the remaining 3 substances not addressed in the SCCS (2012) opinion, a complete literature search has been performed.
Then relevant information regarding skin sensitisation has been retrieved by searching literature databases such as SciFinder, PubMed and Scopus, as well as by searching IPCS INCHEM and Google for relevant international / national assessments and reports.

For the substances registered under REACH (27 substances, 28 CAS RN), the REACH registrations in the publicly accessible part of the REACH Registration Dossier Database, hosted on the ECHA website, were checked in order to identify eventual additional relevant information regarding skin sensitisation.

Additional information from the National Allergy Research Centre has been included if not already located from the above-mentioned sources.

The LOUS survey report (DK EPA, 2015) was also checked for relevant information; however, no relevant information for the purpose of the screening in this project was located.

The relevant information for the purpose of the screening in this project, i.e. for classification for skin sensitisation, including a possible classification in sub-category 1A, is human data and animal data (local lymph node assay, guinea pig maximization test and the Buehler assay). *In vitro* data and non-test data are also included in Phase 2 if retrieved.

3.1.1 Human data

Human evidence for classification of a substance for its skin sensitising potential can be based on positive data from patch testing, epidemiological studies showing allergic contact dermatitis caused by the substance, positive data from experimental studies in man and/or well documented episodes of allergic contact dermatitis, using a weight of evidence approach.

3.1.1.1 Epidemiological studies

The subjects examined are eczema patients, selected occupational groups, other selected groups, or general population, and the endpoint studied is elicitation. Large general population studies are scarce. Focused studies in selected populations are more common and provide insights on frequency of sensitisation compared to exposure.

3.1.1.2 Studies based on diagnostic patch testing

Diagnostic patch testing is conducted in order to diagnose allergic contact dermatitis (ACD) to a substance and is performed according to international standards by dermatologists. The subjects examined are eczema patients attending dermatology clinics and the endpoint studied is elicitation (as an indicator of previous sensitisation). Studies of diagnostic patch testing is usually reported as positive patch test frequencies, e.g. number of patients having a positive patch test result in relation to the total number of patients tested, as well as the percentage of positives. It is important to note how patients or individuals have been selected for patch testing; if all patients at a clinic with suspected ACD are patch tested they are often called consecutive or unselected patients at the clinic. Sometimes more aimed patch testing is performed among selected patients from a certain work environment or where exposure to certain groups of allergens, such as preservatives, fragrances or pigments, is suspected. Patch testing in selected patients usually results in higher frequencies of positive patch tests compared to tests performed in consecutive or unselected patients. This is to be considered under the evaluation of the results.

3.1.1.3 Case reports

The subjects examined are eczema patients diagnosed with contact allergy to a particular substance and the endpoint studied is elicitation. Individual cases are reported and are often the first reports made. Usually there are more details than in larger data-sets. They are useful in early detection of skin sensitisers and classification.

3.1.1.4 Experimental dose-response elicitation studies

This type of studies includes serial dilution patch tests or repeated open application tests (ROAT). The subjects examined are sensitised individuals (usually from diagnostic patch tests) and the endpoint studied is elicitation. Several protocols exist. This type of study provides an indication of the degree of sensitivity and of safe limits of exposure for induction as well as elicitation.

3.1.1.5 Experimental induction tests

This type of studies includes the Human Repeated Insult Patch Test (HRIPT) and the Human Maximization Test (HMT). The subjects examined are healthy volunteers and the endpoint studied is induction of sensitisation. For ethical reasons, such studies are no longer to be performed for EU regulations, including the CLP Regulation, but historical data may exist.

3.1.2 Animal data

There are three common animal test methods used to evaluate the potential of a substance to cause skin sensitisation:

- The mouse local lymph node assay (LLNA)
- The guinea pig maximisation test (GPMT)
- The Buehler assay

3.1.2.1 Mouse Local Lymph Node Assay

The mouse local lymph node assay (LLNA) (OECD TG 429) is used both for determination of skin sensitising potential (hazard identification) and for determination of relative skin sensitisation potency (hazard characterisation). In both instances the metric is cellular proliferation induced in the draining lymph nodes following topical exposure to a chemical, lymph node cell proliferation being causally and quantitatively correlated with the acquisition of skin sensitisation.

The test is considered positive when one of the doses results in a stimulation index (SI) ≥ 3. Potency is measured as a function of derived EC3-values. The EC3-value is the amount of test chemical (% concentration, molar value or dose per unit area) calculated from the dose-response data to elicit a stimulation index of 3. An inverse relationship exists between EC3-value and potency meaning that extremely potent sensitisers have extremely low EC3-values.

It is known that the choice of vehicle may provide a variable EC3 value, which may significantly influence the skin sensitising potency and make it difficult to categorise/subcategorise the substance.

Different variants of the LLNA exist, namely the reduced LLNA (rLLNA) which has been added as an option in the amended OECD TG 429 in 2010, the LLNA: DA (OECD TG 442A), and the LLNA: BrdU-ELISA (OECD TG 442B).
The rLLNA uses only a negative control group and the equivalent of the high-dose group from the full LLNA. The rLLNA does not allow the determination of the potency of a sensitising chemical as only one dose is tested. The rLLNA also uses fewer animals than the full LLNA and should only be used in those circumstances where dose-response information are not required (e.g. to confirm a negative prediction of skin sensitising potential) and thus should not be used for sub-categorisation of skin sensitisers.
The test is considered positive in the LLNA: DA when the stimulation index is ≥ 1.8 and in the LLNA: BrdU-ELISA when the stimulation index is ≥ 1.6. There is no guidance on how the LLNA: DA or the LLNA: BrdU-ELISA can be used for sub-categorisation.

3.1.2.2 Guinea Pig Maximisation Test

The guinea pig maximisation test (GPMT) (OECD TG 406) has been used for over 40 years to detect the skin sensitising potential of chemicals through a test system maximizing the sensitivity by both intradermal and epidermal induction and use of an adjuvant (Freund's Complete Adjuvant). The intradermal induction is made by injection. Consequently the test is not suited for substances which cannot be made up into a liquid formulation.

The GPMT was originally designed to maximise the ability to identify a sensitisation hazard, rather than to determine skin sensitisation potency. Yet, potency categorisation is possible on the basis of the concentration of test material used for intradermal induction and the percentage of guinea pigs sensitised. However, it should be recognised that there is often a degree of uncertainty associated with the derivation of allergenic potencies from the GPMT.

3.1.2.3 Buehler assay

The Buehler assay (OECD TG 406) has been in use for the last 40 years to detect the skin sensitising potential of chemicals using epidermal occluded exposure. The skin barrier of the test species (guinea pig) is kept intact in this assay.

The Buehler test was originally designed to identify a sensitisation hazard, rather than to determine skin sensitisation potency. Yet, potency can be categorised using the results of the Buehler assay on the basis of the number of animals sensitised and the concentration of the test material used for the epidermal induction. However, it should be recognised that there is often a degree of uncertainty associated with the derivation of allergenic potencies from the Buehler assay.

3.1.2.4 Non-compliant skin sensitisation tests

For the 42 selected substances, several older animal studies have been retrieved from the various literature sources. Many of these studies have not been performed according to the present internationally accepted test guidelines.

In vivo test methods which do not comply with recognised test guidelines are strongly discouraged in the CLP Guidance for the identification of skin sensitisers or assessment of skin sensitising potency. The results of such tests have to be well-validated with scientific justification and evaluated carefully, but may provide supportive evidence. If doubts exist about the validity and the interpretation of the results, the evaluation needs to be taken by using a weight of evidence approach.

The results of such older animal studies have been included in both the Phase 1 and Phase 2 evaluations for transparency reasons, but only as supplementary evidence.

3.1.3 *In vitro* data

Two *in vitro* skin sensitisation methods have recently (February 2015) been adopted as OECD test guidelines.

One test is the "*in chemico*" skin sensitisation 'Direct Peptide Reactivity Assay' (DPRA) (OECD TG 442C). This method measures the ability of chemicals to react with proteins (haptenation), a determinant step in the induction of skin sensitisation. It is based on the chemical reactivity of the compound under investigation, with lysine and cysteine residues. The test method, however, is not proposed as a stand-alone full replacement test for the *in vivo* animal studies since the DPRA test is covering only one single biological step in the skin sensitisation pathway and does not consider metabolic capacity. DPRA information may also have the potential to contribute to potency assessment.

Another test is the *in vitro* skin sensitisation 'ARE-Nrf2 luciferase test' (OECD TG 442D). This method measures activation of keratinocytes and determines the direct reactivity of sensitising material to key cysteine residues of Keap1, a regulator of Nrf2. The Nrf2-Keap1-ARE regulatory pathway is considered one of the most relevant pathways for the identification of potential skin sensitisers. The test method, however, is not proposed as a stand-alone full replacement test for the *in vivo* animal studies since it addresses only one single biological step in the overall mechanism of skin sensitisation. Considering the known limitations of this test such as the limited consideration of metabolic aspects and the ability to detect only cysteine-reactive chemicals, it has been recommended that the method should only be used in combination with other information sources.

In vitro data are currently not part of the classification criteria for skin sensitisers according to the CLP Regulation and Guidance.

3.1.4 Non-testing data

At present no formally validated non-testing systems exist to predict skin sensitising potential. However, data such as structural alert data or data to show that the chemical structure of a molecule is similar to that of known sensitisers (e.g. QSARs or expert systems) may form part of the weight of evidence for classification.

3.2 Evaluation of data

Phase 1 consisted of a screening of the available data for the selected 42 substances with the purpose of performing a preliminary assessment if the data could be sufficient for a classification in sub-category 1A according to the CLP criteria, i.e. an identification of possible sub-category 1A candidates. Therefore, a thorough evaluation of the retrieved data was not performed in Phase 1.

In Phase 2, the quality of the data for the selected substances was assessed as described in section 3.2.1 and the data considered valid for the purpose of the screening in this project were then assessed against the CLP classification criteria for skin sensitisation with special focus on whether classification in sub-category 1A is justified. The CLP classification criteria are summarised in section 3.2.2.

3.2.1 Quality of data

For each substance taken forward to Phase 2 the quality of the relevant data was assessed according to the Klimisch criteria (Klimisch et al., 1997).

Each reference was given a score 1-4:

- 1 = reliable without restrictions: “studies or data generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline or in which all parameters described are closely related/comparable to a guideline method.”
- 2 = reliable with restrictions: “studies or data (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.”
- 3 = not reliable: “studies or data in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g. un-physiological pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment.”
- 4 = not assignable: “studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.).”

Then, ‘reliable’ data (assigned score 1 or 2) were then assessed against the CLP classification criteria for skin sensitisation with special focus on whether classification in sub-category 1A is justified.
Other data (assigned score 4) were also included in the assessment.
Not ‘reliable’ data (assigned score 3) were not included in the assessment.

3.3 Comparison with classification criteria according to the CLP Regulation

The available data for the substances evaluated in Phase 1 and Phase 2 were compared with the criteria for classification as skin sensitisers in sub-category 1A according to the CLP Regulation (EC no. 1272/2008). The classification criteria are presented below. The criteria are complex and further guidance for the use of the criteria and on how to evaluate the data are found in the CLP Guidance document "Guidance on the application of the CLP criteria" which is available from the European Chemicals Agency website (ECHA, 2015).

3.3.1 Classification criteria for sub-category 1A

According to Annex I, section 3.4.2.2.1.3 in the CLP regulation (EC no. 1272/2008): "*Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for skin sensitisers as described in section 3.4.2.2.2. Substances may be allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in Table 3.4.2 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals according to the guidance values provided in sections 3.4.2.2.2.1 and 3.4.2.2.3.2 for sub-category 1A and in sections 3.4.2.2.2.2 and 3.4.2.2.3.3 for sub-category 1B.*".

3.3.1.1 Criteria given in Table 3.4.2 for sub-category 1A

Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.

When considering human evidence, it is necessary to take into account the size of the population exposed and the extent of exposure and frequency, and thus the consideration is on a case by case basis. Human data should be incorporated with animal data to decide the sub-categorisation.

3.3.1.2 Criteria for sub-category 1A listed in CLP Annex I, 3.4.2.2.2.1 – human data:

Human evidence for sub-category 1A can include:

- Positive responses at ≤ 500 μg/cm² (HRIPT, HMT – induction threshold);
- Diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;
- Other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

High and substantial incidence of reactions:
For human diagnostic patch test data, only one or two types of the following information regarding a relative high and substantial incidence of reactions in a defined population may be sufficient for sub-categorisation:

Human diagnostic patch test data	High frequency	Low / moderate frequency
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Work place studies: **1: all or randomly selected workers** **2: selected workers with known exposure or dermatitis**	≥ 0.4 % ≥ 1.0 %	< 0.4 % < 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

Relatively low exposure:
Relatively high or low exposure relates to the concentrations people are exposed to in their daily lives, in the workplace, or other conditions and resulting in sensitisation. The exposure index is the sum of the scores obtained from the information in each row of the table below, i.e. a response in each row is necessary. A relatively low exposure is indicated if the exposure index is between 1 and 4.

Exposure data	Relatively low exposure	Relatively high exposure
Concentration / dose	< 1.0 % < 500 µg/cm² (score 0)	≥ 1.0 % ≥ 500 µg/cm² (score 2)
Repeated exposure	< once/daily (score 1)	≥ once/daily (score 2)
Number of exposures (irrespective of concentration of sensitiser)	< 100 exposures (score 0)	≥ 100 exposures (score 2)

3.3.1.3 Criteria for sub-category 1A listed in CLP Annex I, 3.4.2.2.3.2 – non-human data:

Animal test results for sub-category 1A:

- Local lymph node assay: EC3 value ≤ 2 %
- Guinea pig maximisation test: ≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose
- Buehler assay: ≥ 15 % responding at ≤ 0,2 % topical induction dose or ≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose

3.3.1.4 Weight of evidence

For classification of a substance, evidence shall include any or all of the following using a weight of evidence approach:

- Positive data from patch testing, normally obtained in more than one dermatology clinic
- Epidemiological studies showing allergic contact dermatitis caused by the substance
- Positive data from appropriate animal studies
- Positive data from experimental studies in man
- Well documented episodes of allergic contact dermatitis, normally obtained in more than one dermatology clinic
- Severity of reaction may also be considered

Positive effects seen in either humans or animals for skin sensitisation will normally justify classification. In cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to decide on the classification on a case-by-case basis.

4. Results, Phase 1

Phase 1 consisted of a screening of the available data for the selected 42 substances with the purpose of performing a preliminary assessment if the available data could be sufficient for a classification in sub-category 1A according to the CLP criteria, i.e. an identification of possible sub-category 1A candidates.

Based on this preliminary assessment, each of the 42 selected substances was given a priority 1 or 2:

- Priority 1 was given to those substances which were considered as possible sub-category 1A candidates, in total 20 substances.
- Priority 2 was given to those substances which were not considered as possible sub-category 1A candidates, in total 22 substances.

The justification for the prioritisation of the substances as 1 or 2 is presented in Appendix 3.

The 20 substances given a priority 1 are listed in the table below:

Substances	CAS RN	Substances of special concern according to SCCS (2012)[3]
Butyl methacrylate	97-88-1	
Citral	5392-40-5	X
Turpentine oil	8006-64-2	X
Cinnamaldehyde	104-55-2	X
Cinnamyl alcohol	104-54-1	X
Coumarin	91-64-5	X
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
7-Hydroxycitronellal	107-75-5	X
Limonene	138-86-3	X
Methyl oct-2-ynoate	111-12-6	
Ylang ylang ext. / Ylang ylang oil	83863-30-3 / 8006-81-3	X
***Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext.**	8007-80-5 / 84649-98-9	

[3] Special concern in the SCCS opinion (SCCS, 2012) is defined as due to the high number of reported cases, (>100 cases).

Substances	CAS RN	Substances of special concern according to SCCS (2012)[3]
Clove leaf oil	8000-34-8	X
***Evernia furfuracea*, ext.**	90028-67-4	X
***Evernia prunastri*, ext.**	90028-68-5	X
Jasmine, *Jasminum grandiflorum*, ext. / Jasmine, *Jasminum officinale*, ext. / Extract Jasmine (oil), Jasmine, *Jasminum grandiflorum*, ext.	84776-64-7 / 90045-94-6 / 8022-96-6	X
Balsams, Peru (*Myroxylon pereirae*)	8007-00-9	X
Sandalwood, ext. / Sandalwood oil	84787-70-2 / 8006-87-9	X

Among these 20 substances, five were identified in the LOUS report as having a harmonised classification for skin sensitisation in Category 1 (H317) according to the CLP criteria. These substances are: Butyl methacrylate, citral, d-limonene, l-limonene, and turpentine oil.

Among these 20 substances, 17 substances were identified in the SCCS (2012) opinion as established contact allergens in humans and considered to be of special concern as they have given rise to at least 100 reported cases (the substances marked having assigned an 'X' in the right column in the table above).

The remaining three substances considered to be of special concern in the SCCS (2012) opinion (hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), iseugenol and linalool) were not prioritised further in this project. HICC and iseugenol were among the 42 substances originally selected for the Phase 1 preliminary assessment. These two substances were, however, not given a priority 1 in the Phase 1 assessment as a harmonised classification in sub-category 1A already has been proposed, i.e. the purpose of the project has already been fulfilled. Linalool was not selected for this project as a harmonised classification in sub-category 1B recently has been endorsed by the EU Risk Assessment Committee (RAC).

Among the 20 identified possible sub-category 1A candidates, a further prioritisation was performed for the selection of the Phase 2 substances, i.e. the substances to go through the more detailed assessment of the relevant data.

The highest priority was given to the substances identified in the SCCS (2012) opinion as established contact allergens in humans and considered to be of special concern (marked with an 'X' in the right column of the table) and/or based on the clinical experience from the National Allergy Research Centre.

The 11 substances taken forward to Phase 2 are listed in the table below:

Substances	CAS RN	Substances of special concern according to SCCS (2012)
Citral	5392-40-5	X
Cinnamaldehyde	104-55-2	X
Cinnamyl alcohol	104-54-1	X
Coumarin	91-64-5	X
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
7-Hydroxycitronellal	107-75-5	X
Methyl oct-2-ynoate	111-12-6	
***Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext.**	8007-80-5 / 84649-98-9	
***Evernia prunastri*, ext.**	90028-68-5	X

5. Results, Phase 2

This chapter presents the results of the detailed assessment performed for the 11 substances selected for Phase 2. The results are presented as a summary of the available data, comparison with CLP Regulation criteria for classification in sub-category 1A, and conclusion on classification for each substance. Appendices 4-14 present the full overview of the available studies/data for the substances and the evaluation of their skin sensitising potential.

Most of the studies included in the 11 substance evaluations have been cited from secondary literature, i.e. SCCS/SCCNFP opinions, REACH-RD and reviews and therefore assigned reliability score 4 “Not assignable” according to the Klimisch criteria (Klimisch et al., 1997) as described above in Section 3.2.1. The remaining part of the studies included in the 11 substance evaluations have been available in form of publications from the open literature and therefore assigned reliability score 2 “Reliable with restrictions” according to the Klimisch criteria. A substantial part of those studies cited from secondary literature are unpublished data from the Industry.

The studies have been assessed against the CLP classification criteria for skin sensitisation with special focus on whether classification in sub-category 1A is justified.

One of the elements in the criteria for classification in sub-category 1A based on human patch test data is ‘relatively low exposure’. Relatively high or low exposure relates to the concentrations of the substances in cosmetics and other consumer products which individuals are exposed to in their daily lives, in the workplace, or other conditions and resulting in sensitisation. A cut-off concentration of 1 % has been set in order to discriminate between relatively high exposure (≥ 1.0 %) and relatively low exposure (< 1.0 %).
In the SCCS opinion on fragrance allergens in cosmetic products (SCCS, 2012) it is mentioned that based on data from the fragrance industry (International Fragrance Association, IFRA), 80 % of the total fragrance chemical volume is used in cosmetics and 20 % in household products such as e.g., detergents. However, no quantitative information on the concentrations of fragrances in cosmetics and other consumer products is available in the SCCS opinion. Whether the exposure for the 11 Phase 2 substances is relatively high or relatively low from use as fragrance in cosmetics and in other consumer products has therefore been evaluated based on the IFRA standard limit of each substance in each of 11 finished product categories (IFRA, 2015), i.e., if the IFRA standard limit is < 1.0 % the exposure is thus considered as being relatively low. The IFRA standard limits have generally been set based on quantitative risk assessment (QRA).
For the application of QRA, consumer product types were grouped according to key parameters identified within the QRA approach, i.e., sensitisation assessment factors (SAFs) and consumer product exposure. By using these parameters, 11 different IFRA QRA categories for dermal sensitisation were specified by the QRA Expert Group. For many categories there is generally a wide diversity of product types including cosmetics as well as other consumer products. This is because the categories are based on SAFs and consumer product exposure, not on the functional similarity of each product type.
The overall ‘category consumer exposure level’ is driven by the product type in that category with the combined highest consumer exposure level and highest SAF. These data are used with the WoE NESIL (Weight of Evidence No Expected Sensitization Induction Level) to calculate the ‘acceptable exposure level’ (AEL) for individual fragrance ingredients (AEL is the NESIL divided by the SAF and multiplied by the consumer exposure level).

A default maximum level of the fragrance ingredients identified as dermal sensitisers has been set for practical considerations. This 'maximum pragmatic level' is defined as the level not exceeding the usual concentration of the fragrance in the finished product. If the AEL derived from QRA is less than the 'maximum pragmatic level', the AEL is applied as the IFRA standard limit. Otherwise, the 'maximum pragmatic level' is applied as the IFRA standard limit.
The tables in the individual substance evaluations (Appendices 4-14) present the IFRA standard limit for each of the 11 IFRA QRA categories, as well as the product type that drives the 'category consumer exposure level'.

5.1 Citral

5.1.1 Summary of the available data

5.1.1.1 Human data

A total of 30 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies were identified for citral. The positive patch test frequencies from all of the reported patch test population studies vary between 0.3 and 16.7% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.3 and 4.8% (8 studies) and in studies with selected dermatitis patients positive reactions range between 0.3 and 16.7% (22 studies). The total number of published cases is > 300. Sensitisation was reported in 3/7 HRIPT studies after exposure to 3876 µg/cm² (5%) and in 13/14 HMT studies after exposure to 1379 µg/cm².

5.1.1.2 Non-human information

A total of 12 LLNAs, 6 GPMTs and 1 Buehler test were identified testing skin sensitising effects of citral. The reported EC3 values for citral ranged between 1.2% and 15% in different vehicles. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the other GPMTs sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.1% and in 60-100% of the animals after an intradermal induction dose of 25% citral. Sensitisation was also observed but not quantified (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral.

No relevant *in vitro* studies on citral (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.1.1.3 Exposure

According to data from IFRA (2013a) the exposure of citral when used as fragrance in cosmetics and in other consumer products appears to be low.

5.1.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 4.8% with 3/8 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.3 and 16.7% with 14 out of 22 studies reporting frequencies higher than 2%. In addition to this there are more than 300 published cases of positive patch test reactions to citral. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that citral causes a high frequency of occurrence of skin sensitisation based on patch test data from a minority of unselected dermatitis patient studies and a majority of selected dermatitis patient studies and the number of published cases.

In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 14 LLNAs EC3 values between 1.2 and 15% were reported for citral. Two out of the 14 LLNAs reported an EC3 value <2%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, these two studies indicate classification of citral in sub-category 1A. However, in the other 12 LLNAs, the EC3 value was >2% with only one of these 12 LLNAs reporting an EC3 value (2.1%) borderline to the cut-off criteria for classification in sub-category 1A or 1B indicating classification of citral in sub-category 1B. Based on a weight of evidence for the LLNAs, classification of citral in sub-category 1B seems justified.

In 1/6 of the GPMTs sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.1%. According to the CLP criteria a positive response ≥ 30% of the animals responding at ≤ 0.1% intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of citral into sub-category 1A. In 2/6 GPMTs sensitisation was observed in 60-100% of the animals after an intradermal induction dose of 25% citral. According to the CLP criteria a positive response ≥ 30% responding at > 1% intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.); thus, these two studies indicate classification of citral in sub-category 1B. In 3/6 the GPMTs sensitisation was reported to be observed but not quantified (i.e. number of animals affected) with intradermal induction doses of 0.4, 5 and 10% citral; therefore, these GPMTs cannot be compared with the classification criteria.

Sensitisation was also reported to be observed but not quantified (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral; therefore, this study cannot be compared with the classification criteria.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. The results from the animal studies are equivocal, mainly indicating classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is thus warranted for citral.

5.1.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure, a classification of citral as a skin sensitiser in sub-category 1A is justified.

5.2 Cinnamaldehyde

5.2.1 Summary of the available data

5.2.1.1 Human data

A total of 52 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies were identified with cinnamaldehyde. The positive patch test frequencies from all of the reported patch test population studies vary between 0.93 and 90% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.93 and 32.5% (8 studies) and in studies with selected dermatitis patients positive reactions range between 1.2 and 90% (44 studies). A single study in workers reported positive patch test reactions in 1%. The total number of published cases is > 2300. A LOEL-HRIPT/HMT (induction) of 775 µg/cm² was established for cinnamaldehyde by the RIFM Expert Panel based on unpublished reports.

5.2.1.2 Non-human information

A total of 22 LLNAs and 2 LLNA BrdU-ELISA tests were identified testing skin sensitising effects of cinnamaldehyde. The reported EC3 values for cinnamaldehyde ranged between 0.2% and 3.1% in different vehicles.

No relevant *in vitro* studies on cinnamaldehyde (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.2.1.3 Exposure

According to data from IFRA (2013b) the exposure of cinnamaldehyde when used as fragrance in cosmetics and in other consumer products appears to be low.

5.2.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.93 and 32.5% with 7/8 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 1.2 and 90% with 37 out of 44 studies reporting frequencies higher than 2%. A single study in workers reported positive patch test reactions in 1%. In addition to this there are more than 2300 published cases of positive patch test reactions to cinnamaldehyde. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or ≥ 1% for selected workers with known exposure or dermatitis and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that cinnamaldehyde causes a *high frequency* of occurrence of skin sensitisation based on these four types of information.
In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 22 LLNAs EC3 values between 0.2 and 3.1% were reported for cinnamaldehyde with 21/22 EC3 values <2%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.). Hence, data from LLNAs indicate classification of cinnamaldehyde in sub-category 1A.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from the LLNAs support a sub-category 1A classification. A classification as a skin sensitiser in sub-category 1A is thus warranted for cinnamaldehyde.

5.2.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure and supported by data from LLNAs, a classification of cinnamaldehyde as a skin sensitiser in sub-category 1A is justified.

5.3 Cinnamyl alcohol

5.3.1 Summary of the available data

5.3.1.1 Human data

A total of 34 patch test population studies, 3 HRIPTs, 25 HMTs and 4 case studies, were identified with cinnamyl alcohol. The positive patch test frequencies from all of the reported patch test studies vary between 0.56 and 100% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% (4 studies) and in studies with selected dermatitis patients positive reactions range between 1.5 and 100% (30 studies). The total number of published cases is > 600. A LOEL (induction) of 4724 µg/cm² was derived from the HRIPT/HMT studies.

5.3.1.2 Non-human information

A total of 5 LLNAs (two of which were reported to be conducted in accordance with OECD TG 429), 2 GPMT, 1 Freund's complete adjuvant test (FCAT) and 1 Buehler test were identified testing the skin sensitisation of cinnamyl alcohol. EC3 values between 17.9 and 30% were reported for cinnamyl alcohol in the LLNAs and positive reactions were observed in a GPMT (30% positive) and FCAT (15% positive) at intradermal induction doses of 25 and 100%, respectively. No positive reactions were observed in the Buehler test.

No relevant *in vitro* studies on cinnamyl alcohol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.3.1.3 Exposure

According to data from IFRA (2008a) the exposure of cinnamyl alcohol when used as fragrance in cosmetics and in other consumer products appears to be low.

5.3.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% with 1/4 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 1.5 and 100% with 28 out of 30 studies reporting frequencies higher than 2%. In addition to this there are more than 600 published cases of positive patch test reactions to cinnamyl alcohol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that cinnamyl alcohol causes a high frequency of occurrence of skin sensitisation based on frequencies in selected dermatitis patients and total number of cases.
In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNAs EC3 values between 17.9 and 30% were reported for cinnamyl alcohol. According to the CLP Regulation an EC3 value larger than 2% indicates placement of cinnamyl alcohol into sub-category 1B (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 30% of the animals after an intradermal induction dose of 25% cinnamyl alcohol. According to the CLP criteria a positive response ≥ 30% of the animals responding at >1% intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.).

No sensitisation was observed in the Buehler test after an induction dose of 30% cinnamyl alcohol.

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies with selected dermatitis patients and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. All animal studies indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for cinnamyl alcohol.

5.3.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure a classification of cinnamyl alcohol as a skin sensitiser in sub-category 1A is justified.

5.4 Coumarin

5.4.1 Summary of the available data

5.4.1.1 Human data

A total of 25 patch test population studies and 2 case studies, one of which included a ROAT, were identified with coumarin. The positive patch test frequencies from all of the reported patch test studies vary between 0 and 10% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% (7 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 10% (19 studies). The total number of published cases is > 200. A LOEL (induction) of 8858 μg/cm² was derived from the HRIPT/HMT studies.

5.4.1.2 Non-human information

A total of 20 LLNAs and 1 GPMT were identified testing skin sensitising effects of coumarin. The collected evidence from the LLNAs indicates an EC3 for coumarin of ca. 50%. Sensitisation was not observed in the GPMT after an intradermal induction dose of 0.5% coumarin.

Contaminants in coumarin may act as weak or moderate sensitizers (SCCS, 2012).

No relevant *in vitro* studies on coumarin (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.4.1.3 Exposure

According to data from IFRA (2008b) the exposure of coumarin when used as fragrance in cosmetics and in other consumer products appears to be low.

5.4.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% i.e. all 7 studies reporting frequencies lower than 1%. For selected dermatitis patients positive reactions range between 0 and 10% with 9 out of 19 studies reporting frequencies higher than 2%. In addition to this there are more than 200 published cases of positive patch test reactions to coumarin. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that coumarin causes a low/moderate frequency of occurrence of skin sensitisation based on unselected/consecutive dermatitis patients and 10/19 studies with selected dermatitis patients. The remaining studies with selected dermatitis patients (9/19) and number of published cases shows that coumarin causes a high frequency of occurrence of skin sensitisation in humans.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate classification of coumarin in sub-category 1B according to Annex I: 3.4.2.2.2.2.

The collected evidence from the LLNAs indicates an EC3 for coumarin of ca. 50%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, all studies indicate classification of coumarin in sub-category 1B.

The single GPMT with an intradermal induction dose of 0.5% gave no positive reactions which does not justify sub-categorisation (Table 3.4.3).

Overall, there is evidence for classification in sub-category 1A based on the number of cases combined with the estimated low exposure and supported by patch test data from selected dermatitis patients. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for coumarin.

5.4.3 Conclusions on classification and labelling

Based on the number of cases combined with the estimated low exposure and supported by patch test data from selected dermatitis patients, a classification of coumarin as a skin sensitiser in sub-category 1A is justified.

5.5 Eugenol

5.5.1 Summary of the available data

5.5.1.1 Human data

A total of 36 patch test population studies, 1 ROAT and 1 case study were identified with eugenol. The positive patch test frequencies from all of the reported patch test studies vary between 0.3 and 55.4% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% (5 studies) and in studies with selected dermatitis patients positive reactions range between 0.62 and 55.4% (31 studies). The total number of published cases is > 700. A NESIL from HRIPT studies of 5900 µg/cm² was derived based on weight of evidence by the RIFM Expert Panel.

5.5.1.2 Non-human information

A total of 15 LLNAs and one GPMT were identified testing the skin sensitisation of eugenol. EC3 values were reported in 13 studies and ranged between 4.2 and 25.1%. Positive reactions (20%) were observed in the GPMT at an intradermal induction dose of 5%.

No relevant *in vitro* studies on eugenol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.5.1.3 Exposure

According to data from IFRA (2008c) the exposure of eugenol when used as fragrance in cosmetics and in other consumer products appears to be low.

5.5.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% with 2 out of 5 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.62 and 55.4% with 25 out of 31 studies reporting frequencies higher than 2%. In addition to this there are more than 700 published cases of positive patch test reactions to eugenol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that eugenol causes a high frequency of occurrence of skin sensitisation based on the frequency of positive reactions mainly in selected dermatitis patients (>2% in 21/27 studies) and the total number of cases.
In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNA tests, EC3 values between 4.2 and 25.1% (13 studies) were reported for eugenol. According to the CLP Regulation an EC3 value larger than 2 indicates placement of eugenol into sub-category 1B (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 20% of the animals after an intradermal induction dose of 5% which does not justify sub-categorisation into either sub-category 1A or 1B (Annex I: 3.4.2.2.3.2.).

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies mainly with selected dermatitis patients and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for eugenol.

5.5.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases, combined with the estimated low exposure, a classification of eugenol as a skin sensitiser in sub-category 1A is justified.

5.6 Farnesol

5.6.1 Summary of the available data

5.6.1.1 Human data

A total of 20 patch test population studies, 3 HRIPTs, 11 HMTs and 2 case studies were identified with farnesol. The positive patch test frequencies from all of the reported patch test population studies vary between 0.02 and 13.2% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% (4 studies) and in studies with selected dermatitis patients positive reactions range between 0.02 and 13.2% (16 studies). The total number of published cases is > 250. Positive responses after farnesol were seen at concentrations ≥ 6900 μg/cm² in 5/11 HMTs.

5.6.1.2 Non-human information

A total of two LLNAs and four GPMTs were identified testing skin sensitising effects of farnesol. EC3 values were 5.5 and 4.1%. In the GPMTs no positive reactions were observed after intradermal induction doses of 0.16, 5 and 10% farnesol.

No relevant *in vitro* studies on farnesol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.6.1.3 Exposure

According to data from IFRA (2006) the exposure of farnesol when used as fragrance in cosmetics and in other consumer products appears to be low.

5.6.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% i.e. all 4 studies reporting frequencies lower than 1%. For selected dermatitis patients positive reactions range between 0.02 and 13.2% with 7 out of 16 studies reporting frequencies higher than 2%. In addition to this there are more than 250 published cases of positive patch test reactions to farnesol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that farnesol causes a low/moderate frequency of occurrence of skin sensitisation based on unselected/consecutive dermatitis patients and 9/16 studies with selected dermatitis patients. The remaining studies with selected dermatitis patients (7/16) and number of published cases shows that farnesol causes a high frequency of occurrence of skin sensitisation in humans.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate classification of farnesol in sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the two LLNAs the lowest EC3 value for farnesol was 4.1%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, both studies indicate classification of farnesol in sub-category 1B.

Sensitisation was not observed in the four GPMTs with intradermal induction doses of 0.16, 5 and 10% farnesol which do not justify sub-categorisation (Table 3.4.3).

Overall, there is evidence for classification in sub-category 1A based on the number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-

category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for farnesol.

5.6.3 Conclusions on classification and labelling

Based on the number of cases combined with the estimated low exposure, a classification of farnesol as a skin sensitiser in sub-category 1A is justified.

5.7 Geraniol

5.7.1 Summary of the available data

5.7.1.1 Human data

A total of 84 results from patch test population studies, 7 HRIPTs, 4 HMTs and 2 case studies were identified with geraniol. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 40% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 1.2% (10 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 40% (74 studies). The total number of published cases is > 900. Sensitisation was reported in 2/4 HRIPT studies after exposure to 10% geraniol (11 810 µg/cm²) and in 1/4 HMT studies after exposure to 4140 µg/cm².

5.7.1.2 Non-human information

A total of 9 LLNAs, 5 GPMTs and 1 Buehler test were identified testing skin sensitising effects of geraniol. The reported EC3 values for geraniol ranged between 5.6% and 25.8% in different vehicles. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol. No sensitisation was observed in 1/5 GMPTs with an induction concentration of 50% geraniol and in the Buehler test with an induction concentration of 15% geraniol.

No relevant *in vitro* studies on geraniol (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.7.1.3 Exposure

According to data from IFRA (2007) the exposure of geraniol when used as fragrance in cosmetics and in other consumer products appears to be relatively low. A recent study has indicated that up to 0.86% of the population might be exposed to geraniol from personal care products and household cleaning agents at levels exceeding the estimated Acceptable Exposure Level of 55 µg/cm² (Nijkamp et al., 2015).

5.7.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0 and 1.2% with 2/10 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 40% with 44 out of 74 studies reporting frequencies higher than 2%. In addition to this there are more than 900 published cases of positive patch test reactions to geraniol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that geraniol causes a *high frequency* of occurrence of skin sensitisation based on patch test data mainly from selected dermatitis patients and the number of published cases.
In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate classification of geraniol in sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNAs EC3 values between 5.6 (vehicle: ethanol) and 25.8% (vehicle: ethanol:diethyl phthalate 1:3) were reported for geraniol. According to the CLP Regulation an EC3 value larger than 2% indicates classification of geraniol in sub-category 1B.

In the GPMTs sensitisation was reported to be observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol, therefore, these GPMTs cannot be compared with the classification criteria.

No sensitisation was observed in the Buehler test with an induction concentration of 15% geraniol. Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies mainly with selected dermatitis patients and the total number of cases combined with the estimated relatively low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for geraniol.

5.7.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases, combined with the estimated relatively low exposure, a classification of geraniol as a skin sensitiser in sub-category 1A is justified.

5.8 7-Hydroxycitronellal

5.8.1 Summary of the available data

5.8.1.1 Human data

A total of 39 results from patch test population studies, 4 modified HRIPTs, 15 HMTs and 3 case studies were identified with 7-hydroxycitronellal. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 55% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.9 and 2.6% (4 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 55% (35 studies). The total number of published cases is > 800. A LOEL-HRIPT/HMT (induction) of 5906 μg/cm² was established for 7-hydroxycitronellal by the RIFM Expert Panel.

5.8.1.2 Non-human information

A total of 7 LLNAs including 1 LLNA *ex vivo* BrdU, 1 GPMT and 1 Buehler test were identified testing skin sensitising effects of 7-hydroxycitronellal. The reported EC3 values for 7-hydroxycitronellal range between 9.8 and 33%. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the GPMT sensitisation in 60% of the animals (number of animals not reported) after an intradermal induction dose of 0.5% 7-hydroxycitronellal. Sensitisation was also observed in 38% of the animals in the Buehler test with an induction concentration of 30% 7-hydroxycitronellal.

No relevant *in vitro* studies on 7-hydroxycitronellal (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.8.1.3 Exposure

According to data from IFRA (2013c) the exposure of 7-hydroxycitronellal when used as fragrance in cosmetics and in other consumer products appears to be low.

5.8.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.9 and 2.6% with 1/4 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 55% with 29 out of 33 studies reporting frequencies equal to or higher than 2%. In addition to this there are more than 800 published cases of positive patch test reactions to 7-hydroxycitronellal. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b). The collected data described above from patch test studies show that 7-hydroxycitronellal causes a *high frequency* of occurrence of skin sensitisation based on these three types of information.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the seven LLNAs EC3 values between 9.8 and 33% were reported for 7-hydroxycitronellal. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, all seven studies indicate classification of 7-hydroxycitronellal in sub-category 1B.

In the GPMT sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.5% 7-hydroxycitronellal. According to the CLP criteria a positive response ≥ 60% of the animals responding at >0.1% to ≤ 1% intradermal induction dose indicates classification of a substance in

sub-category 1A (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of 7-hydroxycitronellal into sub-category 1A.

Sensitisation was also observed in 38% of the animals in a Buehler test with an induction concentration of 30% 7-hydroxycitronellal. According to the CLP criteria a positive response ≥ 15% of the animals responding at >20% topical induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.3.) and thus, this study indicates classification of 7-hydroxycitronellal into sub-category 1B.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. Except from the GMTP study, which supports a sub-category 1A classification, the remaining animal studies (LLNA and Buehler) indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is thus warranted for 7-hydroxycitronellal.

5.8.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure a classification of 7-hydroxycitronellal as a skin sensitiser in sub-category 1A is justified.

5.9 Methyl oct-2-ynoate

5.9.1 Summary of the available data

5.9.1.1 Human data

A total of 11 results from patch test population studies and 5 case studies were identified with methyl oct-2-ynate. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 2.9% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 1.67% (3 studies) and in studies with selected dermatitis patients positive reactions range between 0.1 and 2.9% (8 studies). The total number of published cases is > 25. A LOEL-HRIPT/HMT (induction) of 194 µg/cm² was established for methyl oct-2-ynate by the RIFM Expert Panel based on unpublished reports.

5.9.1.2 Non-human information

A total of 2 LLNAs (OECD TG 429), 1 GPMT and 1 Buehler test were identified testing skin sensitising effects of methyl oct-2-ynate. In both LLNA studies an EC3 value of <0.5% was reported. In the GPMT sensitisation was observed in 90 % of the animals after an intradermal induction dose of 0.625% methyl oct-2-ynate. Sensitisation was also observed in the Buehler test with positive reactions in 45-70% of the animals after an induction dose of 2.5%.

No relevant *in vitro* studies on methyl oct-2-ynate (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.9.1.3 Exposure

According to data from IFRA (2008d) the exposure of methyl oct-2-ynate when used as fragrance in cosmetics and in other consumer products appears to be low.

5.9.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0 and 1.67% with 1/3 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.1 and 2.9% with 1 out of 8 studies reporting frequencies higher than 2%. In addition to this there are more than 25 published cases of positive patch test reactions to methyl oct-2-ynate. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that methyl oct-2-ynate causes a *low/moderate frequency* of occurrence of skin sensitisation based on these three types of information. In regard to HRIPT studies positive responses were observed at exposure to 194 µg/cm² methyl 2-ocytnoate. A positive response at ≤ 500 µg/cm² in a HRIPT or HMT suggests categorisation into sub-category 1A according to Annex I: 3.4.2.2.2.1 and 3.4.2.2.2.2.

In the 2 LLNAs EC3 values were <0.5%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 90% of the animals after an intradermal induction dose of 0.625%. According to the CLP criteria a positive response ≥ 60% of the animals responding at > 0.1% to ≤ 1% intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

In the Buehler test sensitisation was observed in 45-70% of the animals after an induction does of 2.5%. According to the CLP criteria a positive response ≥ 60% of the animals responding at > 0.2%

to ≤ 20% topical induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

Overall, there is clear evidence for classification in sub-category 1A based on the very low EC3 values from the LLNAs. The results from GPMT and the Buehler test also supports sub-category 1A. Data from human patch test studies and the number of published cases justify classification of methyl oct-2-ynate in sub-category 1B while data from HRIPT studies justify classification of methyl oct-2-ynate in sub-category 1A. A classification as a skin sensitiser in sub-category 1A is thus warranted for methyl oct-2-ynate.

5.9.3 Conclusions on classification and labelling

Based on HRIPT data, the very low EC3 value from LLNAs and results from GPMT and Buehler tests a classification of methyl oct-2-ynate as a skin sensitser in sub-category 1A is justified.

5.10 *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext.

5.10.1 Summary of the available data

5.10.1.1 Human data

A total of 27 positive cases and frequencies between 1 and 27.8% in selected dermatitis patients tested with “cassia” essential oil or “cinnamon oil” were observed.

5.10.1.2 Non-human information

No animal studies with *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. have been identified.

5.10.1.3 Exposure

It has not been possible to identify any data on exposure to *Cinnamomum cassia* leaf oil or *Cinnamomum zeylanicum*, ext.

5.10.2 Comparison with criteria

One out of two studies with “cassia” essential oil gave a frequency of positive patch tests in selected patients of 27.8% i.e. ≥ 2%, which indicate categorisation into sub-category 1A.
No animal studies with *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. have been identified.

5.10.3 Conclusions on classification and labelling

Data on *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. alone is insufficient for sub-categorisation of *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. according to CLP criteria. It may be possible to sub-categorise *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. based on their constituents by read across to the major compounds such as cinnamaldehyde (for Cassia bark extract, Cassia oil, Cinnamon bark extract and Cinnamon bark oil) and eugenol (for Cinnamon leaf oil).

5.11 *Evernia prunastri* extract

5.11.1 Summary of the available data

5.11.1.1 Human data

A total of 35 results from patch test population studies, 5 HRIPTs and 2 case studies were identified with *Evernia prunastri* ext. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 64% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 1.8 and 6.8% (7 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 64% (28 studies). The total number of published cases is > 1900. A LOEL-HRIPT/HMT (induction) of 1417 μg/cm² was established for *Evernia prunastri* ext. by the RIFM Expert Panel based on unpublished reports.

5.11.1.2 Non-human information

A total of 1 LLNA, 1 LLNA *ex vivo* BrdU and 1 GPMT were identified testing skin sensitising effects of *Evernia prunastri* ext. The reported EC3 value for *Evernia prunastri* ext. was 3.9% in the LLNA and 3.4% in the LLNA *ex vivo* BrdU. In the GPMT sensitisation was observed in 63% of the animals after an intradermal induction dose of 20% *Evernia prunastri* ext.

No relevant *in vitro* studies on *Evernia prunastri* ext. (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.11.1.3 Exposure

According to data from IFRA (2008e) the exposure of *Evernia prunastri* ext. when used as fragrance in cosmetics and in other consumer products appears to be low.

5.11.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 1.81 and 6.8% i.e. all studies are reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 64% with 23 out of 28 studies reporting frequencies higher than 2%. In addition to this there are more than 1900 published cases of positive patch test reactions to *Evernia prunastri* ext. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that *Evernia prunastri* ext. causes a *high frequency* of occurrence of skin sensitisation based on these three types of information.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 2 LLNAs EC3 values were 3.4 and 3.9%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, both studies indicate classification of *Evernia prunastri* ext. in sub-category 1B.
In the GPMT sensitisation was observed in 63% of the animals after an intradermal induction dose of 20%. According to the CLP criteria a positive response ≥ 30% of the animals responding at > 1% intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of *Evernia prunastri* ext. into sub-category 1B.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases

combined with the estimated low exposure. The results from the animal studies indicate classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is thus warranted for *Evernia prunastri* ext.

5.11.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure, a classification of *Evernia prunastri* ext. as a skin sensitiser in sub-category 1A is justified.

5.12 Conclusion

For all 11 substances selected for detailed assessment in Phase 2, a classification as a skin sensitiser in sub-category 1A is justified based on the available data.

For nine of the substances, sub-category 1A is justified based on human patch test data i.e. frequencies in unselected and/or selected dermatitis patients and/or a high number of cases.
For methyl oct-2-ynate, sub-category 1A is justified primarily based on non-human data (very low EC3 values in the two LLNAs and results from GPMT and Buehler tests, and supported by human evidence from HRIPT studies).
For *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext.), sub-category 1A is justified based on their constituents by read across to the major compounds such as cinnamaldehyde (for Cassia bark extract, Cassia oil, Cinnamon bark extract and Cinnamon bark oil) and eugenol (for Cinnamon leaf oil).

6. Discussion

6.1 Sub-category 1A candidates (the 11 substances in Phase 2)

For all 11 substances selected for detailed assessment in Phase 2, a classification as a skin sensitiser in sub-category 1A is justified based on the available data.

Generally, sub-category 1A is justified based on the available human data:

For nine of the 11 substances selected for detailed assessment in Phase 2 (i.e. not including methyl oct-2-ynate and *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext.), sub-category 1A is justified based on human patch test data i.e. frequencies in unselected and/or selected dermatitis patients and/or a high number of cases. For two of the substances, farnesol and coumarin, sub-category 1A is justified solely by the high number of cases from human diagnostic patch test studies. For human diagnostic patch test data, only one or two types of information in terms of frequency in defined populations / number of cases may be sufficient for sub-categorisation. Combined with the estimated low exposure for all these substances it seems clear that sub-category 1A is justified for these nine substances based on the available data.

Generally, when sub-category 1A is justified based on the available human data this is not supported by non-human data:

For only two of the nine substances where sub-category 1A is justified based on the available human data, sub-category 1A is also supported by non-human data. For cinnamaldehyde, 21/22 LLNAs showed an EC3 value <2% and thus, justifying sub-category 1A. For 7-hydroxycitronellal, one GPMT supported sub-category 1A, whereas 7 LLNAs and one Buehler test indicated a classification in sub-category 1B.

For the remaining substances, non-human data, if available, do not support sub-category 1A. As a substance may be allocated to sub-category 1A on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals it seems clear that sub-category 1A is justified for these nine substances based on the available data, i.e. despite that the available non-human data do not support classification in sub-category 1A.

The fact that data from human and non-human studies only support each other for a few substances may be explained by the difference in exposure. As described by Anderson et al. (2011) the elicitation thresholds may be lower than those required for induction and the dose required for induction may depend on duration frequency and site of exposure. Humans could be exposed to these substances repeatedly, thus causing a relatively weak sensitiser, as measured by the EC3-value, to be of greater risk for allergic contact dermatitis in humans due to frequent exposure.

Only in some cases, sub-category 1A is justified based on the available non-human data:

Only for one of the 11 substances selected for detailed assessment in Phase 2, methyl oct-2-ynate, sub-category 1A is justified primarily based on non-human data, especially a very low EC3 value in the two LLNAs, but also the results from GPMT and Buehler tests. Furthermore, sub-category 1A is supported by human evidence in form of results in the HRIPT studies.

The reason for the low/moderate frequency of occurrence of skin sensitisation in human diagnostic patch test data with methyl oct-2-ynate is probably the observation that patch test reactions after methyl oct-2-ynate occurs relatively late (after 2-4 weeks) indicating active sensitisation (i.e. the subjects were sensitised by the patch test) (Heisterberg et al. 2010).

Sub-category 1A is justified based on read across:
For one of the 11 substances selected for detailed assessment in Phase 2, *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. it is not possible to suggest a classification in sub-category 1A based on the limited available data. In contrast to the other 10 substances selected for detailed assessment in Phase 2, this fragrance is a mixture of substances and the available data did not include specific information on composition and purity of the constituents in the fragrance. It may, however, be possible to sub-categorise *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. based on their constituents by read across to the major constituents, i.e. cinnamaldehyde (for Cassia bark extract (44%), Cassia oil (87%), Cinnamon bark extract (38%) and Cinnamon bark oil (75%)) and eugenol (for Cinnamon leaf oil (70%)). Both cinnamaldehyde and eugenol are among the 11 substances selected for detailed assessment in Phase 2 for which sub-category 1A is justified. Based on a read across it seems clear that sub-category 1A is justified for *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. based on the available data.

6.2 Other possible sub-category 1A candidates (9 substances given priority 1 in Phase 1)

In addition to the 11 substances selected for detailed assessment in Phase 2, nine other substances (butyl methacrylate, limonene, turpentine oil, Ylang ylang ext./oil, clove leaf oil, *Evernia furfuracea* ext., jasmine, Peru balsam, Sandalwood ext./oil) were given a priority 1 in Phase 1, i.e. considered as possible sub-category 1A candidates.

Seven of these substances (i.e. not including butyl methacrylate and limonene) were considered as possible sub-category 1A candidates based on human patch test data, i.e. frequencies in unselected and/or selected dermatitis patients and/or a high number of cases.
Limonene was considered as a possible sub-category 1A candidate as it is among the substances considered by SCCS (2012) as a substance of special concern, defined as due to the high number of reported cases (>100 cases).
Butyl methacrylate was considered as a possible sub-category 1A candidate based on high frequency of occupational allergic contact dermatitis due to (meth)acrylates in general.

Overall, all these nine substances were considered as possible sub-category 1A candidates based on the available human data. A detailed assessment of these substances was, however, not part of the current project.

6.3 Remaining substances (22 substances given priority 2 in Phase 1)

The remaining 22 substances selected for the preliminary assessment in Phase 1 were given a priority 2, i.e. not considered as possible sub-category 1A candidates.

For 5 substances (d-limonene, (l-limonene), carvone, HICC, isoeugenol), a proposal for a harmonised classification as skin sensitiser in Category 1 H317, or sub-category 1A or 1B has already been submitted.

For 10 substances (benzaldehyde, 2-methyl-4-phenylpentanol, acetylcedrene, trans-anethole, anise alcohol, benzyl salicylate, 2-(4-tert-butylbenzyl)propionaldehyde, citronellol, peppermint ext./oil, spearmint ext.), sub-category 1A is not justified based on human patch test data, i.e. due to a low frequency and low number of cases in the human studies, as well as no/equivocal sensitisation was noted in the non-human studies.

For the remaining 7 substances (mixture (of trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran), Damascone, pin-2(3)-ene/pin-2(10)-ene, p-mentha-1,4(8)-diene, Neroli oil / Orange sour ext., lemon ext., orange sweet ext.), the

available data were too limited for an indication as a possible sub-category 1A candidate. Thus, it cannot be excluded whether these substances would turn out to be sub-category 1A candidates should additional human studies and/or non-human data become available.

6.4 Exposure assessment

One of the elements in the criteria for classification in sub-category 1A based on human patch test data is 'relatively low exposure'. Relatively high or relatively low exposure relates to the concentrations of the substances in cosmetics and other consumer products which individuals are exposed to in their daily lives, in the workplace, or other conditions and resulting in sensitisation. A cut-off concentration of 1 % has been set in order to discriminate between relatively high exposure (≥ 1.0 %) and relatively low exposure (< 1.0 %).

For the 11 substances selected for Phase 2, relatively high or relatively low exposure from use as fragrance in cosmetics and in other consumer products has been evaluated based on the IFRA standard limit of each substance in each of the 11 IFRA QRA product categories (IFRA, 2015), i.e., if the IFRA standard limit is < 1.0 % the exposure is thus considered as being relatively low. This is generally the case for the 11 Phase 2 substances for most of the IFRA QRA product categories where the IFRA standard limits have been set based on QRA.
The SCCS has recently published a 'Memorandum on use of Human Data in risk assessment of skin sensitisation' (SCCS, 2015). It is mentioned that the data used in the QRA approach is animal data and/or results from predictive tests in humans, i.e., experimental induction tests such as the Human Repeated Insult Patch Test (HRIPT) and the Human Maximization Test (HMT). However, it is also mentioned that it would be more appropriate to consider the epidemiological and diagnostic patch test data as these represent the relevant end-point. Furthermore, it is noted that the QRA approach is still under evaluation.
If the QRA approach would be revised in order to include also the epidemiological and diagnostic patch test data the current IFRA standard limits based on the QRA approach might change. If the current IFRA standard limits below 1 % would change to values above this cut-off concentration for relatively high/low exposure this could have an impact on the classification as a skin sensitiser in sub-category 1A for the 11 Phase 2 substances.

6.5 Data quality

A relatively large proportion of the cited studies have only been available from secondary sources, mainly attributed to unpublished data by the industry. Inclusion of these studies in the preliminary assessment of the 42 selected substances in Phase 1, as well as for the detailed assessment of the 11 substances in Phase 2 is justified due to the purpose of the current project, i.e. a screening of the available data for a preliminary assessment whether the criteria for classification in sub-category 1A according to the CLP criteria are fulfilled.
Secondary sources have a reliability score 4 "not assignable" according to the reliability criteria proposed by Klimisch et al. (1997). For a genuine assessment of studies only available from secondary sources, the original study report should be available for the assessment.

6.6 Conclusion

For all 11 substances selected for detailed assessment in Phase 2, a classification as a skin sensitiser in sub-category 1A is justified based on the available data.

Based on the assessments in this project, a decision whether a classification of a substance as a skin sensitiser in sub-category 1A is justified is generally based on the available human data.

For the 11 substances selected for Phase 2, relatively low exposure from use as fragrance in cosmetics and in other consumer products has been evaluated based on the IFRA standard limit of each substance in each of the 11 IFRA QRA product categories, i.e., if the IFRA standard limit is < 1.0 % the exposure is thus considered as being relatively low. This is generally the case for the 11 substances for most of the IFRA QRA product categories where the IFRA standard limits have been set based on QRA. Data for the QRA are generally animal data and/or results from predictive tests in humans (HRIPT and HMT). As it would be more appropriate to consider the epidemiological and diagnostic patch test data the current IFRA standard limits might change if the QRA approach would be revised in order to include also the epidemiological and diagnostic patch test data. This could have an impact on the classification as a skin sensitiser in sub-category 1A for the 11 substances if the current IFRA standard limits below 1 % would change to values above this cut-off concentration for relatively high/low exposure.

A relatively large proportion of the cited studies have only been available from secondary sources, mainly attributed to unpublished data by the industry. For a genuine assessment of such studies, the original study reports should be available for the assessment. Thus, it cannot be excluded whether the outcome of the assessments performed in this project could turn out differently should the original study reports become available for the final assessment.

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Appendix 1 List of abbreviations and acronyms

AEL	Acceptable Exposure Level
AOO	Acetone:Olive oil
BHT	Butylated hydroxytoluene
BrdU-ELISA	5-Bromo-2-deoxyuridine-enzyme-linked immunosorbent assay
CAS RN	Chemical Abstract Service Registration Number
DEP	Diethyl Phthalate
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
FM	Fragrance mix
EC3 value	Effective Concentration inducting a stimulation index of 3 in the LLNA test.
EtOH	Ethanol
FM	Fragrance Mix
GPMT	Guinea Pig Maximisation Test
IFRA	International Fragrance Association
LLNA	Local Lymph Node Assay
MEK	Methyl ethyl ketone
NESIL	No Expected Sensitization Induction Level
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
PEG	Polyethylene glycol
Pet.	Petrolatum
QRA	Quantitative Risk Assessment
REACH RD	Registration, Evaluation, Authorisation and Restriction of Chemicals Registration Dossier
RIFM	Research Institute for Fragrance Materials, Inc.
SAF	Sensitisation Assessment Factors
SCCNFP	Scientific Committee on Cosmetic products and Non-Food Products intended for consumers
SCCS	Scientific Committee on Consumer Safety
SSO	Sorbitan sesquioleate
WoE	Weight of Evidence

Appendix 2 List of substances for evaluation in Phase 1

CAS	Stofnavn	Skin sens 1 Harm. Klass.	Skin sens 1 Selvklass. (også 1A eller 1B)	Andel virks. med Skin sens. selvkl. (ca.)	REACH registr.	Prioritet
97-88-1	Butyl methacrylate	x	x		Ja	1a
100-52-7	Benzaldehyde		x	>90%	Ja	1a
5392-40-5	Citral	x	x		Ja	1a
5989-27-5	(R)-p-mentha-1,8-diene (d-limonene)	x	x		Ja	1a
5989-54-8	(S)-p-mentha-1,8-diene (l-limonene)	x	x		Ja	1a
92585-24-5	2-methyl-4-phenylpentanol	x	x		Ja	1a
131766-73-9	A mixture of: trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran	x	x		Ja	1a
8006-64-2	Turpentine oil	x	x		Ja	1a
32388-55-9	[3R-(3α,3aβ,7β,8aα)]-1-(2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)ethan-1-one (Acetylcedrene)		x	>90%	Ja	1a
4180-23-8	(E)-anethole (trans-anethole)		x	>90%	Ja	1a
105-13-5	4-methoxybenzyl alcohol (Anise alcohol)		x	>90%	Ja	1a
118-58-1	Benzyl salicylate		x	>90%	Ja	1a
80-54-6	2-(4-tert-butylbenzyl)propionaldehyde (Butylphenyl methylproprional)		x	>90%	Ja	1a
99-49-0/	d-p-mentha-1(6),8-dien-2-one (Carvone) /		x	>90%	Nej	2

CAS	Stofnavn	Skin sens 1 Harm. Klass.	Skin sens 1 Selvklass. (også 1A eller 1B)	Andel virks. med Skin sens. selvkl. (ca.)	REACH registr.	Prioritet
6485-40-1/	l-p-mentha-1(6),8-dien-2-one (Carvone) /		x	>90%	Ja	1a
2244-16-8	(S)-2-methyl-5-(1-methylvinyl)cyclohex-2-en-1-one (Carvone)		x	ca 50%	Nej	2
104-55-2	cinnamaldehyde		x	>90%	Ja	1a
104-54-1	cinnamyl alcohol		x	>90%	Ja	1a
106-22-9/	citronellol		x	>90%	Ja	1a
1117-61-9/	(R)-3,7-dimethyloct-6-en-1-ol		x	>90%	Nej	2
7540-51-4	(-)-3,7-dimethyloct-6-en-1-ol		x	>85%	Nej	2
91-64-5	coumarin		x	>90%	Ja	1a
57378-68-4	1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-2-buten-1-one (delta-DAMASCONE)		x	100%	Nej	1b
97-53-0	eugenol		x	>90%	Ja	1a
4602-84-0	farnesol		x	>85%	Ja	1a
106-24-1	geraniol		x	>90%	Ja	1a
31906-04-4/	4-(4-hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde (Hydroxyisohexyl 3-cyclohexene carboxaldehyde, HICC)		x	>90%	Nej	1d
51414-25-6			x	100%	Nej	1d
107-75-5	7-hydroxycitronellal		x	100%	Ja	1a
97-54-1	isoeugenol		x	100%	Nej	1b
138-86-3	limonene	x	x		Nej	1c
111-12-6	methyl oct-2-ynoate		x	>90%	Nej	1b
80-56-8/	pin-2(3)-ene /		x	>75%	Ja	1a
127-91-3	pin-2(10)-ene		x	>90%	Ja	1a

CAS	Stofnavn	Skin sens 1 Harm. Klass.	Skin sens 1 Selvklass. (også 1A eller 1B)	Andel virks. med Skin sens. selvkl. (ca.)	REACH registr.	Prioritet
586-62-9	p-mentha-1,4(8)-diene (terpinolene)		x	> 75%	Ja	1a
83863-30-3/	Ylang ylang ext. /		x	>90%	Nej	1d
8006-81-3	Ylang ylang oil		x	>90%	Nej	1d
8007-80-5/	Cinnamomum cassia leaf oil /		x	ca. 80%	Nej	2
84649-98-9	Cinnamomum zeylanicum, ext.		x	>90%	Ja	1a
8016-38-4/	Neroli Oil /		x	>95%	Nej	2
72968-50-4	Orange, sour, ext.		x	>95%	Ja	1a
84929-31-7	Lemon, ext.		x	>95%	Ja	1a
97766-30-8/	Orange, sweet, Valencia, ext. /		x	>95%	Nej	2
8028-48-6	Orange, sweet, ext.		x	>95%	Ja	1a
8000-34-8	Clove leaf oil		x	>80%	Nej	1d
90028-67-4	Evernia furfuracea, ext.		x	>95%	Nej	1d
90028-68-5	Evernia prunastri, ext.		x	>95%	Nej	1d
84776-64-7/	Jasmine, Jasminum grandiflorum, ext. /		x	>95%	Nej	1d
90045-94-6/	Jasmine, Jasminum officinale, ext. /		x	>95%	Nej	1d
8022-96-6	Extract Jasmine (oil), Jasmine, Jasminum grandiflorum, ext.		x	ca. 30%	Nej	1d
8006-90-4/	Oils, peppermint (Mentha piperita) /		x	ca. 65%	Nej	2
84082-70-2	Peppermint, ext.		x	ca. 25%	Ja	1??
84696-51-5	Spearmint, ext. (Mentha spicita)		x	>95%	Nej	2
8007-00-9	Balsams, Peru (Myroxylon pereirae)		x	>95%	Nej	1d
84787-70-2/	Sandalwood, ext. /		x	10 af >900	Nej	1d
8006-87-9	Sandalwood oil		x	1	Nej	1d

Appendix 3 Justification for the prioritisation of the substances

The justification for prioritisation of the substances into group 1 or 2 is based on studies identified in the literature search, REACH RD and in the SCCS (2012) opinion.

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
97-88-1	Butyl methacrylate	x	OECD TG 429: EC3 > 2% (1 study).	GPMT OECD TG 406: 80% responding after intradermal induction dose of 5% (1 study). Sensitization in 1/8 non TG.	Unselected dermatitis patients frequency ≥ 1% (0/1 study). Selected dermatitis patients frequency ≥ 2% (3/8 studies). Workplace selected workers frequency ≥ 1% (1/1 study). $N_{(cases)}$ < 100.	1 Based on the high frequency of occupational allergic contact dermatitis among dentists, nail technicians, fibreglass workers etc. due to (meth)acrylates.
100-52-7	Benzaldehyde		OECD TG 429: Not sensitising (1 study).	Not sensitising in 3/3 GPMTs similar to TG.	Selected dermatitis patients frequency ≥ 2% (1/4 studies). $N_{(cases)}$ < 100. Human maximisation test (HMT): No conclusion (1 study).	2 Based on the low frequency and number of cases in human studies and no sensitisation in non-human studies.
5392-40-5	Citral	x	OECD TG 429: EC3 ≤ 2% (2 studies). EC3 > 2% (8 studies).	GPMT similar to OECD TG 406: 60 and 100% responding after intradermal induction dose of ?% (2 studies).	Unselected dermatitis patients frequency ≥ 1% (5/11 studies). Selected dermatitis patients frequency ≥ 2% (6/8	1 Based on the high frequency and number of cases in human studies and sensitisation in non-human

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
					studies). $N_{(cases)}$ > 100. Human Repeat Insult Patch Test (HRIPT): Mixed results (2 studies).	studies. SCCS substance of special concern[d)].
5989-27-5	(R)-p-Mentha-1,8-diene (d-limonene)	x	OECD TG 429: EC3 > 2% (6 studies).	_	Unselected dermatitis patients frequency ≥ 1% (5/5 studies – two with mixed results). Selected dermatitis patients frequency ≥ 2% (6/8 studies). Workplace selected workers frequency ≥ 1% (1/1 studies). Workplace all or randomly selected workers frequency ≥ 0.4% (2/2 studies). $N_{(cases)}$ < 100.	2 Harmonised classification sub-category 1B proposal.
5989-54-8	(S)-p-Mentha-1,8-diene (l-limonene)	x	_	_	Unselected dermatitis patients frequency ≥ 1% (1/1). $N_{(cases)}$ < 100.	2 Read-across to d-limonene.
92585-24-5	2-methyl-4-phenylpentanol	x	OECD TG 429: Not sensitising (1 study).	Buehler test non TG: ambiguous.	Selected dermatitis patients frequency ≥ 2% (0/2 studies).	2 Based on the low frequency and number of cases in

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
					$N_{(cases)}$ < 100.	human studies and no sensitisation in non-human studies.
131766-73-9	A mixture of: trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran	x	OECD TG 429: Not sensitising (1 study).	GPMT non TG: 70% responding after intradermal induction dose of ?% (1 study).	_	2 Based on limited data.
8006-64-2	Turpentine oil	x	_	GPMT non TG: Sensitising.	General population studies frequency ≥ 0.2% (1/1 study). Unselected dermatitis patients frequency ≥ 1% (8/10 studies). Selected dermatitis patients frequency ≥ 2% (1/1 study (prisoners)). Workplace selected workers frequency ≥ 1% (1/1 studies). $N_{(cases)}$ > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern[d)].
32388-55-9	[3R-(3α,3aβ,7β,8aα)]-1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-		OECD TG 429: EC3 > 2% (1 study).	_	Unselected dermatitis patients frequency ≥ 1% (0/3 studies). $N_{(cases)}$ < 100.	2 Based on the low frequency and number of cases in human studies and no

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
	tetramethyl-1H-3a,7-methanoazulen-5-yl)ethan-1-one (Acetylcedrene)					sensitisation in non-human studies.
4180-23-8	(E)-Anethole (trans-anethole)		_	GPMT OECD TG 406(?): 100% responding after intradermal induction dose of 2% (1 study).	Two case stories. $N_{(cases)}$ < 100.	2 Based on the low frequency and number of cases in human studies and one non-human study not warranting sub-category 1A.
105-13-5	4-Methoxybenzyl alcohol (Anise alcohol)		OECD TG 429: EC3 > 2% (1 study).	_	Unselected dermatitis patients frequency ≥ 1% (0/5 studies). $N_{(cases)}$ < 100. Human maximisation test (HMT): Not sensitising (1 study).	2 Based on the low frequency and number of cases in human studies and no sensitisation in non-human studies.
118-58-1	Benzyl salicylate		OECD TG 429: EC3 > 2% (1 study).	_	Unselected dermatitis patients frequency ≥ 1% (1/9 studies). Selected dermatitis patients frequency ≥ 2% (2/2 studies). $N_{(cases)}$ < 100.	2 Based on the low frequency and number of cases in human studies and no sensitisation in non-human studies.
80-54-6	2-(4-tert-		OECD TG 429:	GPMT OECD TG 406: Not	Unselected dermatitis	2

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
	Butylbenzyl)propionaldehyde (Butylphenyl methylproprional)		EC3 > 2% (6 studies).	sensitising (2 studies). GPMT non TG: Strong sensitiser (1 study).	patients frequency ≥ 1% (0/12 studies). Selected dermatitis patients frequency ≥ 2% (1/1 studies). $N_{(cases)}$ < 100. Human maximisation test (HMT): Mixed results (1 study).	Based on the low frequency and number of cases in human studies and equivocal results from non-human studies.
99-49-0 / 6485-40-1 / 2244-26-8	d-p-Mentha-1(6),8-dien-2-one (Carvone) / l-p-Mentha-1(6),8-dien-2-one (Carvone) / (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one (Carvone)		OECD TG 429: EC3 > 2% (3 studies).	_	Selected dermatitis patients frequency ≥ 2% (1/1 studies). $N_{(cases)}$ < 100.	2 Harmonised classification category 1 proposal.
104-55-2	Cinnamaldehyde		OECD TG 429: EC3 ≤ 2% (19 studies) EC3 > 2% (1 study).	GPMT non TG: Sensitising (4 studies).	Unselected dermatitis patients frequency ≥ 1% (12/12 studies). Selected dermatitis patients frequency ≥ 2% (6/7 studies). Workplace all or randomly selected workers frequency ≥	1 Based on the high frequency and number of cases in human studies and sensitisation in non-human studies. SCCS substance of special

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
					0.4% (1/1 studies). $N_{(cases)}$ > 100.	concern[d)].
104-54-1	Cinnamyl alcohol		OECD TG 429: EC3 > 2% (5 studies).	GPMT OECD TG 406: 30% responding after intradermal induction dose of ?% (1 study). GPMT non TG: Not sensitising (1 study). Buehler OECD TG 406: Not sensitising after topical induction dose of ?% (1 study).	Unselected dermatitis patients frequency ≥ 1% (6/9 studies). Selected dermatitis patients frequency ≥ 2% (6/7 studies). $N_{(cases)}$ > 100. Human maximisation test (HMT): Not sensitising (1 study). Human Repeat Insult Patch Test (HRIPT): Sensitising (1 study).	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern[d)].
106-22-9 / 1117-61-9 / 7540-51-4	Citronellol / (R)-3,7-dimethyloct-6-en-1-ol / (-)-3,7-dimethyloct-6-en-1-ol		OECD TG 429: EC3 > 2% (1 study).	GPMT non TG: Not sensitising after topical induction dose of 6% (1 study). Buehler OECD TG 406: Not sensitising (1 study).	Unselected dermatitis patients frequency ≥ 1% (2/8 studies). Selected dermatitis patients frequency ≥ 2% (3/4 studies). $N_{(cases)}$ < 100. Human maximisation test (HMT): Not sensitising (1 study). Human Repeat Insult Patch	2 Based on the low frequency and number of cases in human studies and no sensitisation in non-human studies.

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
					Test (HRIPT): Not sensitising (1 study).	
91-64-5	Coumarin		OECD TG 429: EC3 > 2% (1 study).	GPMT non TG: Not sensitising (1 study).	Unselected dermatitis patients frequency ≥ 1% (1/8 studies). Selected dermatitis patients frequency ≥ 2% (3/7 studies). $N_{(cases)}$ < 100.	1 SCCS substance of special concern[d)].
57378-68-4	1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one (delta-DAMASCONE)		OECD TG 429(?): EC3 > 2% (2 studies).	_	Human Repeat Insult Patch Test (HRIPT): Several positive and one negative study according to SCCS (2012). $N_{(cases)}$ < 100.	2 Based on limited data.
97-53-0	Eugenol		OECD TG 429: EC3 > 2% (10 studies).	GPMT similar to OECD TG 406: 20% responding after intradermal induction dose of 5% (1 study).	Unselected dermatitis patients frequency ≥ 1% (7/11 studies). Selected dermatitis patients frequency ≥ 2% (6/6 studies). $N_{(cases)}$ > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern[d)].
4602-84-0	Farnesol		OECD TG 429: EC3 > 2% (2 studies).	_	Unselected dermatitis patients frequency ≥ 1% (1/7 studies – with a frequency of	1 Based on very high frequencies in a limited

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
					13%). Selected dermatitis patients frequency ≥ 2% (2/4 studies – with frequencies of 10 and 12%). $N_{(cases)}$ > 100.	number of studies and the number of cases in human studies. SCCS substance of special concern[d)].
106-24-1	Geraniol		OECD TG 429: EC3 > 2% (5 studies).	GPMT non TG: Not sensitising in four studies. Sensitising in three studies.	Unselected dermatitis patients frequency ≥ 1% (6/16 studies). Selected dermatitis patients frequency ≥ 2% (3/7 studies). $N_{(cases)}$ > 100. Human maximisation test (HMT): Not sensitising (2 studies).	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern[d)].
31906-04-4 / 51414-25-6	4-(4-Hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde (Hydroxyisohexyl 3-cyclohexene carboxaldehyde, HICC)	RAC 1A	OECD TG 429: EC3 > 2% (1 study).	_	Unselected dermatitis patients frequency ≥ 1% (17/19 studies). Selected dermatitis patients frequency ≥ 2% (3/3 studies). Workplace selected workers frequency ≥ 1% (0/1 study). $N_{(cases)}$ > 100.	Harmonised classification sub-category 1A proposal.
107-75-5	7-hydroxycitronellal		OECD TG 429: EC3 > 2% (7 studies).	GPMT non TG: 60% responding after	Unselected dermatitis patients frequency ≥ 1% (7/8	1 Based on the high frequency

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
				intradermal induction dose of 0.5% (1 study). Buehler non TG: ambiguous.	studies). Selected dermatitis patients frequency ≥ 2% (4/6 studies). $N_{(cases)}$ > 100.	and number of cases in human studies. SCCS substance of special concern[d)].
97-54-1	Isoeugenol	RAC 1A	OECD TG 429: EC3 ≤ 2% (17 studies) EC3 > 2% (6 studies).	_	Unselected dermatitis patients frequency ≥ 1% (9/9 studies). Selected dermatitis patients frequency ≥ 2% (8/8 studies). Workplace selected workers frequency ≥ 1% (0/1 study). $N_{(cases)}$ > 100.	Harmonised classification sub-category 1A proposal.
138-86-3	Limonene	x	_	_	Unselected dermatitis patients frequency ≥ 1% (3/10 studies). $N_{(cases)}$ > 100 (mainly due to hydroperoxides of limonene).	1 SCCS substance of special concern[d)].
111-12-6	Methyl oct-2-ynoate		OECD TG 429: EC3 ≤ 2% (2 studies).	_	Unselected dermatitis patients frequency ≥ 1% (2/6 studies). Selected dermatitis patients frequency ≥ 2% (0/1 studies). $N_{(cases)}$ < 100.	1 Based on the very low EC3 value in LLNAs.

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
80-56-8 / 127-91-3	Pin-2(3)-ene / Pin-2(10)-ene		OECD TG 429: EC3 > 2% (1 study). Test substance beta-pinene (CAS 127-91-2).	_	Selected dermatitis patients frequency ≥ 2% (2/2 studies). Workplace selected workers frequency ≥ 1% (1/1 studies). $N_{(cases)}$ < 100.	2 Based on limited data.
586-62-9	p-Mentha-1,4(8)-diene (Terpinolene)		OECD TG 429: EC3 > 2% (1 study).	_	Selected dermatitis patients frequency ≥ 2% (1/1 study). $N_{(cases)}$ < 100.	2 Based on limited data.
83863-30-3 / 8006-81-3	Ylang ylang ext. / Ylang ylang oil		OECD TG 429: EC3 > 2% (1 study).	_	Unselected dermatitis patients frequency ≥ 1% (9/10 studies). Selected dermatitis patients frequency ≥ 2% (6/7 studies). $N_{(cases)}$ > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern[d)].
8007-80-5 / 84649-98-9	*Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext.		_	_	Unselected dermatitis patients frequency ≥ 1% (1/1 study). Selected dermatitis patients frequency ≥ 2% (1/1 study). $N_{(cases)}$ < 100.	1 Based on the high content of cinnamaldehyde, cinnamyl alcohol and/or eugenol (all SCCS substances of special concern[d)]).
8016-38-4 / 72968-50-4	Neroli Oil / Orange, sour, ext.		_	_	Unselected dermatitis patients frequency ≥ 1% (1/2 studies). Selected dermatitis patients	2 Based on limited data.

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
					frequency ≥ 2% (2/2 studies). $N_{(cases)}$ < 100. Human maximisation test (HMT): Not sensitising (1 study).	
84929-31-7	Lemon, ext.		_	_	Unselected dermatitis patients frequency ≥ 1% (0/1 studies). Selected dermatitis patients frequency ≥ 2% (3/5 studies). $N_{(cases)}$ < 100.	2 Based on limited data.
97766-30-8 / 8028-48-6	Orange, sweet, Valencia, ext. / Orange, sweet, ext.		_	_	Unselected dermatitis patients frequency ≥ 1% (0/3 studies). Selected dermatitis patients frequency ≥ 2% (3/4 studies). $N_{(cases)}$ < 100.	2 Based on limited data.
8000-34-8	Clove leaf oil		_	_	Unselected dermatitis patients frequency ≥ 1% (2/2 studies). Selected dermatitis patients frequency ≥ 2% (3/5 studies). $N_{(cases)}$ > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern[d)].
90028-67-4	*Evernia furfuracea,*		OECD TG 429:	_	Unselected dermatitis	1

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
	ext.		EC3 > 2% (2 studies).		patients frequency ≥ 1% (5/5 studies). Selected dermatitis patients frequency ≥ 2% (2/2 studies). $N_{(cases)}$ > 100.	Based on the high frequency and number of cases in human studies. SCCS substance of special concern[d)].
90028-68-5	*Evernia prunastri,* ext.		OECD TG 429: EC3 > 2% (1 study).	_	Unselected dermatitis patients frequency ≥ 1% (10/10 studies). Selected dermatitis patients frequency ≥ 2% (6/6 studies). Workplace selected workers frequency ≥ 1% (0/1 studies). $N_{(cases)}$ > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern[d)].
84776-64-7 / 90045-94-6 / 8022-96-6	Jasmine, *Jasminum grandiflorum*, ext. / Jasmine, *Jasminum officinale*, ext. / Extract Jasmine (oil), Jasmine, *Jasminum grandiflorum*, ext.		OECD TG 429: EC3 > 2% (1 study).	_	Unselected dermatitis patients frequency ≥ 1% (5/7 studies). Selected dermatitis patients frequency ≥ 2% (7/11 studies). $N_{(cases)}$ > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern[d)].
8006-90-4 / 84082-70-2	Oils, peppermint (*Mentha piperita*) / Peppermint, ext.		_	GPMT non TG: 10-40% responding after intradermal induction dose of 0.25 -0.5% (4 studies).	Unselected dermatitis patients frequency ≥ 1% (0/3 studies). Selected dermatitis patients frequency ≥ 2% (3/5 studies).	2 Based on the low frequency and number of cases in human studies, and mixed results in the non-TG-non-

CAS RN	Name	Harm class	LLNA[a)]	GPMT/Buehler[b)]	Human diagnostic patch test data[c)]	Prioritisation
					$N_{(cases)}$ < 100.	human studies.
84696-51-5	Spearmint, ext. (*Mentha spicita*)		_	_	Unselected dermatitis patients frequency ≥ 1% (0/1 study). Selected dermatitis patients frequency ≥ 2% (1/1 study). $N_{(cases)}$ < 100.	2 Based on the low frequency and number of cases in human studies and limited data.
8007-00-9	Balsams, Peru (*Myroxylon pereirae*)		OECD TG 429: EC3 > 2% (1 study).	_	General population studies frequency ≥ 0.2% (1/1 study – dependent on gender and year). Unselected dermatitis patients frequency ≥ 1% (17/17 studies). Selected dermatitis patients frequency ≥ 2% (2/3 studies). Workplace selected workers frequency ≥ 1% (0/1 studies). $N_{(cases)}$ > 100.	1 Based on the high frequency and number of cases in human studies and borderline result from the LLNA. SCCS substance of special concern[d)].
84787-70-2 / 8006-87-9	Sandalwood, ext. / Sandalwood oil		_	_	Unselected dermatitis patients frequency ≥ 1% (4/7 studies). Selected dermatitis patients frequency ≥ 2% (5/7 studies). $N_{(cases)}$ < 100.	1 Based on the high frequency of cases in human studies. SCCS substance of special concern[d)].

LLNA: Local Lymph Node Assay; **GPMT OECD TG**: Guinea Pig Maximisation Test OECD Test Guideline. **SCCS (2012)**: Scientific Committee on Consumer Safety, Opinion on Fragrance allergens in cosmetic products (2012).

a) Criteria for LLNA sub-categorisation 1A: EC3 value ≤ 2%.
Criteria for LLNA sub-categorisation 1B: EC3 value > 2%.

b) Criteria for GPMT sub-categorisation 1A: ≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0.1% to ≤ 1% intradermal induction dose.
Criteria for GPMT sub-categorisation 1B: ≥ 30% to < 60% responding at > 0.1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose.
Criteria for Buehler assay sub-categorisation 1A: ≥ 15% responding at ≤ 0.2% topical induction dose or ≥ 60% responding at > 0.2% to ≤ 20% topical induction dose.
Criteria for Buehler assay sub-categorisation 1B: ≥ 15% to < 60% responding at > 0.2% to ≤ 20% topical induction dose or ≥ 15% responding at > 20% topical induction dose.

c) Human diagnostic patch test data: General population studies: ≥ 0.2% = high frequency; < 0.2% = low/moderate frequency.
Human diagnostic patch test data: Dermatitis patients (unselected, consecutive): ≥ 1% = high frequency; < 1% = low/moderate frequency.
Human diagnostic patch test data: Selected dermatitis patients: ≥ 2% = high frequency; < 2% = low/moderate frequency.
Human diagnostic patch test data: Work place studies: selected workers with known exposure or dermatitis: ≥ 1% = high frequency; < 1% = low/moderate frequency.
Human diagnostic patch test data: Work place studies: all or randomly selected workers: ≥ 0.4% = high frequency; < 0.4% = low/moderate frequency.
Human diagnostic patch test data: Number of published cases ($N_{(cases)}$) ≥ 100 cases = high frequency; < 100 cases = low/moderate frequency.

d) Substances of special concern according to SCCS (2012) defined as more than 100 cases from human studies.

Appendix 4 Citral CAS RN 5392-40-5

Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial, i.e. the aldehyde of geraniol, which is a hapten by itself with a moderate sensitisation potency). Geranial and neral have been identified as metabolites of geraniol and have been both been identified as secondary oxidation products when geraniol autoxidises (SCCS, 2012).

Non-human information

Table 1 summarises relevant animal studies with citral i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with citral.

Method	Results	Remarks/Comments	Reference
LLNA (OECD TG 429): 5, 10 and 25% citral. Vehicle: Acetone:Olive oil (AOO).	Citral was shown to have an EC3 value of 12.6%.		Basketter (2012).
LLNA: LLNA:BrdU-FCM. 5, 10 and 25% citral. Vehicle: AOO.	Citral was shown to have an EC3 value of 9.2%.	Citral was classified as a moderate skin sensitizer by Jung (2012).	Jung (2012).
LLNA: 0.4, 2, 4, 8 and 20% citral. Vehicle: 1:3 ethanol:diethyl phthalate (EtOH:DEP).	Citral was shown to have an EC3 value of 1.2% (0.079 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2004b) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.1% α-tocopherol in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 1.5% (0.099 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003k) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.3% antioxidant mix (equal parts butylated hydroxytoluene (BHT), tocopherol and eugenol) in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 2.1% (0.14 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003l) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.1% Trolox C in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 3.7% (0.24 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003m) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 4.6% (0.3 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003n) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral.	Citral was shown to have an EC3 value of	According to SCCS (2012) there were no	Unpublished summary report by RIFM 2009

Method	Results	Remarks/Comments	Reference
Vehicle: 0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP.	4.6% (0.3 M).	reported deviations from OECD TG 429.	(RIFM 2003o) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 5.3% (0.35 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003p) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.1% Trolox C in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 5.8% (0.38 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003q) cited from SCCS (2012).
LLNA: 2.5, 5, 10, 25 and 50% citral. Vehicle: 1:3 EtOH:DEP.	Citral was shown to have an EC3 value of 6.3% (0.41 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003r) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.1% α-tocopherol in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 6.8% (0.44 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003s) cited from SCCS (2012).
LLNA: (concentration not reported) citral. Vehicle: AOO.	Citral was shown to have an EC3 value of 13%.		Basketter et al., 2002a cited from Lalko and Api (2008).
LLNA: 5, 10 and 25% citral. Vehicle: 4:1 AOO.	Citral was shown to have an EC3 value between 7 and 15%.	According to REACH- RD (2015c) the study was reliable with restrictions (reliability 2) and performed equivalent or similar to OECD TG 429.	Basketter and Scholes 1992 cited from REACH-RD (2015c).
GPMT: Intradermal induction 0.1% citral; Topical induction 5% citral; Challenge dose 0.5%. Vehicle not reported.	6/10 (60%) animals were positive.	According to REACH- RD (2015d) the study is reliable with restrictions (reliability 2) and performed equivalent or similar to OECD TG 406.	Basketter and Allenby 1991; Basketter et al., 1991 and Basketter & Scholes, 1992 cited from REACH-RD (2015d).
GPMT: Intradermal induction 10% citral; Topical induction 10% citral; Challenge dose 10%. Vehicle not reported.	Sensitization observed.	No further information is available from Lalko and Api (2008).	Ishihara et al., 1986a cited from Lalko and Api (2008).
GPMT: Intradermal induction 0.4% citral;	Sensitization observed.	No further information is available from Lalko and Api (2008).	Goodwin and Johnson 1985 cited from Lalko and Api (2008).

Method	Results	Remarks/Comments	Reference
Topical induction 1% citral; Challenge dose 0.25%. Vehicle not reported.			
GPMT: Intradermal induction 25% citral in paraffin oil DAB7 or Freund's adjuvant/aqua dest. (1:1); Topical induction 25% citral in paraffin oil DAB7; Challenge doses 10, 5 and 5% citral in paraffin oil DAB7.	100% positive reactions.	According to REACH-RD (2015e) the study is reliable with restrictions (reliability 2) and performed equivalent or similar to OECD TG 406.	Study report 1978-11-15 cited from REACH-RD (2015e).
GPMT: Intradermal induction 25% citral in paraffin oil DAB7 or Freund's adjuvant/aqua dest. (1:1); Topical induction 25% citral in paraffin oil DAB7; Challenge doses 10, 5 and 5% citral in paraffin oil DAB7.	100% positive reactions except for after 144 hours after a 5% rechallenge where it was 60%.	According to REACH-RD (2015f) the study is reliable with restrictions (reliability 2) and performed equivalent or similar to OECD TG 406.	Study report 1978-11-12 cited from REACH-RD (2015f).
GPMT: Intradermal induction 5% citral; Topical induction 25% citral in pet.; Challenge dose: subirritant. Vehicle not reported.	Sensitization observed.	No further information is available from Lalko and Api (2008).	Klecak et al., 1977 cited from Lalko and Api (2008).
Buehler: Induction concentration 20% citral in petrolatum (pet.); Challenge dose 20% in pet.	Sensitization observed.	No further information is available from Lalko and Api (2008).	Unpublished report by RIFM 1973 cited from Lalko and Api (2008).

A total of 14 LLNAs, 6 GPMTs and 1 Buehler test are summarised in table 1. The reported EC3 values for citral range between 1.2% (vehicle: 1:3 ethanol:diethyl phthalate) and 15% (vehicle: Acetone:Olive oil 4:1). In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the other GPMTs 60% of the animals responded after an intradermal induction dose of 0.1% while 60-100% of the animals responded after an intradermal induction dose of 25% citral. Sensitisation was also observed but not quanitfied (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral.

No relevant *in vitro* studies on citral (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on citral involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with citral.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 324 selected patients patch tested with citral 2% in petrolatum (pet.).	42/324 (13%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Prospective study of 655 consecutive patients patch tested with citral 3.5% in pet.	6/655 (0.92%) patients were positive.	A prospective study of patch test data at the Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall and Brared Christensson (2014).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with citral 2% in pet.	20/1951 (1%, 95% CI: 0.6-1.4%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Prospective study of 1055 consecutive patients patch tested with citral 1.5% in pet.	7/1055 (0.66%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2006-2010).	Hagvall et al. (2012).
Patch test: Prospective study of 565 selected patients patch tested with citral 2% in vaseline.	19/565 (3.4%) patients were positive.	A prospective study on patch test data from multicentre study, Hungary (2009-2010).	Ponyai et al. (2012).
Patch test: Retrospective study of 205 selected patients tested with citral 2% in pet.	23/205 (11.2%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study	9/100 (9%, 95% CI: 4.2-16.4%)	Single-centre, double-blind prospective	Nagtegaal et al. (2012).

Method	Results	Remarks/Comments	Reference
of 100 selected patients with contact allergy patch tested with citral 2% in pet.	patients were positive.	experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	
Patch test: Retrospective study of 1502 consecutive eczema patients patch tested with citral 2% in pet.	5/1502 (0.3 %) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011, 2012).
Patch test: Prospective study of 30 selected patients (with positive reactions to ascaridole 1 and 5 %) patch tested with citral 2% in pet.	2/30 (7%) patients were positive.	A prospective study of patch test data at the Department of Dermatology, University Medical Centre Groningen, The Netherlands (2008-2011).	Bakker et al. (2011).
Patch test: Retrospective study of 86 selected patients patch tested with citral 2% in pet.	2/86 (2.3%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 367 selected fragrance mix (FM) II positive patients patch tested with citral 2% in pet.	59/367 (16.1%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2005-2008).	Krautheim et al. (2010).
Patch test: Prospective study of 320 selected eczema patients patch tested with citral 2% in pet.	2/320 (0.6%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 2021 consecutive	12/2021 (0.6%, 95% CI: 0.3-1.1%) patients were	A retrospective study on patch test data from multicentre	Schnuch et al. (2007).

Method	Results	Remarks/Comments	Reference
patients patch tested with citral 2% in pet.	positive.	project IVDK (2003-2004).	
Patch test: Prospective study of 422 selected patients with suspected contact allergy patch tested with citral 2% in pet.	5/422 (1.2%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Prospective study of 1701 consecutive patients patch tested with citral 2% in pet.	12/1701 (0.7%) patients were positive.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al., 2005 cited from SCCS (2012).
Patch test: Study of 1701 patients patch tested with citral 1%. Vehicle not reported.	6/1701 (0.35%) patients were positive.		Frosch et al., 2004 and 2005 cited from Lalko and Api (2008).
Patch test: Study of 586 consecutive patients patch tested with citral 2% in pet.	28/586 (4.8%) patients were positive.	According to SCCS 2012 irritant reactions were observed in 82/586 (14%).	Heydorn et al., 2003 cited from SCCS (2012).
Patch test: Prospective study of 1855 consecutive patients patch tested with citral 2% in pet.	21/1855 (1.1%) patients were positive.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al. (2002a).
Patch test: Prospective study of 1825 consecutive patients patch tested with citral 2% in pet.	19/1825 (1%) patients were positive.	Multicenter study of patch test data in The Netherlands (1998-1999).	de Groot et al. (2000).
Patch test: Study of 192 patients patch tested with citral 1% in pet.	8/192 (4.2%) patients were positive.		Frosch et al., 1995 cited from Lalko and Api (2008).
Patch test: Study of	1/192 (0.5%)	According to Lalko and	Frosch et al.,

Method	Results	Remarks/Comments	Reference
192 patients patch tested with citral 0.1%. Vehicle not reported.	patients were positive.	Api (2008) the reaction was questionable.	1995 cited from Lalko and Api (2008).
Patch test: Study of 78 selected patients sensitive to FM patch tested with citral 2% in pet.	13/78 (16.7%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Study of 310 cosmetic dermatitis patients; 408 non-cosmetic patients and 122 control subjects patch tested with citral in 5%. Vehicle not reported.	8/310 (2.6%) cosmetic dermatitis patients; 9/408 (2.2%) non-cosmetic patients and 1/122 (0.8%) control subjects were positive.		Itoh et al., 1986 and 1988 and Nishimura et al., 1984 cited from Lalko and Api (2008).
Patch test: Study of 240 cosmetic dermatitis patients; 584 non-cosmetic patients and 105 control subjects patch tested with citral 2%. Vehicle not reported.	1/240 (0.4%) cosmetic dermatitis patients; 2/584 (0.3%) non-cosmetic patients and 0/105 (0%) control subjects were positive.		Itoh et al., 1986 and 1988 and Nishimura et al., 1984 cited from Lalko and Api (2008).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with citral 2% in pet.	5/182 (2.6%) patients were positive.		Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 228 patients patch tested with citral 1% in pet.	4/228 (1.7%) patients were positive.	North American Contact Dermatitis Research Group (1973-1974).	Michell et al., 1982 cited from SCCNFP (1999).
Patch test: Study of 155 cosmetic dermatitis patients and 159 eczema/dermatitis patients patch tested with citral 5% in pet.	4/155 (2.6%) cosmetic dermatitis patients and 5/159 (3.1%) eczema/dermatitis patients were positive.	According to Lalko and Api (2008) a total of 48 control subjects were also tested with citral (5% pet) with no positive reactions.	Ishihara et al., 1981 cited from Lalko and Api (2008).
Patch test:	1/4 (25%) patients		Malten 1979

Method	Results	Remarks/Comments	Reference
Occupational study of 4 bakers with hand eczema patch tested with citral 0.5% in pet.	were positive.		cited from SCCNFP (1999).

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with citral.

Table 3. HRIPT and HMT studies with citral adapted from Lalko and Api (2008).

Method	Results	Remarks/Comments	Reference
HRIPT: Citral concentration: 1.2%. Vehicle: 3:1 ethanol:diethyl phthalate (EtOH:DEP) with 0.2% tocopherol.	2/101 (2%) tests were positive.	According to REACH-RD (2015g) the study is reliable with restrictions (reliability 2) and performed according to HRL Protocol #100RIFM and HRL Standard Operating Procedures.	Study report 2004-09-11 cited from REACH-RD (2015g).
HRIPT: Citral concentration: 1.2% (1400 µg/cm^2). Vehicle: 1:3 EtOH:DEP.	0/101 (0%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 2004b cited from Lalko and Api (2008).
HRIPT: Citral concentration: 4-8%. Vehicle: not reported.	19/40 (48%) tests were positive.		Opdyke 1979 cited from SCCFNP (1999).
HRIPT: Citral concentration: 4% (1240 µg/cm^2). Vehicle: petrolatum (pet.).	0/50 (0%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1971a cited from Lalko and Api (2008).
HRIPT: Citral concentration: 1% (775 µg/cm^2). Vehicle: alcohol SDA39C.	0/40 (0%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1965 cited from Lalko and Api (2008).

Method	Results	Remarks/Comments	Reference
HRIPT: Citral concentration: 5% (3876 µg/cm^2). Vehicle: alcohol SDA39C.	5/8 (62.5%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1964a cited from Lalko and Api (2008).
HRIPT: Citral concentration: 0.5% (388 µg/cm^2). Vehicle: alcohol SDA39C.	0/41 (0%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1964b cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm^2). Vehicle: pet.	16/25 (64%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974a cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm^2). Vehicle: pet.	14/25 (56%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974c cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm^2). Vehicle: pet.	12/25 (48%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974c cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm^2). Vehicle: pet.	8/25 (32%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974c cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm^2). Vehicle: pet.	11/24 (45.8%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974d cited from Lalko and Api (2008).
HMT: Citral	0/25 (0%) tests were positive.	No further information available from Lalko	Unpublished report from RIFM

Method	Results	Remarks/Comments	Reference
concentration: 5% (3448 µg/cm^2). Vehicle: butylene glycol.		and Api (2008).	1974e cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm^2) Vehicle: pet.	3/25 (12%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1972b cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm^2). Vehicle: pet.	3/25 (12%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1972c cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm^2). Vehicle: pet.	5/25 (20%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1972c cited from Lalko and Api (2008).
HMT: Citral concentration: 2% (1379 µg/cm^2). Vehicle: pet.	2/24 (8.3%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1972d cited from Lalko and Api (2008).
HMT: Citral concentration: 8% (5517 µg/cm^2) Vehicle: pet.	8/24 (33.3%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1971b cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm^2). Vehicle: pet.	9/25 (36%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1971c cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm^2). Vehicle: pet.	4/25 (16%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1971c cited from Lalko and Api (2008).
HMT:	5/25 (20%)	No further information	Unpublished

Method	Results	Remarks/Comments	Reference
Citral concentration: 4% (2759 µg/cm^2). Vehicle: pet.	tests were positive.	available from Lalko and Api (2008).	report from RIFM 1971c cited from Lalko and Api (2008).

HRIPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Test.

Case studies

Table 4 summarises case reports with ACD where citral has been found to be among the causative agents.

Table 4. Case studies with citral.

Method	Results	Remarks/Comments	Reference
Patch test: A 30-year old female patient with recurrent allergic contact cheilitis was patch tested with fragrance mix (FM) II and citral.	Strong positive reaction to FM II and citral. The cheilitis was attributed to a lip salve containing citral.	Case study (year not reported).	Hindle et al., 2007 cited from SCCS (2012).
Patch test: Over a period of 2 years a total of 9 beauticians with bilateral hand dermatitis were patch tested with the British baseline series, FM I and II, cosmetics and own products.	Positive reactions in 5 of the 9 beauticians were observed.	Multible case study (UK).	De Mozzi and Johnston (2014).

A total of 30 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies with citral are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0.3 and 16.7% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0.3 and 4.8% (8 studies) and for selected dermatitis patients positive reactions range between 0.3 and 16.7% (22 studies). The total number of published cases is > 300. Sensitisation was reported in 3/7 HRIPT studies at a citral concentration of 3876 µg/cm² (5%). In the HMT studies 13/14 studies with citral showed a positive result after 1379 µg/cm². Based on these data the Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT[4] (induction) of 1400 µg/cm² and a LOEL-HRIPT/HMT[5] (induction) of 3876 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 1400 µg/cm² was established for citral by the RIFM Expert Panel (IFRA, 2013a).

[4] NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test

[5] LOEL-HRIPT/HMT: Lowest Observed Effect Level- Human Repeat Insult Patch Test/Human Maximisation test

Citral is a ”top 100” substance and has a harmonised classification for skin sensitisation (SCCS, 2012; DK-EPA, 2015).

According to SCCS (2012) citral is used in volumes greater than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 11.6% of a total of 516 consumer products; 25% of a total of 300 consumer products; ca. 12% of 3000 products and 8.2% of children's cosmetics were labelled to contain citral (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 26.1% of 88 tested deodorants were labelled to contain citral and the fragrance was detected in 44% (range: 39-554 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for citral in different IFRA QRA product categories reported by IFRA (2013a and 2015) are shown in table 5.

Table 5. The IFRA standard limits for citral in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.04%
Category 2	Deodorants/antiperspirants	0.05%
Category 3	Hydroalcoholics for shaved skin	0.2%
Category 4	Hydroalcoholics for unshaved skin	0.6%
Category 5	Hand cream	0.3%
Category 6	Mouthwash	1.0%
Category 7	Intimate wipes	0.1%
Category 8	Hair styling aids	1.4%
Category 9	Rinse-off hair conditioners	5.0%*
Category 10	Hard surface cleaners	2.5%*
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.
*Maximum pragmatic level.

Citral is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 30 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies were identified for citral. The positive patch test frequencies from all of the reported patch test population studies vary between 0.3 and 16.7% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.3 and 4.8% (8 studies) and in studies with selected dermatitis patients positive reactions range between 0.3 and 16.7% (22 studies). The total number of published cases is > 300. Sensitisation was reported in 3/7 HRIPT studies after exposure to 3876 µg/cm² (5%) and in 13/14 HMT studies after exposure to 1379 µg/cm².

Non-human data
A total of 12 LLNAs, 6 GPMTs and 1 Buehler test were identified testing skin sensitising effects of citral. The reported EC3 values for citral ranged between 1.2% and 15% in different vehicles. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the other GPMTs sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.1% and in 60-100% of the animals after an intradermal induction dose of 25% citral. Sensitisation was also observed but not quantified (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral.

No relevant *in vitro* studies on citral (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure
According to data from IFRA (2013a) the exposure of citral when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria
For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 4.8% with 3/8 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.3 and 16.7% with 14 out of 22 studies reporting frequencies higher than 2%. In addition to this there are more than 300 published cases of positive patch test reactions to citral. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that citral causes a *high frequency* of occurrence of skin sensitisation based on patch test data from a minority of unselected dermatitis patient studies and a majority of selected dermatitis patient studies and the number of published cases.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 14 LLNAs EC3 values between 1.2 and 15% were reported for citral. Two out of the 14 LLNAs reported an EC3 value <2%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, these two studies indicate classification of citral in sub-category 1A. However, in the other 12 LLNAs, the EC3 value was >2% with only one of these 12 LLNAs reporting an EC3 value (2.1%) borderline to the cut-off criteria for classification in sub-category 1A or 1B indicating classification of citral in sub-category 1B. Based on a weight of evidence for the LLNAs, classification of citral in sub-category 1B seems justified.

In 1/6 of the GPMTs sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.1%. According to the CLP criteria a positive response ≥ 30% of the animals responding at ≤ 0.1% intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of citral into sub-category 1A. In 2/6 GPMTs sensitisation was observed in 60-100% of the animals after an intradermal induction dose of 25% citral. According to the CLP criteria a positive response ≥ 30% responding at > 1% intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.); thus, these two studies indicate classification of citral in sub-category 1B. In 3/6 the GPMTs sensitisation was reported to be observed but not quantified (i.e. number of animals affected) with intradermal induction doses of 0.4, 5 and 10% citral; therefore, these GPMTs cannot be compared with the classification criteria.

Sensitisation was also reported to be observed but not quantified (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral; therefore, this study cannot be compared with the classification criteria.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. The results from the animal studies are equivocal, mainly indicating classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is thus warranted for citral.

Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure, a classification of citral as a skin sensitiser in sub-category 1A is justified.

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Appendix 5 Cinnamaldehyde CAS RN 104-55-2

Non-human information

Table 1 summarises relevant animal studies with cinnamaldehyde i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with cinnamaldehyde.

Method	Results	Remarks/Comments	Reference
LLNA: BrdU-ELISA *in vivo* and *ex vivo* BrdU. 1, 5 and 10% cinnamaldehyde.	Cinnamaldehyde was shown to have an EC2 value of 6.1% in the BrdU-ELISA *in vivo* and an EC2 value of 6.9 in the *ex vivo* BrdU.	Cinnamaldehyde was classified as positive for skin sensitisation by Williams et al. (2015).	Williams et al. (2015).
LLNA: 0.1, 0.99, 3.3, 9.9 and 19.8% cinnamaldehyde.	Cinnamaldehyde was shown to have an EC3 value of 0.75% (57 mM).	Cinnamaldehyde was classified as a strong skin sensitizer by Niklasson et al. (2013).	Niklasson et al. (2013).
LLNA: *Ex vivo* BrdU. 0.5, 1, 5 and 10% cinnamaldehyde. Vehicle: Acetone: Olive oil (AOO).	Cinnamaldehyde was shown to have an EC3 value of 1.91%.	Cinnamaldehyde was classified as a moderate skin sensitizer by Ulker et al. (2013).	Ulker et al. (2013).
LLNA: LLNA:BrdU-ELISA. 1, 3 and 10%. Vehicle: AOO.	Cinnamaldehyde was shown to have an EC2 value of 2.2%.	Cinnamaldehyde was classified as positive for skin sensitisation by Kojima (2011).	Kojima (2011).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 3:1 ethanol:diethyl phthalate (EtOH:DEP).	Cinnamaldehyde was shown to have an EC3 value of 0.2% (0.015 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003a) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.1% α-tocopherol in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.2% (0.015 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003b) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 2% α-	Cinnamaldehyde was shown to have an EC3 value of 0.6%	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM

Method	Results	Remarks/Comments	Reference
tocopherol in 3:1 EtOH:DEP.	(0.045 M).		2003c) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.3% antioxidant mix (equal parts Butylated hydroxytoluene (BHT), tocopherol and eugenol) in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.7% (0.053 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003d) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.1% Trolox C in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.7% (0.053 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003e) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 2% α-tocopherol in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.8% (0.06 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003f) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.9% (0.068 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003g) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.1% α-tocopherol in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 1.1% (0.083 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003h) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.3% antioxidant mix (equal parts BHT, tocopherol and	Cinnamaldehyde was shown to have an EC3 value of 1.3% (0.098 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003i) cited from SCCS (2012).

Method	Results	Remarks/Comments	Reference
eugenol) in 3:1 EtOH:DEP.			
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.1% Trolox C in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 1.4% (0.11 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003j) cited from SCCS (2012).
LLNA: concentration in vehicle not reported. Vehicle: 4:1 AOO.	Cinnamaldehyde was shown to have an EC3 value of 1.3% (0.10 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Elahi et al 2004 cited from SCCS (2012).
LLNA: 0.5, 1, 2.5, 5 and 10% cinnamaldehyde. Vehicle: 4:1 AOO.	Cinnamaldehyde was shown to have an EC3 value of 3.1% (0.23 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Basketter et al., 2001 cited from SCCS (2012).
LLNA: 1 and 2.5% cinnamaldehyde. Vehicle: 4:1 AOO.	Cinnamaldehyde was shown to have an EC3 value of 1.4% (0.11 M).	Too few concentrations tested and few details in reference according to SCCS (2012).	Smith and Hotchkiss 2001 cited from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: 50:50 EtOH:water.	Cinnamaldehyde was shown to have an EC3 value of 1.2% (0.091 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 2001 cited from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: 90:10 EtOH:water.	Cinnamaldehyde was shown to have an EC3 value of 1.6% (0.12 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 2000 cited from SCCS (2012).
LLNA: 0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: Dimethyl sulfoxide (DMSO).	Cinnamaldehyde was shown to have an EC3 value of 0.9% (0.068 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 1999 cited from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: propylene glycol.	Cinnamaldehyde was shown to have an EC3 value of 1.4% (0.11 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 1998 cited from SCCS (2012).
LLNA: 0.1, 0.25, 0.5, 1, 2.5, 5, 10 and	Cinnamaldehyde was shown to	According to SCCS (2012) there were no	Wright et al., 1997 cited

Method	Results	Remarks/Comments	Reference
25% cinnamaldehyde. Vehicle: Dimethylformamide (DMF).	have an EC3 value of 0.5% (0.038 M).	reported deviations from OECD TG 429.	from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: Methyl ethyl ketone (MEK).	Cinnamaldehyde was shown to have an EC3 value of 1.1% (0.083 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al 1996 cited from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: 4:1 AOO.	Cinnamaldehyde was shown to have an EC3 value of 1.7% (0.13 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 1995 cited from SCCS (2012).
GPMT: Concentration: 0.75% cinnamaldehyde (2 samples). Vehicle: not reported.	9/10 (90%) and 10/10 (100%) animals were positive.		Basketter 1992 cited from Bickers et al. (2005).
GPMT: Concentration: 0.75% cinnamaldehyde. Vehicle: 70:30 acetone/polyethylene glycol (PEG) 400.	100% of the animals were sensitised.		Basketter and Scholes 1992 cited from Bickers et al. (2005).
GPMT: Concentration: 3% cinnamaldehyde. Vehicle: not reported.	Strong sensitisation effects reported (no further details).		Ishihara et al., 1986 cited from Bickers et al. (2005).

A total of 22 LLNAs, 2 LLNA BrdU-ELISA tests and 3 GPMTs are summarised in table 1. The reported EC3 values for cinnamaldehyde range between 0.2% (vehicle: 3:1 ethanol:diethyl phthalate with or without α-tocopherol) and 3.1% (vehicle: Acetone:Olive oil 4:1). In the LLNA BrdU-ELISA tests EC2 values were reported to be between 2.2 and 6.9%. Positive reactions were observed in all GPMTs with cinnamaldehyde concentrations down to 0.75%. It is, however, not clear from the review by Bickers et al. (2005) whether the concentration was the intradermal induction dose.

No relevant *in vitro* studies on cinnamaldehyde (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on cinnamaldehyde involving several thousand dermatitis patients from various countries in Europe and the US. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with cinnamaldehyde

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with cinnamaldehyde 1% in petrolatum (pet.).	76/806 (9.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with cinnamaldehyde 1% in pet.	27/1951 (1.4%, 95% CI: 0.9-1.9%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 41 selected children age 0-5 years; 838 selected children age 6-18 years and selected adults > 18 years patch tested with cinnamaldehyde 1% in pet.	2/41 (4.9%) children age 0-5 years; 10/838 (1.2%) children age 6-18 years and 516/17213 (3%) adults >18 years were positive.	A retrospective study of pooled patch test data from patients collected by the North American Contact Dermatitis Group (NACDG) (2005-2012).	Zug et al. (2014).
Patch test: Retrospective study of selected allergic contact dermatitis (ACD) patients patch tested with cinnamaldehyde 1% in pet.	122/5079 (2.4%) patients were positive.	A retrospective study of pooled patch test data from patients collected by the North American Contact Dermatitis Group (NACDG) (2007-2008).	Fransway et al. (2013).
Patch test: Retrospective study of 940 selected patients tested with cinnamaldehyde 1% in pet.	66/940 (7%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Retrospective study of 164 hairdressers and hairdressing apprentices with eczema were tested	3/164 (1%) patients were positive.	A retrospective study of patch test data at Department of Occupational Dermatology Research and Education Centre,	Lyons et al. (2013).

Method	Results	Remarks/Comments	Reference
with cinnamaldehyde 1% in pet.		Australia (1993-2010).	
Patch test: Prospective study of 23 selected patients with chronic idiopathic urticarial patch tested with cinnamaldehyde 1% in pet.	3/23 (13%) patients were positive.	A prospective longitudinal study at Tufts Medical Center, USA. Year not stated.	Hession and Scheinman (2012).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with cinnamaldehyde 1% in pet.	10/100 (10%, 95% CI: 4.9-17.62%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1503 consecutive eczema patients patch tested with cinnamaldehyde 1% in pet.	20/1503 (1.3%, 95% CI: 0.8-2%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with cinnamaldehyde 1% pet.	Ca. 24/157 (ca. 15%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).
Patch test: Retrospective study of 1214 consecutive patients and 4527 selected patients patch tested with cinnamaldehyde in 1% pet.	17/1214 (1.43%, 95% CI: 0.67-2.18%) and 120/4527 (2.64%, 95% CI: 2.16-3.13%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Retrospective study	7/86 (8.1%) patients were	A retrospective and descriptive analysis of	Cuesta et al. (2010).

Method	Results	Remarks/Comments	Reference
of 86 selected patients patch tested with cinnamaldehyde 2% in pet.	positive.	a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	
Patch test: Retrospective study of 774 consecutive eczema patients patch tested with cinnamaldehyde 1% in pet.	66/774 (8.5%) patients were positive.	Retrospective study of patch test data from Odense University Hospital, Denmark (1995-2007).	Andersen et al. (2009).
Patch test: Prospective study of 18 selected cinnamon-sensitive patients patch tested with cinnamaldehyde 2% in pet.	4/18 (22%) patients were positive.	Prospective study of cinnamon-sensitive patients at the Department of Dermatology of the VU University Medical Centre, The Netherlands (year not stated).	Pentinga et al. (2009).
Patch test: Prospective study of 320 selected eczema patients patch tested with cinnamaldehyde 1% in pet.	5/320 (1.6%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 364 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with cinnamaldehyde 1% in pet.	a) 38/364 (10.4%) and b) 67/364 (19.2%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Retrospective study of selected ACD patients patch tested with cinnamaldehyde 1% in pet. between year 2003-2004: 5138 patients and year 2005-2006: 4435 patients.	Year 2003-2004: 123/5138 (2.4%) and year 2005-2006: 138/4435 (3.1%) patients were positive.	A retrospective study of pooled patch test data from patients collected by the North American Contact Dermatitis Group (NACDG) (2005-2006).	Zug et al. (2009).

Method	Results	Remarks/Comments	Reference
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with cinnamaldehyde 1% in pet.	1/15 (6.7%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).
Patch test: Retrospective study of selected ACD patients patch tested with cinnamaldehyde 1% in pet. over two decades. Year 1984-1985: 1199 patients; year 1985-1989: 3964 patients; year 1992-1994: 3528 patients; year 1994-1996: 3112 patients; year 1996-1998: 3443 patients and year 1998-2000: 4735 patients.	Year 1984-1985: 71/1199 (5.9%); year 1985-1989: 123/3964 (3.1%); year 1992-1994: 95/3528 (2.7%); year 1994-1996: 75/3112 (2.4%); year 1996-1998: 96/3443 (2.8%) and year 1998-2000: 170/4735 (3.7%) patients were positive.	A retrospective study of pooled patch test data from patients collected by the North American Contact Dermatitis Group (NACDG) (1970-2002).	Nguyen et al. (2008).
Patch test: Retrospective study of 2063 unselected patients patch tested with cinnamaldehyde 1% in pet.	21/2063 (1%, 95% CI: 0.5-1.5%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Prospective study of 1603 selected patients with eczematous dermatitis patch tested with cinnamaldehyde 1% in pet.	27/1603 (1.7%) patients were positive.	A prospective analysis of multicentre data on patients from five US sites and one Canadian site (year not reported)	Belsito et al. (2006).
Patch test: Study of 30 selected patients with a positive patch test to their own	6/30 (20%) patients were positive.		Vocanson et al. (2006).

Method	Results	Remarks/Comments	Reference
perfumed product patch tested with cinnamaldehyde. Concentration and vehicle not reported.			
Patch test: Prospective study of 422 selected patients with suspected contact allergy patch tested with cinnamaldehyde 1% in pet.	7/422 (1.7%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Retrospective study of 4900 unselected patients patch tested with cinnamaldehyde 1% in pet.	93/4900 (1.9%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Prospective study of 747 selected patients with suspected fragrance allergy patch tested with cinnamaldehyde 1% in pet.	14/747 (1.9%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).
Patch test: Study of 226 selected patients sensitive to FM patch tested with cinnamaldehyde 1% in pet.	30/226 (13%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999)	Brites et al. (2000).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with cinnamaldehyde 2% in 1% sorbitan sesquioleate.	10/50 (20%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with	5/40 (12.5%) patients were positive.		Katsarma and Gawkrodger (1999).

Method	Results	Remarks/Comments	Reference
cinnamyl alcohol in pet. Concentration not reported.			
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with cinnamaldehyde 1% in pet.	24/167 (14.4%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Prospective study of 1072 consecutive patients patch tested with cinnamaldehyde 1% in pet.	10/1072 (0.93%) patients were positive.	Prospective study of patients in a multicentre study involving 9 European centres. Year not stated.	Frosch et al., 1995 cited from SCCNFP (1999).
Patch test: Retrospective study of 2447 consecutive patients from three age groups patch tested between 1979-1983 with 2% pet. and 3440 consecutive patients from three age groups patch tested between 1988-1992 with cinnamaldehyde 1% in pet.	Between 1979 and 1983 754-795/2447 (30.8-32.5%) and between 1988 and 1992 313-440/3440 (9.1-12.8%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	Johansen and Menne (1995).
Patch test: Prospective study of 61 selected patients sensitive to FM patch tested with cinnamaldehyde 2% in pet.	21/61 (34%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with cinnamaldehyde 2% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Prospective study of 162 selected patients positive to a	34/162 (21%) patients were positive.	Retrospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany	Enders et al., 1989 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
fragrance mix patch tested with cinnamaldehyde 1%. Vehicle not reported.		(1987).	
Patch test: Study of 78 selected patients positive to a fragrance mix patch tested with cinnamaldehyde 1%. Vehicle not reported.	10/78 (12.8%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Retrospective study of 1200 selected patients with dermatitis patch tested between 1983 and 1984 with cinnamaldehyde 2% in pet. and 1500 selected patients with dermatitis patch tested between 1984 and 1985 with cinnamaldehyde 1% in pet.	Between 1983 and 1984 9/63 (14.3%) and between 1984 and 1985 3/54 (5.6%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with cinnamaldehyde. Concentration and vehicle not reported.	6/403 (1.5%) patients were positive.	It is unclear from the reference exactly how many patients were tested with cinnamyl alcohol.	Adams and Maibach (1985).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with cinnamaldehyde 0.5% in pet.	7/182 (3.7%) patients were positive.		Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 20 selected perfume allergic patients	6/20 (30%) patients were positive.		Larsen et al., 1977 cited from SCCNFP

Method	Results	Remarks/Comments	Reference
patch tested with cinnamaldehyde 1%. Vehicle not reported.			(1999).
Patch test and ROAT[1]: 17 cinnamaldehyde-allergic patients (20 controls) were tested with a dilution series of cinnamaldehyde in a patch test and a ROAT.	No threshold could be established as all tested doses gave positive reactions in the ROAT. Minimum tested dose was 0.26 µg/cm^2.	Copenhagen, Denmark and Malmö, Sweden. Year not stated.	Bruze et al. (2003).
Patch test and ROAT[1]: 22 cinnamaldehyde-allergic patients (20 controls) were tested with a dilution series of cinnamaldehyde in a patch test and a ROAT.	The ROAT threshold in percentage was higher than the patch test threshold.	Clinical study at Gentofte Hospital and Odense University Hospital, Denmark. Year not stated.	Johansen et al. (1996).

[1]ROAT: Repeat Open Application Test.

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with cinnamaldehyde.

Table 3. HRIPT and HMT studies with cinnamaldehyde adapted from Bickers et al. (2005).

Method	Results	Remarks/Comments	Reference
HRIPT: Cinnamaldehyde concentration: 0.5% Vehicle: 3:1 diethyl phthalate:ethanol (DEP:EtOH).	0/94 (0%) tests were positive.		Unpublished report (RIFM 2004) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 3% Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol.	4/28 (14%) tests were positive.		Unpublished report (RIFM 2003b) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 3%	Study aborted during induction phase due to the		Unpublished report (RIFM 2003b) cited

Method	Results	Remarks/Comments	Reference
Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol.	number or irritant reactions.		from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 0.5% Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol.	0/22 (0%) tests were positive.		Unpublished report (RIFM 2002a) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 0.5% Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol.	0/19 (0%) tests were positive.		Unpublished report (RIFM 2002b) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 0.1, 0.5, 1 or 1.25% Vehicle: EtOH.	0/41 (0%), 0/38 (0%), 5/41 (12%) and 5/10 (50%) were positive after 0.1, 0.5, 1 or 1.25% cinnamaldehyde, respectively.		Danneman et al., 1983 cited from Cocchiara et al. (2005).
HRIPT: Cinnamaldehyde concentration: 1% Vehicle: EtOH or petrolatum (pet.).	1/55 (2%) tests were positive with ethanol as vehicle, no reactions with petrolatum as vehicle.		Marzulli and Maibach 1976 and 1980 cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 1% Vehicle: alcohol SDA 39C.	5/41 (12%) tests were positive.		Unpublished report (RIFM 1973d) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 0.5% Vehicle: EtOH.	0/38 (0%) tests were positive.		Unpublished report (RIFM 1965) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration:	0/41 (0%) tests were positive.		Unpublished report (RIFM 1964a) cited

Method	Results	Remarks/Comments	Reference
0.125% Vehicle: EtOH.			from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 1.25% Vehicle: EtOH.	5/10 (50%) tests were positive.		Unpublished report (RIFM 1964b) cited from Bickers et al. (2005).
HMT: Cinnamaldehyde concentration: 3% Vehicle: butylene glycol.	3/25 (12%) tests were positive.		Unpublished report (RIFM 1974a) cited from Bickers et al. (2005).
HMT: Cinnamaldehyde concentration: 2% Vehicle: pet.	11/25 (44%) tests were positive.		Unpublished report (RIFM 1973c) cited from Bickers et al. (2005).

HRIPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Tests.

Case studies

Table 4 summarises case reports with allergic contact dermatitis in different clinics in Europe and the US where cinnamaldehyde has been found as a causative agent.

Table 4. Case studies with cinnamaldehyde

Method	Results	Remarks/Comments	Reference
Patch test: A 33-year old man with itching eczematous lesions was patch tested with cinnamaldehyde. Concentration and vehicle not reported.	Positive reaction on day 2 and day 4 was observed.	Case study, Italy (year not stated).	Guarneri (2010).
Patch test: A 47-year old man with dermatitis was patch tested with cinnamaldehyde. Concentration and vehicle not reported.	Positive reaction on day 2 was observed.	Case study, USA (year not stated).	Decapite and Anderson (2004).
Patch test: A 42-year old woman with rash on her arms was patch tested with cinnamaldehyde. Concentration and vehicle not reported.	Positive reaction after 20 min (anaphylaxis) was observed.	Case study, UK (year not stated).	Diba and Statham (2003).

A total of 52 results from patch test population studies, 2 repeat open application tests (ROATs), 10 HRIPTs, 2 HMTs and 3 case studies with cinnamaldehyde are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0.93 and 38% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0.93 and 32.5% (8 studies) and for selected dermatitis patients positive reactions range between 1.2 and 90% (44 studies). A single study in workers reported positive patch test reactions in 1%. The total number of published cases is > 2300. Sensitisation was reported in 6/12 HRIPT studies at cinnamaldehyde concentrations between 1 and 3%. Both HMT studies reported positive reactions after 2-3% cinnamaldehyde. The Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT[6] (induction) of 591 µg/cm² and a LOEL-HRIPT/HMT[7] (induction) of 775 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 590 µg/cm² was established for cinnamaldehyde by the RIFM Expert Panel (IFRA, 2013b).

[6] NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test

[7] LOEL-HRIPT/HMT: Lowest Observed Effect Level- Human Repeat Insult Patch Test/Human Maximisation test

Two ROATs with cinnamaldehyde are summarised in table 2 (Johansen et al., 1996; Bruze et al., 2003). Results from these studies may be used for establishing a specific concentration limit for cinnamaldehyde in consumer products.

According to SCCS (2012) cinnamaldehyde is used in volumes less than 175 ton per year in perfume formulations. It has been reported that 2.5% of a total of 516 consumer products; 6% of a total of 300 fragrance products; ca. 2% of 3000 products and 1% of children cosmetics were labelled to contain cinnamaldehyde (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 1.1% of 88 tested deodorants were labelled to contain cinnamaldehyde and the fragrance was detected in 4% (range: 5 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)). Besides exposure from use of cosmetic products, cinnamaldehyde exposure also occurs from clothing, candles and food (SCCS, 2012).

The IFRA standard limits for cinnamaldehyde in different IFRA QRA product categories reported by IFRA (2013b and 2015) are shown in table 5.

Table 5. The IFRA standard limits for cinnamaldehyde in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.02%
Category 2	Deodorants/antiperspirants	0.02%
Category 3	Hydroalcoholics for shaved skin	0.05%
Category 4	Hydroalcoholics for unshaved skin	0.05%
Category 5	Hand cream	0.05%
Category 6	Mouthwash	0.4%
Category 7	Intimate wipes	0.04%
Category 8	Hair styling aids	0.05%
Category 9	Rinse-off hair conditioners	0.05%
Category 10	Hard surface cleaners	0.05%
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Cinnamaldehyde is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 52 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies were identified with cinnamaldehyde. The positive patch test frequencies from all of the reported patch test population studies vary between 0.93 and 90% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.93 and 32.5% (8 studies) and in studies with selected dermatitis patients positive reactions range between 1.2 and 90% (44 studies). A single study in workers reported positive patch test reactions in 1%. The total number of published cases is > 2300. A LOEL-HRIPT/HMT (induction) of 775 µg/cm² was established for cinnamaldehyde by the RIFM Expert Panel based on unpublished reports.

Non-human data
A total of 22 LLNAs and 2 LLNA BrdU-ELISA tests were identified testing skin sensitising effects of cinnamaldehyde. The reported EC3 values for cinnamaldehyde ranged between 0.2% and 3.1% in different vehicles.

No relevant *in vitro* studies on cinnamaldehyde (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure
According to IFRA (2013b) the exposure of cinnamaldehyde when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria
For unselected/consecutive dermatitis patients positive reactions range between 0.93 and 32.5% with 7/8 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 1.2 and 90% with 37 out of 44 studies reporting frequencies higher than 2%. A single study in workers reported positive patch test reactions in 1%. In addition to this there are more than 2300 published cases of positive patch test reactions to cinnamaldehyde. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or ≥ 1% for selected workers with known exposure or dermatitis and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015).The collected data described above from patch test studies show that cinnamaldehyde causes a *high frequency* of occurrence of skin sensitisation based on these four types of information.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 22 LLNAs EC3 values between 0.2 and 3.1% were reported for cinnamaldehyde with 21/22 EC3 values <2%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.). Hence, data from LLNAs indicate classification of cinnamaldehyde in sub-category 1A.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from the LLNAs support a sub-category 1A classification. A classification as a skin sensitiser in sub-category 1A is thus warranted for cinnamaldehyde.

Conclusions on classification and labelling
Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure and supported by data from LLNAs, a classification of cinnamaldehyde as a skin sensitiser in sub-category 1A is justified.

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Appendix 6 Cinnamyl alcohol CAS RN 104-54-1

According to SCCS (2012) cinnamyl alcohol is a fragrance compound known to be a prohapten and to form sensitising compounds by metabolic transformation, which increases the likelihood for cross-reactivity between cinnamyl alcohol and cinnamaldehyde.

Non-human information

Table 1 summarises relevant animal studies with cinnamyl alcohol i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with cinnamyl alcohol.

Method	Results	Remarks/Comments	Reference
LLNA: *Ex vivo* LLNA-BrdU ELISA. 0, 0.5, 1, 5 and 10% cinnamyl alcohol. Vehicle: Acetone: olive oil (AOO).	Cinnamyl alcohol was shown to have an EC3 value of 17.9%.	Cinnamyl alcohol was classified as a weak skin sensitizer by Ulker et al. (2014).	Ulker et al. (2014).
LLNA (OECD TG 429): 10, 25 and 50% cinnamyl alcohol. Vehicle: AOO	Cinnamyl alcohol was shown to have an EC3 value of 25.2%.		Basketter (2012).
LLNA: LLNA:BrdU-FCM. 10, 25 and 50% cinnamyl alcohol. Vehicle: 4:1 AOO.	Cinnamyl alcohol was shown to have an EC3 value of 21%.	Cinnamyl alcohol was classified as positive for skin sensitisation by Jung (2012).	Jung (2012).
LLNA (OECD TG 429): Cinnamyl alcohol (concentration not reported). Vehicle: 4:1 AOO.	Cinnamyl alcohol was shown to have an EC3 value of 20.1% (1.5 M).		Elahi et al. (2004).
LLNA: 10, 25, 50 and 90% cinnamyl alcohol. Vehicle: 4:1 AOO.	Cinnamyl alcohol was shown to have an EC3 value of 21%.	Cinnamyl alcohol was classified as a weak skin sensitizer by Gerberick et al. (2005).	Estrada et al., 2003 cited from Gerberick et al. (2005).
GPMT: Intradermal induction 25%; topical induction 25%; challenge dose 3% cinnamyl alcohol. Vehicle: not reported.	Positive reactions in 3/10 (30%) of the tested animals.		Modjtahedi (2011).

Method	Results	Remarks/Comments	Reference
GPMT: Concentration: 10% cinnamyl alcohol. Vehicle: not reported.	Strong sensitisation effects reported (no further details).		Ishihara et al., 1986 cited from Bickers et al. (2005).
Freund's complete adjuvant test: Intradermal induction dose 5% with challenge doses of 3, 10, 30 and 100% cinnamyl alcohol. Vehicle: not reported.	Positive reactions in 0/20, 0/20, 0/20 and 3/20 (15%) animals, respectively.		Study report 1986-01-06 cited from REACH-RD (2015k).
Buehler test: Induction dose 3, 10 and 30% with a challenge dose of 3% cinnamyl alcohol. Vehicle: not reported.	Positive reactions in 0/11, 0/13 and 0/15 animals, respectively.		Modjtahedi (2011).

A total of five LLNAs (two of which were reported to be conducted in accordance with OECD TG 429), two GPMTs, one Freund's complete adjuvant test (FCAT) and one Buehler test were identified testing the skin sensitisation of cinnamyl alcohol. EC3 values between 17.9 and 30% were reported for cinnamyl alcohol in the LLNAs. Sensitisation was observed in 30% of the animals in a GPMT after an intradermal induction dose of 25%. Another GPMT reported strong sensitisation effects but without specifying the number of affected animals. In the Freund's complete adjuvant test 15% of the animals had positive reactions after an intradermal induction dose of 100% cinnamyl alcohol. No positive reactions were observed in the Buehler test after induction doses of 3, 10 and 30% cinnamyl alcohol.

No relevant *in vitro* studies on cinnamyl alcohol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on cinnamyl alcohol involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with cinnamyl alcohol.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients	66/806 (8.2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information	Schnuch et al. (2015).

Method	Results	Remarks/Comments	Reference
patch tested with cinnamyl alcohol 1% in petrolatum (pet.).		Network of Departments of Dermatology) (2007-2009).	
Patch test: Retrospective study of 1951 selected eczema patients patch tested with cinnamyl alcohol 2% in pet.	48/1951 (2.5%, 95% CI: 1.8-3.1%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 940 selected patients tested with cinnamyl alcohol 1% in pet.	129/940 (13.7%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with cinnamyl alcohol 1% in pet.	13/100 (13%, 95% CI: 7.11-21.20%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1501 consecutive eczema patients patch tested with cinnamyl alcohol 1% in pet.	10/1501 (0.7%, 95% CI: 0.3-1.9%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with cinnamyl alcohol 5% in pet.	54/157 (34.4%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 86 selected patients patch tested with cinnamyl alcohol 2% in pet.	12/86 (13.9%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Prospective study of 18 selected cinnamon-sensitive patients patch tested with cinnamyl alcohol 2% in pet.	5/18 (28%) patients were positive.	Prospective study of cinnamon-sensitive patients at the Department of Dermatology of the VU University Medical Centre, The Netherlands (year not stated).	Pentinga et al. (2009).
Patch test: Prospective study of 320 selected eczema patients patch tested with cinnamyl alcohol in 2% pet.	8/320 (2.5%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 266 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with cinnamyl alcohol 1% in pet.	a) 24/266 (9.02%) and b) 44/266 (16.54%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with cinnamyl alcohol 2% in pet.	15/15 (100%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).
Patch test: Retrospective study of 2063 unselected	13/2063 (0.6%, 95% CI: 0.2-1.0%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).

Method	Results	Remarks/Comments	Reference
patients patch tested with cinnamyl alcohol 1% in pet.			
Patch test: Retrospective study of a) 29 patients positive to their own deodorant and b) 133 negative to their own deodorant patch tested with cinnamyl alcohol 1% in pet.	a) 3/29 (10%) and b) 2/133 (1.5%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998-2002).	Uter et al. (2007).
Patch test: Study of 30 selected patients with a positive patch test to their own perfumed product patch tested with cinnamyl alcohol. Concentration and vehicle not specified.	6/30 (20%) patients were positive.		Vocanson et al. (2006).
Patch test: Prospective study of 422 selected patients with suspected contact allergy patch tested with cinnamyl alcohol 2% in pet.	13/422 (3.1%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Retrospective study of 4900 unselected patients patch tested with cinnamyl alcohol 1% in pet.	88/4900 (1.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Prospective study of 747 selected patients with suspected	11/747 (1.5%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).

Method	Results	Remarks/Comments	Reference
fragrance allergy patch tested with cinnamyl alcohol 1% in pet.			
Patch test: Study of 226 selected patients sensitive to FM patch tested with cinnamyl alcohol 1% in pet.	18/226 (8%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999)	Brites et al. (2000).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with cinnamyl alcohol 2% in 1% sorbitan sesquioleate.	8/50 (16%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with cinnamyl alcohol in pet. Concentration not reported.	4/40 (10%) patients were positive.		Katsarma and Gawkrodger (1999).
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with cinnamyl alcohol 5% in lanolin.	11/167 (6.6%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Prospective study of 1072 consecutive patients patch tested with cinnamyl alcohol 1% in pet.	6/1072 (0.56%) patients were positive.	Prospective study of patients in a multicentre study involving 9 European centres. Year not stated.	Frosch et al., 1995 cited from SCCNFP (1999).
Patch test: Retrospective study of 367 selected patients patch tested with	40/367 (10.9%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark	Johansen and Menne (1995).

Method	Results	Remarks/Comments	Reference
cinnamyl alcohol 1-2% in pet.		(1979-1983 and 1988-1992).	
Patch test: Prospective study of 50 selected patients positive to a fragrance mix (FM) patch tested with cinnamyl alcohol. Concentration not reported.	17/50 (34%) patients were positive.	Retrospective study of patch test data from Department of Dermatology and Venereology, Hungary. Year not stated.	Becker et al. (1994).
Patch test: Prospective study of 61 selected patients sensitive to a FM patch tested with cinnamyl alcohol 5% in pet.	19/61 (31%) patients were positive. Control tests in 100 patients not allergic to fragrances showed that cinnamyl alcohol was marginally irritant at the concentration chosen.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Study of selected patients positive to a FM patch tested with cinnamyl alcohol. Concentration and vehicle not reported.	14% patients were positive.	Study of patch test data from France. No further information available from SCCNFP 1999.	Artigou et al., 1989 cited from SCCNFP (1999).
Patch test: Prospective study of 162 selected patients positive to a FM patch tested with cinnamyl alcohol 1%. Vehicle not reported.	9/162 (5.6%) patients were positive.	Retrospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1987).	Enders et al. (1989).
Patch test: Study of 78 selected patients positive to a FM patch tested with cinnamyl alcohol 1%.	5/78 (6.4%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
Vehicle not reported.			
Patch test: Study of 119 selected patients with contact allergy to cosmetic products patch tested with cinnamyl alcohol 5% in pet.	2/119 (1.7%) patients were positive.		De Groot et al., 1988 cited from SCCNFP (1999).
Patch test: Study of 156 selected patients with pure contact allergy to cosmetic products patch tested with cinnamyl alcohol Concentration and vehicle not reported.	6/156 (3.8%) patients were positive.		Broneck et al., 1987 cited from SCCNFP (1999).
Patch test: Retrospective study of 63 selected patients with dermatitis tested positive to perfume mixture patch tested between 1983 and 1984 with cinnamyl alcohol 3% in pet. and 54 selected patients with dermatitis tested positive to perfume mixture patch tested between 1984 and 1985 with cinnamyl alcohol 1% in pet.	Between 1983 and 1984 9/63 (14.3%) and between 1984 and 1985 5/54 (9.3%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with cinnamyl alcohol.	17/403 (4.2%) patients were positive.	It is unclear from the reference exactly how many patients were tested with cinnamyl alcohol.	Adams and Maibach (1985).

Method	Results	Remarks/Comments	Reference
Concentration and vehicle not reported.			
Patch test: study of 20 selected perfume allergic patients patch tested with cinnamyl alcohol 5%. Vehicle not reported.	15/20 (75%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with cinnamyl alcohol.

Table 3. HRIPT and HMT studies with cinnamyl alcohol adapted from Letizia et al. (2005).

Method	Results	Remarks/Comments	Reference
HRIPT: Sample: NA[1] 4%. Vehicle: 1:3 ethanol:diethyl phthalate (EtOH:DEP).	2/54 (4%) at re-challenge, subjects reacted under occluded conditions but not under semi-occlusive conditions or in a 5-day repeated open application test.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (2001a, 2002a) cited from Letizia et al. (2005).
HRIPT: Sample: NA 4%. Vehicle: 3:1 EtOH:DEP.	1/55, however as subject also reacted to vehicle control and to neat ethanol, it was concluded that the reaction was caused by the ethanol component and not cinnamyl alcohol	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (2001b, 2002b) cited from Letizia et al. (2005).
HRIPT (modified Draize): Sample: NA 4%. Vehicle: EtOH and petrolatum (pet.).	4/150 (2.7%) reactions with ethanol as vehicle; no reactions with petrolatum as vehicle.		Jordan and King 1977 cited from Letizia et al. (2005).
HMT:	4/28 (14%) were	No further information	Unpublished

Method	Results	Remarks/Comments	Reference
Sample: 82-10-17 (prepared via a borohydride reduction process) 10%. Vehicle: DEP.	positive (virgin panel)	available from Letizia et al. (2005).	report from RIFM (1982a) cited from Letizia et al. (2005).
HMT: Sample: 82-10-654 10%. Vehicle: DEP.	4/27 questionable para-allergic reactions; 3 of these subjects were retested and 2/3 reacted	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1982b) cited from Letizia et al. (2005).
HMT: Sample: 81-10-HR recrystallized sample 10%. Vehicle: DEP.	2/22 (9%) were positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1981a) cited from Letizia et al. (2005).
HMT: Sample: 81-10-HR (retest) 10%. Vehicle: DEP.	2/23 (8.7%) were positive (virgin panel)	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1981b) cited from Letizia et al. (2005).
HMT: Sample: 79-10-ED-UN 10%. Vehicle: DEP.	6/28 (21%) were positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980a) cited from Letizia et al. (2005).
HMT: Sample: 79-10-ED-W (washed with alkali) 10%. Vehicle: DEP.	0/24 (0%) were positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980b) cited from Letizia et al. (2005).
HMT: Sample: 79-10-0 10%. Vehicle: DEP.	2/22 (9%) were positive plus 1 questionable reaction.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980d) cited from Letizia et al. (2005).
HMT: Sample: 79-10-N (pure form of 79-10-0) 10%. Vehicle: DEP.	0/35 sensitisation reactions (0%); 7 irritations reactions and 1 hyper-irritation reaction.	No further information available from Letizia et al. (2005) and Bickers et al 2005.	Unpublished report from RIFM (1980e) cited from Letizia et al. (2005) and Bickers et al. (2005).
HMT: Sample: 79-10-ED-W and F	1/28 (4%) was positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980c) cited

Method	Results	Remarks/Comments	Reference
(washed with alkali) 10%. Vehicle: DEP.			from Letizia et al. (2005).
HMT: Sample: CA (washed with alkali) 10%. Vehicle: DEP	1/21 questionable reactions (virgin panel)	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980f) cited from Letizia et al 2005
HMT: Sample: 79-10-CADEP 10%. Vehicle: DEP.	6/26 (23%) were positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1979a) cited from Letizia et al. (2005).
HMT: Sample: 79-4-5J 4%. Vehicle: pet.	1 irritation reaction in 24 Japanese Americans	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1979b) cited from Letizia et al. (2005).
HMT: Sample: SKK-10-OX 10%. Vehicle: pet.	10/33 (30%) were positive plus 3 questionable and 2 irritant reactions	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1977a) cited from Letizia et al. (2005).
HMT: Sample: CA-10-STY (extracted from styrax) 10%. Vehicle: pet.	5/25 (20%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1977b) cited from Letizia et al. (2005).
HMT: Sample: 76-Bedoukianol-10 (2nd retest) 10%. Vehicle: pet.	3/25 (12%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1977d) cited from Letizia et al. (2005).
HMT: Sample: SKK-10-P (pure form of SKK-10-OX) 10%. Vehicle: pet.	1/24 (4%) was positive plus 3 questionable and 2 irritant reactions.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1977e) cited from Letizia et al. (2005).
HMT: Sample: 35-10-35R (0) (retest) 10%. Vehicle: hydrophilic ointment.	2/25 (8%)were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1976a) cited from Letizia et al. (2005).
HMT:	7/25 (28%) were	No further information	Unpublished

Method	Results	Remarks/Comments	Reference
Sample: 76-Bedoukianol-10 10%. Vehicle: pet.	positive	available from Letizia et al. (2005).	report from RIFM (1976b) cited from Letizia et al. (2005).
HMT: Sample: 76-Bedoukianol-10 (retest) 10%. Vehicle: pet.	9/25 (36%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1976c) cited from Letizia et al. (2005).
HMT: Sample: Cinnamyl alcohol 10%. Vehicle: hydrophilic ointment	2/25 (8%) were positive	No further information available from Bickers et al. (2005).	Unpublished report from RIFM (1976d) cited from Bickers et al. (2005).
HMT: Sample: CA 10%. Vehicle: pet.	5/25 (20%) were positive (virgin panel)	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1976e) cited from Letizia et al. (2005).
HMT: Sample: 76-10FDO-B 10% Vehicle: pet.	1/11 (9%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1976f) cited from Letizia et al. (2005).
HMT: Sample: 35-10-35R (0) 10%. Vehicle: pet.	3/25 (12%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1975) cited from Letizia et al. (2005).
HMT: Sample: NA 4%. Vehicle not reported.	0/25 (0%) were positive.		Greif 1967 cited from Letizia et al. (2005).
Modified HMT: Sample: NA 4%. Vehicle: EtOH and pet.	1 reaction (25-30 subjects) with ethanol as vehicle; no reactions with petrolatum as vehicle.		Jordan and King 1977 cited from Letizia et al. (2005).

[1]NA: Not Available

Case studies

Table 4 summarises case reports with allergic contact dermatitis in different clinics in Europe where cinnamyl alcohol has been found as a causative agent.

Table 4. Case studies with cinnamyl alcohol.

Method	Results	Remarks/Comments	Reference
Patch test: A 33-year old man with itching eczematous lesions was patch tested with cinnamyl alcohol. Concentration and vehicle not reported.	Positive reaction on Day 2 (D2) and D4 was observed.	Case study, Italy (year not stated).	Guarneri (2010).
Patch test: An 18-year old woman with acute eczema was patch tested with cinnamyl alcohol 5% in petrolatum (pet.).	Positive reaction on D2 and D3 was observed.	Case study, Italy (year not stated).	Lauriola et al. (2009).
Patch test: A 74-year old woman with extensive eczematous and bullous dermatitis was patch tested with cinnamyl alcohol 1% in pet.	Positive reaction on D2 and D4 was observed.	Case study, Spain (year not stated).	Garcia-Abujeta et al. (2005).
Patch test: A 47-year old man with vesicular dermatitis was patch tested with cinnamyl alcohol 1% in pet.	Positive reaction on D2 and D3 was observed.	Case study, Germany (year not stated).	Hartmann and Hunzelmann (2004).

A total of 34 patch test population studies, 3 HRIPTs, 25 HMTs and 4 case studies with cinnamyl alcohol are summarised above (Table 2, 3 and 4). As shown in Table 1 the positive patch test frequencies from all of the reported patch test studies vary between 0.56 and 100% in dermatitis patients. For unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% (4 studies) and for selected dermatitis patients positive reactions range between 1.5 and 100% (30 studies). The total number of published cases is > 600. Sensitisation was reported in 2/3 HRIPT studies at a cinnamyl alcohol concentration of 4%. In the HMT studies 17/24 studies with cinnamyl alcohol showed a positive result after 10%. The Research Institute for Fragrance materials, Inc. (RIFM) deducted a NOEL[8]-HRIPT (induction) of 3000 µg/cm², a NOEL-HMT (induction) of 2759 µg/cm² and a LOEL[9] (induction) of 4724 µg/cm² for cinnamyl alcohol. In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 3000 µg/cm² was established for cinnamyl alcohol by the RIFM Expert Panel (IFRA, 2008a).

[8]NOEL: No Observed Effect Level

[9]LOEL: Lowest Observed Effect Level

According to SCCS (2012) cinnamyl alcohol is used in volumes less than 175 ton per year in perfume formulations. It has been reported that 6.4% of a total of 516 consumer products; 8% of a total of 300 fragrance products, ca. 4% or 3000 products and 6.7% of children cosmetics were labelled to contain cinnamyl alcohol (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 12.5% of 88 tested deodorants were labelled to contain cinnamyl alcohol and the fragrance was detected in 48% (range: 2-503 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for cinnamyl alcohol in different IFRA QRA product categories reported by IFRA (2008a and 2015) are shown in table 5.

Table 5. The IFRA standard limits for cinnamyl alcohol in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.09%
Category 2	Deodorants/antiperspirants	0.1%
Category 3	Hydroalcoholics for shaved skin	0.4%
Category 4	Hydroalcoholics for unshaved skin	0.4%
Category 5	Hand cream	0.4%
Category 6	Mouthwash	2.2%
Category 7	Intimate wipes	0.2%
Category 8	Hair styling aids	0.4%
Category 9	Rinse-off hair conditioners	0.4%
Category 10	Hard surface cleaners	0.4%
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Cinnamyl alcohol is registered under the REACH regulation with an annual tonnage band of 100-1000 tonnes/year.

Summary and discussion of skin sensitization

Human data

A total of 34 patch test population studies, 3 HRIPTs, 25 HMTs and 4 case studies, were identified with cinnamyl alcohol. The positive patch test frequencies from all of the reported patch test studies vary between 0.56 and 100% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% (4 studies) and in studies with selected dermatitis patients positive reactions range between 1.5 and 100% (30 studies). The total number of published cases is > 600. A LOEL (induction) of 4724 µg/cm² was derived from the HRIPT/HMT studies.

Non-human data

A total of 5 LLNAs (two of which were reported to be conducted in accordance with OECD TG 429), 2 GPMT, 1 Freund's complete adjuvant test (FCAT) and 1 Buehler test were identified testing the skin sensitisation of cinnamyl alcohol. EC3 values between 17.9 and 30% were reported for cinnamyl alcohol in the LLNAs and positive reactions were observed in a GPMT (30% positive) and FCAT (15% positive) at intradermal induction doses of 25 and 100%, respectively. No positive reactions were observed in the Buehler test.

No relevant *in vitro* studies on cinnamyl alcohol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure
According to data from IFRA (2008a) the exposure of cinnamyl alcohol when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria
For unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% with 1/4 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 1.5 and 100% with 28 out of 30 studies reporting frequencies higher than 2%. In addition to this there are more than 600 published cases of positive patch test reactions to cinnamyl alcohol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that cinnamyl alcohol causes a *high frequency* of occurrence of skin sensitisation based on frequencies in selected dermatitis patients and total number of cases.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNAs EC3 values between 17.9 and 30% were reported for cinnamyl alcohol. According to the CLP Regulation an EC3 value larger than 2% indicates placement of cinnamyl alcohol into sub-category 1B (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 30% of the animals after an intradermal induction dose of 25% cinnamyl alcohol. According to the CLP criteria a positive response ≥ 30% of the animals responding at >1% intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.).

No sensitisation was observed in the Buehler test after an induction dose of 30% cinnamyl alcohol.

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies with selected dermatitis patients and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. All animal studies indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for cinnamyl alcohol.

Conclusions on classification and labelling
Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure a classification of cinnamyl alcohol as a skin sensitiser in sub-category 1A is justified.

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Appendix 7 Coumarin CAS RN 91-64-5

Non-human information

Table 1 summarises relevant animal studies with coumarin i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with coumarin.

Method	Results	Remarks/Comments	Reference
LLNA: 10, 25 and 50% coumarin (purity 99.9%). Vehicle: Dimethyl formamide (DMF).	Coumarin was shown to have a stimulation index (SI) < 3 at all concentrations.	According to SCCS (2012) the study did not deviate from OECD 429 except that coumarin should have been tested in higher concentrations.	Vocanson et al., 2006 cited from SCCS (2012).
LLNA: 5, 10 and 25% Rhodiascent TM Coumarine (purity not reported). Vehicle: 4:1 acetone:olive oil (AOO).	Coumarin was shown to have a stimulation index (SI) < 3 at all concentrations.	According to SCCP (2006) the study was performed in compliance with EEC 96/54/EC Part B, Method B.6. EEC 96/54/EC Part B, Method B.6 is, however, similar to OECD TG 406, which is the GPMT/Buehler test.	CIT 2001 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine Rhodiascent TM (purity not reported). Vehicle: DMF. Three sets of experiments were performed.	Coumarin was shown to have SI < 3 in all experiments except of Experiment 2 at 50% were the SI was 3.1.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2003 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine – Chine 0013090/01 Ex PRC (purity not reported). Vehicle: DMF. Three sets of experiments were performed.	Coumarin was shown to have SI < 3 in all experiments except of Experiment 1 at 50% were the SI was 3.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2003 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine –	Coumarin was shown to have SI < 3 in all	According to SCCP (2006) the study was performed in	INSERM 2003 cited from SCCP (2006).

Method	Results	Remarks/Comments	Reference
Chine Tianjin freeword (purity not reported). Vehicle: DMF. Three sets of experiments were performed.	experiments except of Experiment 2 at 25 and 50% were the SI was 3.7 and 4, respectively.	compliance with OECD draft 429 (2000).	
LLNA: 1, 2.5, 5 and 10% 6-Chloro-Coumarine (purity not reported). Vehicle: DMF. Three sets of experiments were performed.	Coumarin was shown to have SI < 3 in all experiments except of Experiment 1 at 5 and 10% were the SI was 3.4 and 3.3, respectively.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2003 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine Rhodiascent TM (purity not reported). Vehicle: DMF.	Coumarin was shown to have a SI < 3 at all concentrations.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2004 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine – Chine 0013090/01 Ex PRC (purity not reported). Vehicle: DMF.	Coumarin was shown to have SI < 3 except at 50% were the SI was 3.19.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2004 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine – Chine Tianjin freeword (purity not reported). Vehicle: DMF. Two experiments.	Coumarin was shown to have a SI < 3 at all concentrations.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2004 cited from SCCP (2006).
LLNA: 2.5, 5 and 10% 6-Chloro-Coumarine (purity not reported). Vehicle: DMF.	Coumarin was shown to have SI < 3 except at 5% were the SI was 4.94.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2004 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine – SRD aromatics LTD –	Coumarin was shown to have a SI < 3 at all concentrations.	According to SCCP (2006) the study was performed in compliance with OECD	INSERM 2004 cited from SCCP (2006).

Method	Results	Remarks/Comments	Reference
Indian no. 2 (purity not reported). Vehicle: DMF.		draft 429 (2000).	
GPMT: Intradermal induction 0.5%; topical induction 25%; challenge dose 25% coumarin. Vehicle not reported.	No positive reactions were observed in the tested animals.	According to REACH-RD (2015h) the study was performed equivalent or similar to OECD TG 406.	Study report from 1979 cited from REACH-RD (2015h).

A total of 20 LLNAs and 1 GPMT with coumarin is summarised in table 1. The majority of LLNAs reported SI values below 3 at concentrations up to 50% coumarin and the SCCS (2012) established an EC3 > 50% for coumarin. Sensitisation was not observed in the GPMT after an intradermal induction dose of 0.5% coumarin.

According to SCCS (2012) "*Researchers from INSERM and "Rhodia Organique, Lyon , France" observed that pure coumarin is not an allergen in the LLNA, however, commercially available materials, containing "contaminants" (3,4-dihydrocoumarin, 6-chlorocoumarin and 6,12-epoxy-6H,12H-dibenzo[b,f][1,5] dioxocin, were identified as weak and moderate sensitisers, resp.*"

No relevant *in vitro* studies on coumarin (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on coumarin involving several thousand dermatitis patients from various countries in Europe. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with coumarin.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 324 selected patients patch tested with coumarin 5% in petrolatum (pet.).	66/324 (4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with coumarin 5% in pet.	8/1951 (0.41%, 95% CI: 0.1-0.7%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 205 selected patients tested with coumarin 5% in pet.	9/205 (4.4%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with coumarin 5% in pet.	2/100 (2%, 95% CI: 0.24-7.04%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Prospective study of 565 selected patients patch tested with coumarin 5% in vaseline.	29/565 (5.1%) patients were positive. In addtition 8 patients had contact uriticaria.	A prospective study on patch test data from multicentre study, Hungary (2009-2010).	Ponyai et al. (2012).
Patch test: Retrospective study of 1503 consecutive eczema patients patch tested with coumarin 5% in pet.	3/1503 (0.7%, 95% CI: 0.1-0.5%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Retrospective study of 86 selected patients patch tested with coumarin 5% in pet.	1/86 (1.2%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 367 selected FM II positive patients patch tested with coumarin 5% in pet.	10/367 (2.7%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2005-2008).	Krautheim et al. (2010).
Patch test:	1/18 (6%)	Prospective study of	Pentinga et al.

Method	Results	Remarks/Comments	Reference
Prospective study of 18 selected cinnamon-sensitive patients patch tested with coumarin 5% in pet.	patients were positive.	cinnamon-sensitive patients at the Department of Dermatology of the VU University Medical Centre, The Netherlands (year not stated).	(2009).
Patch test: Prospective study of 320 selected eczema patients patch tested with coumarin 5% in pet.	2/320 (0.6%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 2020 consecutive patients patch tested with coumarin 5%. Vehicle not reported.	8/2020 (0.4%, 95% CI: 0.2-0.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Study of 252 selected dermatitis patients patch tested with 2% coumarin and 100 selected dermatitis patients patch tested with 1 and 10% coumarin. Vehicle not reported.	1/252 (0.4%) and 0/100 (0%) patients were positive, respectively.	According to the authors no cases of irritancy was observed at 10% coumarin. The number of reported tested subjects is not consistent and the one positive patch test was dismissed by Vocanson 2006 because the patient had "highly sensitive skin".	Vocanson et al. (2006).
Patch test: Study of 101 selected patients allergic to fragrance mix patch tested with coumarin 2%. Vehicle not reported.	1/101 (1%) patients were positive.		Vocanson et al. (2006).
Patch test: Study of 30 selected patients with a positive patch test to their own	0/30 (0%) patients were positive.		Vocanson et al. (2006).

Method	Results	Remarks/Comments	Reference
perfumed product patch tested with coumarin 2%. Vehicle not reported.			
Patch test: Prospective study of 1701 consecutive patients patch tested with coumarin 5% in pet.	0/1701 (0%) patients were positive while 7 doubtful or irritant reactions were observed.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al. (2005).
Patch test: Prospective study of 1855 consecutive patients patch tested with coumarin 5% in pet.	5/1855 (0.3%) patients were positive.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al. (2002a).
Patch test: Prospective study of 1825 consecutive patients patch tested with coumarin 5% in pet.	13/1825 (0.7%) patients were positive.	Multicenter study of patch test data in The Netherlands (1998-1999).	de Groot et al. (2000).
Patch test: Study of 14 000 consecutive eczema patients patch tested with coumarin 5% or 8% in pet. (8% for a short period only).	58/14 000 (0.4%) patients were positive.		Kunkeler et al 1998 cited from SCCNFP (1999).
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with coumarin 5%. Vehicle not reported.	2/167 (1.2%) patients were positive.		Larsen et al 1996 cited from SCCNFP (1999).
Patch test: Study of 119 selected	1/119 (0.8%) patients were		De Groot et al., 1988 cited from

Method	Results	Remarks/Comments	Reference
patients with contact allergy to cosmetic products patch tested with coumarin 5% in pet.	positive.		SCCNFP (1999).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with coumarin. Concentration and vehicle not reported.	4/403 (1%) patients were positive.	It is unclear from the reference exactly how many patients were tested with coumarin.	Adams and Maibach (1985).
Patch test: Study of 242 randomly selected eczema patients patch tested with coumarin 5.8%. Vehicle not reported.	9/242 (3.7%) patients were positive.		Van Joost et al., 1985 cited from SCCNFP (1999).
Patch test: Study of 241 consecutive patients patch tested with coumarin 5%. Vehicle not reported.	2/241 (0.8%) patients were positive.		Ferguson and Shama 1984 cited from SCCNFP (1999).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with coumarin 8% in pet.	12/182 (6.8%) patients were positive.	Coumarin 8% was tested in 54 controls with no positive reactions.	Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 20 selected perfume allergic patients patch tested with coumarin 5%. Vehicle not reported.	2/20 (10%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).

Case studies

Table 3 summarises case reports with allergic contact dermatitis in different clinics in Europe where coumarin has been found as a causative agent.

Table 3. Case studies with coumarin.

Method	Results	Remarks/Comments	Reference
Patch test: A 44-year old woman with dermatitis after use of deodorant and eau de toilette was tested by ROAT[1] with chemical fractions of perfume concentrate from her eau de toilette.	Coumarin was confirmed as the allergen by ROAT with 1% after having caused dermatitis by the use of a deodorant containing coumarin at 0.23%.	Case study, Europe (year not stated).	Mutterer et al. (1999).
Patch test: A woman with eczema caused by a perfumed lotion was patch tested with coumarin 0.5% in pet and diluent.	Positive reaction was observed.	Case study, Denmark (year not stated).	Johansen et al., 1994 cited from SCCNFP (1999).

[1]ROAT: Repeat Open Application Test

A total of 25 patch test population studies and 2 case studies, one of which included a ROAT, with coumarin are summarised above (Table 2 and 3). As shown in Table 2 the positive patch test frequencies from all of the reported patch test studies vary between 0 and 10% in dermatitis patients. For unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% (7 studies) and for selected dermatitis patients positive reactions range between 0 and 10% (19 studies). The total number of published cases is > 200.

According to IFRA (2008b) a NOEL[10]-HRIPT (induction) of 3543 µg/cm² and a NOEL-HMT (induction) of 5517 µg/cm² has been established for coumarin. On basis of data from HRIPT or HMT (not specified by IFRA (2008b)) a LOEL[11] (induction) of 8858 µg/cm² for coumarin was derived.

Coumarin is a ”top 100” substance and is according to SCCS (2012) classified as a skin sensitiser with R43 (based on the old classification criteria) (note that the substance does not have a harmonised classification as a skin sensitizer).

According to SCCS (2012) coumarin is used in volumes higher than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 17% of a total of 516 products; 30% of a total of 300 products; ca. 11% of

[10] NOEL: No Observed Effect Level

[11] LOEL: Lowest Observed Effect Level

3000 products and 4.8% of children cosmetics were labelled to contain coumarin (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 33% of 88 tested deodorants were labelled to contain coumarin and the fragrance was detected in 52% (range: 3.8-1255 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for coumarin in different IFRA QRA product categories reported by IFRA (2008b and 2015) are shown in table 4.

Table 4. The IFRA standard limits for coumarin in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.1%
Category 2	Deodorants/antiperspirants	0.13%
Category 3	Hydroalcoholics for shaved skin	0.5%
Category 4	Hydroalcoholics for unshaved skin	1.6%
Category 5	Hand cream	0.8%
Category 6	Mouthwash	2.5%
Category 7	Intimate wipes	0.3%
Category 8	Hair styling aids	2.0%*
Category 9	Rinse-off hair conditioners	5.0%*
Category 10	Hard surface cleaners	2.5%*
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

* Maximum pragmatic level.

Coumarin is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 25 patch test population studies and 2 case studies, one of which included a ROAT, were identified with coumarin. The positive patch test frequencies from all of the reported patch test studies vary between 0 and 10% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% (7 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 10% (19 studies). The total number of published cases is > 200. A LOEL (induction) of 8858 µg/cm² was derived from the HRIPT/HMT studies.

Non-human data

A total of 20 LLNAs and 1 GPMT were identified testing skin sensitising effects of coumarin. The collected evidence from the LLNAs indicates an EC3 for coumarin of ca. 50%. Sensitisation was not observed in the GPMT after an intradermal induction dose of 0.5% coumarin.

Contaminants in coumarin may act as weak or moderate sensitizers (SCCS, 2012).

No relevant *in vitro* studies on coumarin (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure
According to data from IFRA (2008b) the exposure of coumarin when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria
For unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% i.e. all 7 studies reporting frequencies lower than 1%. For selected dermatitis patients positive reactions range between 0 and 10% with 9 out of 19 studies reporting frequencies higher than 2%. In addition to this there are more than 200 published cases of positive patch test reactions to coumarin. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that coumarin causes a *low/moderate frequency* of occurrence of skin sensitisation based on unselected/consecutive dermatitis patients and 10/19 studies with selected dermatitis patients. The remaining studies with selected dermatitis patients (9/19) and number of published cases shows that coumarin causes a *high frequency* of occurrence of skin sensitisation in humans.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate classification of coumarin in sub-category 1B according to Annex I: 3.4.2.2.2.2.

The collected evidence from the LLNAs indicates an EC3 for coumarin of ca. 50%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, all studies indicate classification of coumarin in sub-category 1B.

The single GPMT with an intradermal induction dose of 0.5% gave no positive reactions which does not justify sub-categorisation (Table 3.4.3).

Overall, there is evidence for classification in sub-category 1A based on the number of cases combined with the estimated low exposure and supported by patch test data from selected dermatitis patients. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for coumarin.

Conclusions on classification and labelling
Based on the number of cases combined with the estimated low exposure and supported by patch test data from selected dermatitis patients, a classification of coumarin as a skin sensitiser in sub-category 1A is justified.

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Appendix 8 Eugenol CAS RN 97-53-0

According to SCCS (2012) eugenol is a fragrance compound known to be a prohapten and to form sensitising compounds by metabolic transformation.

Non-human information

Table 1 summarises relevant animal studies with eugenol i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with eugenol.

Method	Results	Remarks/Comments	Reference
LLNA: BrdU-ELISA 25% eugenol. Vehicle: acetone:olive oil (AOO).	Eugenol was shown to have a mean SI of 2.37	The aim of the study was to validate the use of BALB/c mice in the LLNA:BrdU-ELISA (OECD TG 442B).	Hou et al. (2015).
LLNA: Concentrations of eugenol not specified. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 4.2%.		Strauss et al. (2015).
LLNA: BrdU-ELISA *in vivo* and *ex vivo* BrdU. 0, 2, 20 and 40% eugenol. Vehicle: AOO.	Eugenol was shown to have an EC2 value of 8.5% in the BrdU-ELISA *in vivo* and an EC2 value of 9.5% in the *ex vivo* BrdU.	Eugenol was classified as positive for skin sensitisation by Williams et al. (2015).	Williams et al. (2015).
LLNA: *Ex vivo* BrdU. 2.5, 10, 20 and 50% eugenol. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 16.6%.	Eugenol was classified as a weak skin sensitizer by Ulker et al. (2013).	Ulker et al. (2013).
LLNA (OECD TG 429): 2.5, 10 and 25% eugenol. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 4.6%.		Basketter (2012).
LLNA: LLNA:BrdU-FCM. 5, 10 and 25% eugenol. Vehicle: AOO (proportion not specified).	Eugenol was shown to have an EC3 value of 10.1%.	Eugenol was classified as a weak skin sensitizer by Jung (2012).	Jung (2012).
LLNA: 0, 5, 10 and 25% eugenol.	Eugenol was shown to have an EC3 value of		Fukuyama et al. (2010).

Method	Results	Remarks/Comments	Reference
Vehicle: 4:1 AOO.	5.28%.		
LLNA: 1, 3, 10, 30 and 50% eugenol. Vehicle: 3:1 Ethanol:Diethyl phthalate (EtOH:DEP).	Eugenol was shown to have an EC3 value of 5.3% (0.32 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001f) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% eugenol. Vehicle: 1:3 EtOH:DEP.	Eugenol was shown to have an EC3 value of 10.5% (0.64 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001g) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% eugenol. Vehicle: EtOH.	Eugenol was shown to have an EC3 value of 10.7% (0.65 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001h) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% eugenol. Vehicle: DEP.	Eugenol was shown to have an EC3 value of 15.1% (0.92 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001i) cited from SCCS (2012).
LLNA: 0, 2.5, 5, 10 and 25% eugenol tested in five different laboratories. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 5.8, 14.5, 8.9, 13.8 and 6%, respectively.		Basketter et al. (2007).
LLNA (OECD TG 429): 0, 2.5, 5, 10, 25 and 50% eugenol. Vehicle: 1:3 EtOH:DEP.	Eugenol was shown to have an EC3 value of 5.4%.		Lalko and Api (2006).
LLNA: BrdU-ELISA 0, 1, 6, 15 and 30% eugenol. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 25.1%.		Takeyoshi et al. (2004).
LLNA: 2.5, 5, 10, 25 and 50% eugenol. Vehicle: 4:1 AOO.	Eugenol was shown to have an EC3 value of 11.9% (0.72 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Basketter et al., 1999 cited from SCCS (2012).
GPMT: Intradermal	Positive reactions in 2/10 (20%) of		Takeyoshi et al. (2004).

Method	Results	Remarks/Comments	Reference
induction 5%; topical induction 5%; challenge dose 5% eugenol. Vehicle not reported.	the tested animals.		

A total of 15 LLNAs are summarised in table 1. One study, validating the use of BALB/c mice in the LLNA:BrdU-ELISA (OECD TG 442B), reported a mean stimulation index (SI) of 2.37 (Hou et al., 2015) and one study reported EC2 values of 8.5 and 9.5% (Williams et al., 2015). The remaining 13 studies reported EC3 values between 4.2 and 25.1%. In the single GPMT study a 5% eugenol induction and challenge concentration resulted in positive reactions in 20% of the tested animals.

No relevant *in vitro* studies on eugenol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on eugenol involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with eugenol.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with eugenol 1% in petrolatum (pet.).	54/806 (6.7%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with cinnamaldehyde 2% in pet.	12/1951 (0.62%, 95% CI: 0.3-1%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 940 selected patients tested with eugenol 1% in pet.	118/940 (12.6%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with eugenol 1% in pet.	7/100 (7%, 95% CI: 2.86-13.89%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical	Nagtegaal et al. (2012).

Method	Results	Remarks/Comments	Reference
		Centre, The Netherlands (2005-2010).	
Patch test: Retrospective study of 1502 consecutive eczema patients patch tested with eugenol 1% in pet.	4/1502 (0.3%, 95% CI: 0.1-0.6%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with eugenol 5% in pet.	87/157 (55.4%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).
Patch test: Retrospective study of 1214 consecutive patients and 4527 selected patients patch tested with eugenol 1%. Vehicle not reported.	5/1214 (0.44%, 95% CI: 0.04-0.84%) and 71/4527 (1.57%, 95% CI: 1.19-1.95%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Retrospective study of 86 selected patients patch tested with eugenol 2% in pet.	12/86 (13.9%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 167 selected patients patch tested with eugenol 2% in pet.	4/167 (2.4%) patients were positive.	A retrospective study at the Division and School of Allergy and Clinical Immunology, Department of Human Pathology, University of Messina, Italy (year not stated).	Minciullo et al. (2010).
Patch test: Prospective study of 18 selected cinnamon-sensitive patients patch tested with eugenol 2% in pet.	3/18 (17%) patients were positive.	Prospective study of cinnamon-sensitive patients at the Department of Dermatology of the VU University Medical Centre, The Netherlands (year not stated).	Pentinga et al. (2009).

Method	Results	Remarks/Comments	Reference
Patch test: Prospective study of 320 selected eczema patients patch tested with eugenol 2% pet.	4/320 (1.3%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 225 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with eugenol 1% in pet.	a) 30/225 (13.3%) and b) 53/225 (23.4%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with eugenol 2%. Vehicle not reported.	2/15 (13.3%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).
Patch test: Retrospective study of 2065 unselected patients patch tested with eugenol 1%. Vehicle not reported.	11/2065 (0.5%, 95% CI: 0.2-0.7%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Study of 30 selected patients with a positive patch test to their own perfumed product patch tested with eugenol. Concentration and vehicle not reported.	6/30 (20%) patients were positive.		Vocanson et al. (2006).
Patch test: prospective study of 422 selected patients with suspected contact allergy patch tested with eugenol 2% pet.	8/422 (1.9%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Retrospective study of 4900 unselected patients patch tested with eugenol 1% in pet.	93/4900 (1.9%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Retrospective study of 1750 selected patients with suspected	21/1750 (1.2%) patients were positive.	A retrospective analysis of patients from Italy (1998-2000).	Giusti et al. (2001).

Method	Results	Remarks/Comments	Reference
fragrance allergic contact dermatitis patch tested with eugenol 1% in pet.			
Patch test: Prospective study of 747 selected patients with suspected fragrance allergy patch tested with eugenol 1% in pet.	19/747 (2.5%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).
Patch test: Study of 226 selected patients sensitive to fragrance mix (FM) patch tested with eugenol 1% in pet.	33/226 (14.6%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999)	Brites et al. (2000).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with eugenol ext. 2% in 1 % sorbitan sesquioleate (SSO).	6/50 (12%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with eugenol in pet. Concentration not reported.	2/40 (5%) patients were positive.		Katsarma and Gawkrodger (1999).
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with eugenol 5%. Vehicle not reported.	13/167 (7.8%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Prospective study of 1072 consecutive patients patch tested with eugenol 1%. Vehicle not reported.	13/1072 (1.2%) patients were positive.	Prospective study of patients in a multicentre study involving 9 European centres. Year not stated.	Frosch et al., 1995 cited from SCCNFP (1999).
Patch test: Retrospective study of 367 selected patients patch tested with eugenol 1-2% in pet.	30/367 (8.2%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	Johansen and Menne (1995).
Patch test: Prospective study of 50 selected patients positive to a FM	3/50 (6%) patients were positive.	Retrospective study of patch test data from Department of	Becker et al. (1994).

Method	Results	Remarks/Comments	Reference
patch tested with eugenol. Concentration and vehicle not reported.		Dermatology and Venereology, Hungary. Year not stated.	
Patch test: Prospective study of 61 selected patients positive to a FM patch tested with eugenol 5% in pet.	12/61 (19.7%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with eugenol 5% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Prospective study of 162 selected patients positive to a FM patch tested with eugenol 1% in pet.	11/162 (6.8%) patients were positive.	Prospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1991).	Enders et al. (1989).
Patch test: Study of 78 selected patients positive to a FM patch tested with eugenol 2%. Vehicle not reported.	8/78 (10.3%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Study of 156 selected patients with pure contact allergy to cosmetic products patch tested with eugenol. Concentration and vehicle not reported.	11/156 (7.1%) patients were positive.		Broneck et al., 1987 cited from SCCNFP (1999)
Patch test: Retrospective study of 63 selected patients with dermatitis tested positive to perfume mixture patch tested between 1983 and 1984 with eugenol 5% in pet. and 54 selected patients with dermatitis tested positive to perfume mixture patch tested between 1984 and 1985 with eugenol 1% in pet.	Between 1983 and 1984 8/63 (12.7%) and between 1984 and 1985 9/54 (16.7%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with eugenol. Concentration and	4/403 (1%) patients were positive.	It is unclear from the reference exactly how many patients were tested with eugenol.	Adams and Maibach (1985).

Method	Results	Remarks/Comments	Reference
vehicle not reported.			
Patch test: Study of 20 selected perfume allergic patients patch tested with eugenol 2%. Vehicle not reported.	4/20 (20%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).
Patch test and ROAT[1]: 5 patients tested positive to FM I and eugenol (2%) were tested with a dilution series of eugenol in patch test (17 dilutions) and a ROAT (0.5, 1 and 2.7% eugenol).	4/5 patients were positive to concentrations down to 1.32% eugenol. 4/5 patients became positive to 2.7% eugenol and 1/5 became positive to 1% eugenol in the ROAT.	A prospective analysis of patients from Sweden (year not stated).	Svedman et al. (2012).

[1]ROAT: Repeated Open Application Test

Case studies

Table 3 summarises the case reports with allergic contact dermatitis where eugenol has been found as a causative agent.

Table 3. Case studies with eugenol.

Method	Results	Remarks/Comments	Reference
Patch test: A 35-year old dental nurse with vesicular hand eczema and rhinitis was patch tested with eugenol (2% in petrolatum) and an intermediate restorative material (IRM® liquid) (10% pet.). The IRM® liquid contained >99% eugenol.	Eugenol (2% pet.): weak positive reaction. IRM® liquid: ++ patch test reaction.	Case study, Finland (year not stated).	Kanerva et al. (1998).

A total of 36 patch test population studies and 1 case study with eugenol are summarised above (Table 2 and 3). As shown in Table 2 the positive patch test frequencies from all of the reported patch test studies vary between 0.3 and 55.4% in dermatitis patients. For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% (5 studies) and for selected dermatitis patients positive reactions range between 0.62 and 55.4% (31 studies). The total number of published cases is > 700.

In a study by Svedman and co-workers it was shown that 4/5 patients tested positive to eugenol concentrations down to 1.32% also tested positive to 2.7% eugenol in a Repeated Open Application Test (ROAT) (Svedman et al., 2012).

The Research Institute for Fragrance Materials, Inc. (RIFM) reported a NOEL-HRIPT[12] (induction) of 5906 µg/cm² for eugenol. In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 5900 µg/cm² was established for eugenol by the RIFM Expert Panel (IFRA, 2008c).

According to SCCS (2012) eugenol is used in volumes greater than 175 ton per year in perfume formulations. It has been reported that 15.7% of a total of 516 consumer products; 27% of a total of 300 consumer products, ca. 7.5% of 3000 consumer products and 7.2% of children cosmetics were labelled to contain eugenol (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen and Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 27.3% of 88 tested deodorants were labelled to contain eugenol and the fragrance was detected in 30% (range: 1-514 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)). Eugenol is a ”top 100” substance and is according to SCCS classified as a skin sensitiser with R43 (based on the old classification criteria) (SCCS, 2012) (note that the substance does not have a harmonised classification as a skin sensitizer).

The IFRA standard limits for eugenol in different IFRA QRA product categories reported by IFRA (2008c and 2015) are shown in table 4.

Table 4. The IFRA standard limits for eugenol in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.2%
Category 2	Deodorants/antiperspirants	0.2%
Category 3	Hydroalcoholics for shaved skin	0.5%
Category 4	Hydroalcoholics for unshaved skin	0.5%
Category 5	Hand cream	0.5%
Category 6	Mouthwash	4.3%
Category 7	Intimate wipes	0.4%
Category 8	Hair styling aids	0.5%
Category 9	Rinse-off hair conditioners	0.5%
Category 10	Hard surface cleaners	0.5%
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Eugenol is registered under the REACH regulation with an annual tonnage band of 100 - 1 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 36 patch test population studies, 1 ROAT and 1 case study were identified with eugenol. The positive patch test frequencies from all of the reported patch test studies vary between 0.3 and

[12] NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test

55.4% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% (5 studies) and in studies with selected dermatitis patients positive reactions range between 0.62 and 55.4% (31 studies). The total number of published cases is > 700. A NESIL from HRIPT studies of 5900 µg/cm² was derived based on weight of evidence by the RIFM Expert Panel.

Non-human data
A total of 15 LLNAs and one GPMT were identified testing the skin sensitisation of eugenol. EC3 values were reported in 13 studies and ranged between 4.2 and 25.1%. Positive reactions (20%) were observed in the GPMT at an intradermal induction dose of 5%.

No relevant *in vitro* studies on eugenol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure
According to data from IFRA (2008c) the exposure of eugenol when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria
For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% with 2 out of 5 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.62 and 55.4% with 25 out of 31 studies reporting frequencies higher than 2%. In addition to this there are more than 700 published cases of positive patch test reactions to eugenol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that eugenol causes a *high frequency* of occurrence of skin sensitisation based on the frequency of positive reactions mainly in selected dermatitis patients (>2% in 21/27 studies) and the total number of cases.
In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNA tests, EC3 values between 4.2 and 25.1% (13 studies) were reported for eugenol. According to the CLP Regulation an EC3 value larger than 2 indicates placement of eugenol into sub-category 1B (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 20% of the animals after an intradermal induction dose of 5% which does not justify sub-categorisation into either sub-category 1A or 1B (Annex I: 3.4.2.2.3.2.).

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies mainly with selected dermatitis patients and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for eugenol.

Conclusions on classification and labelling
Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases, combined with the estimated low exposure, a classification of eugenol as a skin sensitiser in sub-category 1A is justified.

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Appendix 9 Farnesol CAS RN 4602-84-0

Non-human information

Table 1 summarises relevant animal studies with farnesol i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with farnesol.

Method	Results	Remarks/Comments	Reference
LLNA: 5, 10 and 25% farnesol. Vehicle: 4:1 Acetone: Olive Oil (AOO).	Farnesol was shown to have an EC3 value of 5.5% (0.25 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429 except that farnesol should also have been tested at lower concentrations.	Unpublished summary report by RIFM 2009 (RIFM 2004d) cited from SCCS (2012).
LLNA: 5, 10 and 25% farnesol. Vehicle: 4:1 AOO.	Farnesol was shown to have an EC3 value of 4.1% (0.18 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429 except that farnesol should also have been tested at lower concentrations.	Unpublished summary report by RIFM 2009 (RIFM 2004d) cited from SCCS (2012).
GPMT: Intradermal induction 10% farnesol in vaseline diluted in peanut oil; Topical induction 10%; Challenge dose 25, 50 or 100% farnesol in vaseline.	No positive reactions reported.		Unpublished report by RIFM 1983a cited from Lapczynski et al. (2008a).
GPMT: Intradermal induction 5% farnesol in peanut oil; Topical induction 100%; Challenge dose 25% farnesol in peanut oil.	No positive reactions reported.		Unpublished report by RIFM 1995b cited from Lapczynski et al. (2008a).
GPMT: Induction 10%; Challenge dose 10% farnesol.	No positive reactions reported.		Ishihara et al., 1986 cited from Lapczynski et al. (2008a).

Method	Results	Remarks/Comments	Reference
Vehicle not reported.			
GPMT: Induction 0.16%; Challenge dose 0.16% farnesol in acetone.	No positive reactions reported.		Watanbe et al., 1985 cited from Lapczynski et al. (2008a).

A total of two LLNAs and four GPMTs are summarised in table 1. The reported EC3 values in the two LNNAs were 5.5 and 4.1%, respectively. In the GPMTs no positive reactions were observed after intradermal induction doses of 0.16, 5 and 10% farnesol.

No relevant *in vitro* studies on farnesol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on farnesol involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with farnesol.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 324 selected patients patch tested with farnesol 5% in petrolatum (pet.).	39/324 (12%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with farnesol 5% in pet.	8/1951 (0.41%, 95% CI: 0.1-0.7%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 205 selected patients tested with farnesol 5% in pet.	27/205 (13.2%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with farnesol 5% in pet.	10/100 (10%, 95% CI: 4.9-17.62%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU	Nagtegaal et al. (2012).

Method	Results	Remarks/Comments	Reference
		University Medical Centre, The Netherlands (2005-2010).	
Patch test: Prospective study of 565 selected patients patch tested with farnesol 5% in vaseline.	14/565 (2.5%) patients were positive.	A prospective study on patch test data from multicentre study, Hungary (2009-2010).	Ponyai et al. (2012).
Patch test: Retrospective study of 1502 consecutive eczema patients patch tested with farnesol 5% in pet.	6/1502 (0.4%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011, 2012).
Patch test: Retrospective study of 86 selected patients patch tested with farnesol 5% in pet.	1/86 (1.2%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 367 selected fragrance mix (FM) II positive patients patch tested with farnesol 5% in pet.	42/367 (11.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2005-2008).	Krautheim et al. (2010).
Patch test: Prospective study of 320 selected eczema patients patch tested with farnesol 5% in pet.	3/320 (0.9%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 4238 unselected patients patch tested with farnesol 5% in pet.	38/4238 (0.9%, 95% CI: 0.6-1.2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Prospective study of 1701 consecutive	6/1701 (0.35%, 95% CI: 0.13-0.77%) patients were positive.	A prospective analysis of patients from six dermatology departments	Frosch et al. (2005).

Method	Results	Remarks/Comments	Reference
patients patch tested with farnesol 5% in pet.		(Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	
Patch test: Prospective study of 1855 consecutive patients patch tested with farnesol 5% in pet.	10/1855 (0.5%) patients were positive.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al. (2002a).
Patch test: Study of 102 selected patients patch tested with farnesol 5% in pet.	4/102 (4%) patients were positive.		Hausen et al., 2001 cited from Lapczynski et al. (2008a).
Patch test: Study of 1483 selected patients with suspected cosmetic dermatitis patch tested with farnesol 5% in pet.	16/1483 (1.1%) patients were positive.	Nagoya, Japan (year not stated).	Sugiura et al., 2000 cited from SCCS (2012).
Patch test: Retrospective study of 8521 selected patients patch tested with farnesol. Concentration and vehicle not reported.	2/8521 (0.02%) patients were positive.	Retrospective study performed at the Department of Dermatology, University Hospital, Leuven, Belgium (1985-1997).	Goossens and Merckx 1997 cited from SCCNFP (1999).
Patch test: Study of 111 selected patients patch tested with farnesol 1% in lanolin.	8/111 (7.2%) patients were positive.		Goossens and Merckx 1997 cited from SCCNFP (1999).
Patch test: Study of 466 selected patients patch tested with farnesol (2, 5 and 10%). Vehicle not reported.	5/466 (1.1%) patients were positive to 5 or 10% and 1/466 (0.2%) were positive to 2%.	Patch test study performed by the Japanese society of contact dermatitis. Year not stated.	Sugai et al., 1994 cited from SCCNFP (1999).
Patch test: Study of 573 selected patients patch	7/573 (1.2%) patients were positive.		Hirose et al., 1987 cited from Lapczynski et al.

Method	Results	Remarks/Comments	Reference
tested with farnesol 20% in pet.			(2008a).
Patch test: Study of 1367 selected patients patch tested with farnesol 2, 5 or 10% in pet.	11/1367 (0.8%) patients were positive.		Yamamoto et al., 1985 cited from Lapczynski et al. (2008a).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with farnesol 4% in pet.	2/182 (1.1%) patients were positive.	Farnesol 4% was tested in 20 control eczema patients with no positive reactions.	Malten et al., 1984 cited from SCCNFP (1999).

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with farnesol.

Table 3. HRIPT and HMT studies with farnesol adapted from Lapczynski et al. (2008a).

Method	Results	Remarks/Comments	Reference
HRIPT: Farnesol concentration: 5% (2865 µg/cm^2) Vehicle: 3:1 Diethyl phthalate:Ethanol (DEP:EtOH).	0/108 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (2004c) cited from Lapczynski et al. (2008a).
HRIPT: Farnesol concentration: 5% (1529 µg/cm^2) Vehicle: petrolatum (pet.).	0/103 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (2000b) cited from Lapczynski et al. (2008a).
HRIPT: Farnesol concentration: 5% (1529 µg/cm^2) Vehicle: pet.	0/101 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (2000a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm^2) Vehicle: pet.	0/35 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1978) cited from Lapczynski et al. (2008a).

Method	Results	Remarks/Comments	Reference
HMT: Farnesol concentration: 10% (6900 µg/cm^2) Vehicle: pet.	4/25 (16%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1977b) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm^2) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1977a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm^2) Vehicle: pet.	0/26 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1977a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 10% (6900 µg/cm^2) Vehicle: pet.	6/25 (24%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1976b) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 10% (6900 µg/cm^2) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1976a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm^2) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1975d) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm^2) Vehicle: pet.	2/25 (8%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1975c) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm^2) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1975b) cited from Lapczynski et al. (2008a).

Method	Results	Remarks/Comments	Reference
HMT: Farnesol concentration: 12% (8280 µg/cm^2) Vehicle: pet.	7/25 (28%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1975a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm^2) Vehicle: pet.	4/25 (16%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1974a) cited from Lapczynski et al. (2008a).

HRIPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Test.

Case studies

Table 4 summarises case reports with allergic contact dermatitis where farnesol has been found as a causative agent.

Table 4. Case studies with farnesol.

Method	Results	Remarks/Comments	Reference
Patch test: A 48-year old metalworker with recurrent hand dermatitis was patch tested with farnesol and a long list of other allergens.	Farnesol and several other allergens tested positive.	Case study (Germany, 2007-2008).	Tanko et al. (2009).
Patch test: A woman with axillary dermatitis due to a deodorant was patch tested with farnesol.	Positive result at 5% farnesol.	Case study (location and year not stated).	Goossens and Merckx 1997 cited from SCCNFP (1999).

A total of 20 patch test population studies, 3 HRIPTs, 11 HMTs and 2 case studies with farnesol are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0.02 and 13.2% in dermatitis patients. For unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% (4 studies) and for selected dermatitis patients positive reactions range between 0.02 and 13.2% (16 studies). The total number of published cases is > 250. No positive results were reported in the three HRIPTs at farnesol concentrations of 2865 µg/cm² or lower. In the HMTs 2/3 studies with farnesol showed positive results after 6900 µg/cm² and 3/8 studies showed positive results after 8280 µg/cm². Based on these data the Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL[13]-HRIPT (induction) of 2755 µg/cm² and a LOEL[14] (induction) of 68 974

[13] NOEL: No Observed Effect Level.

[14] LOEL: Lowest Observed Effect Level.

µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 2700 µg/cm² was established for farnesol by the RIFM Expert Panel (IFRA, 2006).

According to SCCS (2012) farnesol is used in volumes less than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 3.9% of a total of 516 consumer products; 8% of a total of 300 consumer products; ca. 4% of 3000 products and 2.9% of children's cosmetics were labelled to contain farnesol (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 14.8% of 88 tested deodorants were labelled to contain farnesol and the fragrance was detected in 39% (range: 9-1791 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for farnesol in different IFRA QRA product categories reported by IFRA (2006 and 2015) are shown in table 5.

Table 5. The IFRA standard limits for farnesol in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.08%
Category 2	Deodorants/antiperspirants	0.11%
Category 3	Hydroalcoholics for shaved skin	0.4%
Category 4	Hydroalcoholics for unshaved skin	1.2%
Category 5	Hand cream	0.6%
Category 6	Mouthwash	2.0%
Category 7	Intimate wipes	0.2%
Category 8	Hair styling aids	2.0%*
Category 9	Rinse-off hair conditioners	5.0%*
Category 10	Hard surface cleaners	2.5%*
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.
*Maximum pragmatic level.

Farnesol is not registered under the REACH regulation.

Summary and discussion of skin sensitization

Human data

A total of 20 patch test population studies, 3 HRIPTs, 11 HMTs and 2 case studies were identified with farnesol. The positive patch test frequencies from all of the reported patch test population studies vary between 0.02 and 13.2% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% (4 studies) and in studies with selected dermatitis patients positive reactions range between 0.02 and 13.2% (16 studies). The total number of published cases is > 250. Positive responses after farnesol were seen at concentrations ≥ 6900 µg/cm² in 5/11 HMTs.

Non-human data

A total of two LLNAs and four GPMTs were identified testing skin sensitising effects of farnesol. EC3 values were 5.5 and 4.1%. In the GPMTs no positive reactions were observed after intradermal induction doses of 0.16, 5 and 10% farnesol.

No relevant *in vitro* studies on farnesol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure
According to data from IFRA (2006) the exposure of farnesol when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria
For unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% i.e. all 4 studies reporting frequencies lower than 1%. For selected dermatitis patients positive reactions range between 0.02 and 13.2% with 7 out of 16 studies reporting frequencies higher than 2%. In addition to this there are more than 250 published cases of positive patch test reactions to farnesol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that farnesol causes a *low/moderate frequency* of occurrence of skin sensitisation based on unselected/consecutive dermatitis patients and 9/16 studies with selected dermatitis patients. The remaining studies with selected dermatitis patients (7/16) and number of published cases shows that farnesol causes a *high frequency* of occurrence of skin sensitisation in humans.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate classification of farnesol in sub-category 1B according to Annex I: 3.4.2.2.2.2.
In the two LLNAs the lowest EC3 value for farnesol was 4.1%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, both studies indicate classification of farnesol in sub-category 1B.

Sensitisation was not observed in the four GPMTs with intradermal induction doses of 0.16, 5 and 10% farnesol which do not justify sub-categorisation (Table 3.4.3).

Overall, there is evidence for classification in sub-category 1A based on the number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for farnesol.

Conclusions on classification and labelling
Based on the number of cases combined with the estimated low exposure, a classification of farnesol as a skin sensitiser in sub-category 1A is justified.

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Appendix 10 Geraniol CAS RN 106-24-1

Non-human information

Table 1 summarises relevant animal studies with geraniol i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with geraniol.

Method	Results	Remarks/Comments	Reference
LLNA: *Ex vivo* BrdU. 2.5, 10, 20 and 50% geraniol. Vehicle: 4:1 Acetone:Olive oil (AOO).	Geraniol was shown to have an EC3 value of 13.1%.		Ulker et al. (2014).
LLNA: 0, 5, 10, 15, 20 and 30% geraniol. Vehicle: AOO.	Geraniol was shown to have an EC3 value of 22.4% (1.45 M).		Hagvall et al. (2007).
LLNA: 0, 1, 3, 6, 10 and 20% **air-exposed** geraniol. Vehicle: AOO.	Air-exposed geraniol was shown to have an EC3 value of 4.4% (0.28 M).		Hagvall et al. (2007).
LLNA: 0.5, 1, 3, 6 and 10% **air-exposed** geraniol. Vehicle: AOO.	Air-exposed geraniol was shown to have an EC3 value of 5.8% (0.37 M).		Hagvall et al. (2007).
LLNA: 1, 3, 10, 30 and 50% geraniol. Vehicle: Ethanol (EtOH).	Geraniol was shown to have an EC3 value of 5.6% (0.36 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001j) cited from SCCS (2012).
LLNA: 2.5, 5, 10, 25 and 50% geraniol. Vehicle: 3:1 EtOH:diethyl phthalate (DEP).	Geraniol was shown to have an EC3 value of 11.4% (0.74 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003t) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% geraniol. Vehicle: DEP.	Geraniol was shown to have an EC3 value of 11.8% (0.76 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001k) cited from SCCS (2012).

Method	Results	Remarks/Comments	Reference
LLNA: 1, 3, 10, 30 and 50% geraniol. Vehicle: 1:3 EtOH:DEP.	Geraniol was shown to have an EC3 value of 20.4% (1.32 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001l) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% geraniol. Vehicle: 3:1 EtOH:DEP.	Geraniol was shown to have an EC3 value of 25.8% (1.67 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001m) cited from SCCS (2012).
GPMT: Intradermal induction 0.1% geraniol in Dobs/saline; Topical induction 50% in 70/30 acetone/PEG 400; Challenge dose 10% in 70/30 acetone/PEG 400.	No positive reactions reported.		Unpublished report by RIFM 1989 cited from Lapczynski et al. (2008b).
GPMT: Intradermal induction 0.1% geraniol in Dobs/saline; Topical induction 50% in acetone; Challenge dose 10% in acetone.	Sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Unpublished report by RIFM 1989 cited from Lapczynski et al. (2008b).
GPMT: Intradermal induction 5% geraniol in petrolatum (pet.); Topical induction 30% in pet; Challenge dose 10% in pet.	Sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Unpublished report by RIFM 1977 cited from Lapczynski et al. (2008b).
GPMT: Intradermal induction 5% geraniol in pet.; Topical induction 25% in pet; Challenge dose sub-irritant.	Sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Klecak et al., 1977 cited from Lapczynski et al. (2008b).

Method	Results	Remarks/Comments	Reference
GPMT: Intradermal induction 10% geraniol; Topical induction 10%; Challenge dose 10%. Vehicle not reported.	Sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Ishihara et al., 1986 cited from Lapczynski et al. (2008b).
Buehler: Induction concentration 15% geraniol in DEP ; Challenge dose 2.5, 7.5 or 25% in DEP.	No sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Unpublished report by RIFM 1992 cited from Lapczynski et al. (2008b).

A total of 9 LLNAs, 5 GPMTs and 1 Buehler test are summarised in table 1. The reported EC3 values for geraniol range between 5.6% (vehicle: ethanol) and 25.8% (vehicle: ethanol:diethyl phthalate 1:3). Air-exposed geraniol was tested in two LLNAs with resulting EC3 values of 4.4 and 5.8%. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol. No sensitisation was observed in the Buehler test with an induction concentration of 15% geraniol.

No relevant *in vitro* studies on geraniol (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on geraniol involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with geraniol.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with geraniol 1 % in petrolatum (pet.).	31/806 (3.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with geraniol 2% in pet.	9/1951 (0.46%, 95% CI: 0.2-0.8%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).

Method	Results	Remarks/Comments	Reference
Patch test: Prospective study of 655 consecutive patients patch tested with geraniol 4 and 11% in pet.	1/655 (0.15%) and 7/655 (1.1%) patients were positive after 4 and 11% geraniol, respectively.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall et al. (2013).
Patch test: Prospective study of 649 consecutive patients patch tested with geraniol 6% in pet.	3/649 (0.46%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall et al. (2013).
Patch test: Prospective study of 655 consecutive patients patch tested with **oxidised** geraniol 4 and 6% in pet.	6/655 (0.92%) and 15/655 (2.3%) patients were positive after 4 and 16% geraniol, respectively.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall et al. (2013).
Patch test: Prospective study of 653 consecutive patients patch tested with **oxidised** geraniol 11% in pet.	30/655 (4.6%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall et al. (2013).
Patch test: Prospective study of 2227 consecutive patients patch tested with geraniol 2% in pet.	3/2227 (0.13%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2006-2010).	Hagvall et al. (2012).
Patch test: Prospective study of 2179 consecutive patients patch tested with **oxidised** geraniol 2% in pet.	12/2179 (0.55%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2006-2010).	Hagvall et al. (2012).
Patch test: Retrospective study of 940 selected patients tested with	52/940 (5.5%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël,	Nardelli et al. (2013).

Method	Results	Remarks/Comments	Reference
geraniol 1% in pet.		Belgium (1990-2011).	
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with geraniol. Vehicle and concentration not reported.	9/100 (9%, 95% CI: 4.2-16.4%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1502 consecutive eczema patients patch tested with geraniol 1% in pet.	0/1502 (0 %) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011, 2012).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with geraniol 5% in pet.	Ca. 31/157 (ca. 20%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).
Patch test: Retrospective study of 86 selected patients patch tested with geraniol 2% in pet.	17/86 (19.7%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 1214 consecutive patients and 5695 selected patients patch tested with geraniol in 1% pet.	5/1214 (0.39%, 95% CI: 0.10-0.69%) and 50/5695 (0.87%, 95% CI: 0.63-1.1%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Prospective study	2/320 (0.6%) patients were	A prospective analysis of selected eczema	van Oosten et al. (2009).

Method	Results	Remarks/Comments	Reference
of 320 selected eczema patients patch tested with geraniol 2% in pet.	positive.	patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	
Patch test: Retrospective study of 89 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with geraniol 1% in pet.	a) 15/89 (16.85%) and b) 22/89 (24.72%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with geraniol 2% in pet.	0/15 (0%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).
Patch test: Retrospective study of 2063 unselected patients patch tested with geraniol 1% in pet.	10/2063 (0.5%, 95% CI: 0.1-0.7%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Retrospective study of a) 29 patients positive to their own deodorant and b) 141 negative to their own deodorant patch tested with geraniol 1% in pet.	a) 2/29 (7%) and b) 0/141 (0%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998-2002).	Uter et al. (2007).
Patch test: Study of 30 selected	6/30 (20%) patients were		Vocanson et al. (2006).

Method	Results	Remarks/Comments	Reference
patients with a positive patch test to their own perfumed product patch tested with geraniol. Concentration and vehicle not reported.	positive.		
Patch test: Study of 658 patients patch tested with geraniol 5% in pet.	6/658 (0.9%) patients were positive.		Heydorn et al., 2003 cited from Lapczynski et al. (2008b).
Patch test: Study of 315 patients patch tested with geraniol 5% in pet.	0/315 (0%) patients were positive.		Heydorn et al., 2002 cited from Lapczynski et al. (2008b).
Patch test: Retrospective study of 4900 unselected patients patch tested with geraniol 1% in pet.	59/4900 (1.2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Study of 160 patients sensitive to fragrance mix (FM) patch tested with geraniol. Concentration and vehicle not reported.	12/160 (7.5%) patients were positive.		Temesvari et al., 2002 cited from Hostynek and Maibach (2004).
Patch test: Prospective study of 747 selected patients with suspected fragrance allergy patch tested with geraniol 1% in pet.	7/747 (0.9%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).
Patch test: Study of 226 patients patch tested with geraniol 1% in	19/226 (8.4%) patients were positive.		Brites et al., 2000 cited from Lapczynski et al. (2008b).

Method	Results	Remarks/Comments	Reference
pet.			
Patch test: Study of 934 patients patch tested with geraniol 1% in pet.	67/934 (7.2%) patients were positive.		Buckley et al., 2000 cited from Lapczynski et al. (2008b).
Patch test: Study of 223 patients patch tested with geraniol 2%. Vehicle not reported.	1/223 (0.4%) patients were positive.		Kiec-Swierczynska & Krecisz 2000 cited from Lapczynski et al. (2008b).
Patch test: Study of 1483 selected patients with suspected cosmetic dermatitis patch tested with geraniol 5% in pet.	5/1483 (0.3%) patients were positive.	Nagoya, Japan (year not stated).	Sugiura et al., 2000 cited from SCCS (2012).
Patch test: Ten-centre study of 542 patients sensitive to FM patch tested with geraniol in pet. Concentration not reported.	58/542 (10.7%) patients were positive.		Bordalo et al., 1999 cited from Hostynek and Maibach (2004).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with geraniol 2% in 1% sorbitan sesquioleate.	3/50 (6%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with geraniol in pet. Concentration not reported.	0/40 (0%) patients were positive.		Katsarma and Gawkrodger (1999).
Patch test: Study of 38 patients sensitive to FM	5/38 (13.2%) patients were positive.		Katsarou 1999 cited from Hostynek and

Method	Results	Remarks/Comments	Reference
patch tested with geraniol 1% in pet.			Maibach (2004).
Patch test: Study of 8 patients sensitive to FM patch tested with geraniol 20% in pet.	3/8 (37.5%) patients were positive.		Goossens & Merckx 1997 cited from Hostynek and Maibach (2004).
Patch test: Study of 41 eczema patients patch tested with geraniol 1% in pet.	1/41 (2.4%) patients were positive.		Schauder & Ippen 1997 cited from Hostynek and Maibach (2004).
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with geraniol 5%. Vehicle not specified.	5/167 (3%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Prospective study of 1072 consecutive patients patch tested with geraniol 1% in pet.	4/1072 (0.4%) patients were positive.	Prospective study of patients in a multicentre study involving 9 European centres. Year not stated.	Frosch et al., 1995a cited from SCCNFP (1999).
Patch test: Multicentre study of 702 eczema patients patch tested with geraniol 1% in pet.	5/702 (0.7%) with SSO and 3/702 (0.4%) without SSO patients were positive.	No further information available from Hostynek and Maibach (2004).	Frosch et al., 1995b cited from Hostynek and Maibach (2004).
Patch test: Retrospective study of 367 selected patients patch tested with geraniol in 1-2% pet.	15/367 (4%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	Johansen and Menne (1995).
Patch test: Prospective study	3/50 (6%) patients were	Retrospective study of patch test data from	Becker et al. (1994).

Method	Results	Remarks/Comments	Reference
of 50 selected patients sensitive to FM patch tested with geraniol. Concentration and vehicle not reported.	positive.	Department of Dermatology and Venereology, Hungary. Year not stated.	
Patch test: Prospective study of 61 selected patients sensitive to FM patch tested with geraniol 5% in pet.	8/61 (13%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with geraniol 5% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Study of 103 patients patch tested with geraniol 5% in pet.	4/103 (3.9%) patients were positive.		Haba et al., 1993 cited from Lapczynski et al. (2008b).
Patch test: Study of 20 volunteers (age: 1-18 years) patch tested with geraniol 1% in pet.	0/20 (0%) patients were positive.	The study by Adifadel et al 1992 has a reliability score of 2 according to REACH-RD(2015i).	Abifadel et al., 1992 cited from REACH-RD (2015i).
Patch test: Study of 111 patients patch tested with geraniol 5% pet.	1/111 (0.9%) patients were positive.		Nagareda et al., 1992 cited from Lapczynski et al. (2008b).
Patch test: Study of 115 patients patch tested with geraniol 5% in vaseline.	0/115 (0%) patients were positive.		Remaut 1992 cited from Lapczynski et al. (2008b).
Patch test: Multi-centre study of 17 patients sensitive to FM patch tested with geraniol 1% in pet.	2/17 (12%) patients were positive.		Roesyanto-Mahadi et al., 1990 cited from Hostynek and Maibach (2004).
Patch test: Study of 20 patients sensitive to fragrance patch	2/20 (10%) patients were positive.		Safford et al., 1990 cited from Hostynek and Maibach (2004).

Method	Results	Remarks/Comments	Reference
tested with geraniol 2% in pet.			
Patch test: Prospective study of 162 selected patients sensitive to FM patch tested with geraniol 1%. Vehicle not reported.	4/162 (2.5%) patients were positive.	Retrospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1987).	Enders et al. (1989).
Patch test: Study of 200 patients sensitive to FM patch tested with geraniol 1-3% in pet.	14/200 (7%) patients were positive.		Malanin & Ohela 1989 cited from Hostynek and Maibach (2004).
Patch test: Study of 52 eczema patients patch tested with geraniol 2% in pet.	2/52 (3.8%) patients were positive.		Nethercott et al., 1989 cited from Hostynek and Maibach (2004).
Patch test: Study of 78 selected patients sensitive to FM patch tested with geraniol 1%. Vehicle not reported.	4/78 (5.1%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Study of 119 patients patch tested with geraniol 5% in pet.	2/119 (1.7%) patients were positive.		De Groot et al., 1988 cited from Lapczynski et al. (2008b).
Patch test: Study of 31 patients sensitive to oak moss patch tested with geraniol. Vehicle not reported.	5/31 (16%) patients were positive.		Goncalo et al., 1988 cited from Hostynek and Maibach (2004).
Patch test: Study of 156 selected patients with pure contact allergy to cosmetic products patch tested with	2/156 (1.2%) patients were positive.		Broneck et al., 1987 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
geraniol. Concentration and vehicle not reported.			
Patch test: Study of 574 selected patients patch tested with geraniol 20% in pet.	5/574 (0.9%) patients were positive.		Hirose et al., 1987 cited from Lapczynski et al. (2008b).
Patch test: Retrospective study of 63 selected patients with dermatitis tested positive to perfume mixture patch tested between 1983 and 1984 with geraniol 3% in pet. and 54 selected patients with dermatitis tested positive to perfume mixture patch tested between 1984 and 1985 with geraniol 1% in pet.	Between 1983 and 1984 4/63 (6.3%) and between 1984 and 1985 4/54 (7.4%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Study of 830 patients patch tested with geraniol 5%. Vehicle not reported.	6/830 (0.7%) patients were positive.		Itoh et al., 1986 cited from Lapczynski et al. (2008b).
Patch test: Study of 299 patients sensitive to FM patch tested with geraniol 2% in pet.	10/299 (3.3%) patients were positive.		Rudzki & Grzywa 1986 cited from Hostynek and Maibach (2004).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with	8/403 (2%) patients were positive.	It is unclear from the reference exactly how many patients were tested with geraniol.	Adams and Maibach (1985).

Method	Results	Remarks/Comments	Reference
geraniol. Concentration and vehicle not reported.			
Patch test: Study of 144 patients sensitive to FM patch tested with geraniol 1% in pet.	10/144 (7%) patients were positive.		Angelini et al., 1985 cited from Hostynek and Maibach (2004).
Patch test: Study of 1033 patients patch tested with geraniol 2% pet.	6/1033 (0.6%) patients were positive.		Cronin 1985 cited from Lapczynski et al. (2008b).
Patch test: Study of 179 patients patch tested with geraniol 10% in pet.	11/179 (6.2%) patients were positive.		De Groot et al., 1985 cited from Lapczynski et al. (2008b).
Patch test: Study of 50 cosmetic allergic patients patch tested with geraniol 5% in pet.	20/50 (40%) patients were positive.		Emmons and Marks 1985 cited from Hostynek and Maibach (2004).
Patch test: Study of 242 patients patch tested with geraniol 7%. Vehicle not reported.	1/242 (0.4%) patients were positive.		Van Joost et al., 1985 cited from Lapczynski et al. (2008b).
Patch test: Study of 241 patients patch tested with geraniol 2% in yellow paraffin.	10/241 (4.2%) patients were positive.		Ferguson and Sharma 1984 cited from SCCNFP (1999).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with geraniol 1% in pet.	3/182 (1.6%) patients were positive.		Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 522 patients patch tested with	3/522 (0.6%) patients were positive.		Nishimura et al., 1984 cited from Lapczynski et al.

Method	Results	Remarks/Comments	Reference
geraniol 5%. Vehicle not reported.			(2008b).
Patch test: Study of 242 patients patch tested with geraniol 1%. Vehicle not reported.	1/242 (0.4%) patients were positive.		Van Joost et al., 1984 cited from Lapczynski et al. (2008b).
Patch test: Study of 181 patients patch tested with geraniol 20% in pet.	7/181 (3.9%) patients were positive.		Hayakawa et al., 1983 cited from Lapczynski et al. (2008b).
Patch test: Study of 467 patients patch tested with geraniol 2%. Vehicle not reported.	1/467 (0.2%) patients were positive.		Ohela and Saramies 1983 cited from Lapczynski et al. (2008b).
Patch test: Study of 23 fregrance sensitive patients patch tested with geraniol 1% in pet.	3/23 (13%) patients were positive.		Sugai 1983 cited from Hostynek and Maibach (2004).
Patch test: Study of 539 patients patch tested with geraniol 2% yellow paraffin.	8/539 (1.5%) patients were positive.		Addo et al., 1982 cited from Lapczynski et al. (2008b).
Patch test: Prospective multicentre study of 487 patients allergic to cosmetics patch tested with geraniol. Concentration and vehicle not reported.	5/487 (1%) patients were positive.		Eiermann et al., 1982 cited from Hostynek and Maibach (2004).
Patch test: Study of 155 patients patch tested with geraniol 5% in pet.	1/155 (0.6%) patients were positive.		Itoh 1982 cited from Lapczynski et al. (2008b).
Patch test: Study	28/1277 (2.2%)		Sugai 1982 cited

Method	Results	Remarks/Comments	Reference
of 1277 patients patch tested with geraniol 2% in pet.	patients were positive.		from Lapczynski et al. (2008b).
Patch test: Study of 172 patients patch tested with geraniol 2% pet.	7/172 (4%) patients were positive.		Calnan et al., 1980 cited from Lapczynski et al. (2008b).
Patch test: Study of 198 patients patch tested with geraniol 2% vaseline.	0/198 (0%) patients were positive.		Ishihara et al., 1979 cited from Lapczynski et al. (2008b).
Patch test: Study of 198 patients patch tested with geraniol 5% in vaseline.	3/198 (1.5%) patients were positive.		Ishihara et al., 1979 cited from Lapczynski et al. (2008b).
Patch test: Study of 20 selected perfume allergic patients patch tested with geraniol 5%. Vehicle not reported.	6/20 (30%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).
Patch test: Study of 792 eczema patients patch tested with geraniol 10% in pet.	4/792 (0.5%) patients were positive.		Fregert and Hjorth 1969 cited from Hostynek and Maibach (2004).
Patch test: Study of 15 eczema patients allergic to Balsam of Peru patch tested with geraniol 10% in pet.	2/15 (13%) patients were positive.		Hjorth 1961 cited from Hostynek and Maibach (2004).
Patch test: Study of 3 eczema patients patch tested with geraniol 1% in acetone.	1/3 (33%) patients were positive.		Keil 1947 cited from Hostynek and Maibach (2004).

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with geraniol.

Table 3. HRIPT and HMT studies with geraniol adapted from Lapczynski et al. (2008b).

Method	Results	Remarks/Comments	Reference
HRIPT: Geraniol concentration: 2% (2362 µg/cm^2) Vehicle: 3:1 Diethyl phthalate:Ethanol (DEP:EtOH).	0/110 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (2000) cited from Lapczynski et al. (2008b).
HRIPT: Geraniol concentration: 5% (5905 µg/cm^2) **plus 0.5% tocopherol** Vehicle: 3:1 DEP:EtOH.	1/109 (0.9%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (2002) cited from Lapczynski et al. (2008b).
HRIPT: Geraniol concentration: 10% (11810 µg/cm^2) Vehicle: 3:1 DEP:EtOH.	3/112 (2.7%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (2004) cited from Lapczynski et al. (2008b).
HRIPT: Geraniol concentration: 5% (3876 µg/cm^2) Vehicle: alcohol SDA 39C.	0/40 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (1964) cited from Lapczynski et al. (2008b).
HRIPT: Geraniol concentration: 12.5% (9690 µg/cm^2) Vehicle: EtOH.	0/41 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (1964a) cited from Lapczynski et al. (2008b).
HRIPT (modified): Geraniol concentration: 10% Vehicle: petrolatum (pet.).	0/104 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Marzulli and Maibach 1980 cited from Lapczynski et al. (2008b).
HRIPT (modified): Geraniol concentration: 10% Vehicle: alcohol.	2/73 (2.7%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Marzulli and Maibach 1980 cited from Lapczynski et al.

Method	Results	Remarks/Comments	Reference
			(2008b).
HMT: Geraniol concentration: 6% Vehicle: not reported.	0/25 (0%) tests were positive.	No further information available from REACH-RD (2015j). This study was administered a reliability score of 4 (not assignable) in REACH-RD (2015j).	Study report from 1986 cited from REACH-RD (2015j).
HMT: Geraniol concentration: 6% (4140 µg/cm^2) Vehicle: pet.	0/24 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (1979) cited from Lapczynski et al. (2008b).
HMT: Geraniol concentration: 6% (4140 µg/cm^2) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Grief (1967) cited from Lapczynski et al. (2008b).
HMT: Geraniol concentration: 6% (4140 µg/cm^2) Vehicle: pet.	1/26 (3.8%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (1979a) cited from Lapczynski et al. (2008b).

HRIPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Test.

Case studies

Table 4 summarises case reports with allergic contact dermatitis where geraniol has been found to be among the causative agents.

Table 4. Case studies with geraniol.

Method	Results	Remarks/Comments	Reference
Patch test: A 54-year old female bartender with chronic hand dermatitis was patch tested with geraniol and a long list of other allergens present in her environment.	Geraniol, lime peel, FM I and FM II tested positive.	Case study (year not reported).	Swerdlin et al. (2010).
Patch test: A 48-year old male metalworker with	Geraniol and several other allergens tested	Case study (Germany, 2007-2008).	Tanko et al. (2009).

recurrent hand dermatitis was patch tested with geraniol and a long list of other allergens.	positive.

A total of 84 results from patch test population studies, 7 HRIPTs, 4 HMTs and 2 case studies with geraniol are summarised above (Table 2, 3 and 4). In addition there were four patch test studies with oxidised geraniol. As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0 and 40% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0 and 1.2% (10 studies) and for selected dermatitis patients positive reactions range between 0 and 40% (74 studies). The total number of published cases is > 900.
Sensitisation (2.7%) was reported in a HRIPT at a geraniol concentration of 11 810 μg/cm² (10%) and in a modified HRIPT at 10% geraniol. Geraniol (5905 μg/cm²) and tocopherol (0.5%) lead to sensitisation in 1/109 subjects. In the HMTs 1/4 studies with geraniol showed a positive result after 4140 μg/cm². Based on these data the Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT[15] (induction) of 11 811 μg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 11 800 μg/cm² was established for geraniol by the RIFM Expert Panel (IFRA, 2007).

Geraniol is a ”top 100” substance and is according to SCCS (2012) classified as a skin sensitiser with R43 (based on the old classification criteria) (note that the substance does not have a harmonised classification as a skin sensitizer).

Geraniol is identified as a prehapten (compounds which sensitization potency are markedly increased by air exposure due to oxidation) and forms oxidation products with increased sensitizing capacity both via spontaneous autoxidation at air exposure and via metabolic oxidation (SCCS, 2012).

According to SCCS (2012) geraniol is used in volumes greater than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 22.1% of a total of 516 consumer products; 42% of a total of 300 consumer products; ca. 20% of 3000 products and 12% of children’s cosmetics were labelled to contain geraniol (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 48.9% of 88 tested deodorants were labelled to contain geraniol and the fragrance was detected in 87% (range: 1-399 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

In a recent study the Acceptable Exposure Level (AEL) was estimated for geraniol based on human studies and LLNA data. Comparing the AEL with the estimated aggregate dermal exposure of geraniol from personal care products and household cleaning agents it was shown that between 0.02 and 0.86% of the population may have an aggregated exposure of geraniol which exceeds the lowest AEL of 55 μg/cm² (Nijkamp et al., 2015).

The IFRA standard limits for geraniol in different IFRA QRA product categories reported by IFRA (2007 and 2015) are shown in table 5.

[15] NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test.

Table 5. The IFRA standard limits for geraniol in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.3%
Category 2	Deodorants/antiperspirants	0.4%
Category 3	Hydroalcoholics for shaved skin	1.8%
Category 4	Hydroalcoholics for unshaved skin	5.3%
Category 5	Hand cream	2.8%
Category 6	Mouthwash	8.6%
Category 7	Intimate wipes	0.9%
Category 8	Hair styling aids	2.0%*
Category 9	Rinse-off hair conditioners	5.0%*
Category 10	Hard surface cleaners	2.5%*
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.
*Maximum pragmatic level.

Geraniol is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 84 results from patch test population studies, 7 HRIPTs, 4 HMTs and 2 case studies were identified with geraniol. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 40% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 1.2% (10 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 40% (74 studies). The total number of published cases is > 900. Sensitisation was reported in 2/4 HRIPT studies after exposure to 10% geraniol (11 810 µg/cm²) and in 1/4 HMT studies after exposure to 4140 µg/cm².

Non-human data

A total of 9 LLNAs, 5 GPMTs and 1 Buehler test were identified testing skin sensitising effects of geraniol. The reported EC3 values for geraniol ranged between 5.6% and 25.8% in different vehicles. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol. No sensitisation was observed in 1/5 GMPTs with an induction concentration of 50% geraniol and in the Buehler test with an induction concentration of 15% geraniol.
No relevant *in vitro* studies on geraniol (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure

According to data from IFRA (2007) the exposure of geraniol when used as fragrance in cosmetics and in other consumer products appears to be relatively low. A recent study has indicated that up to 0.86% of the population might be exposed to geraniol from personal care products and household cleaning agents at levels exceeding the estimated Acceptable Exposure Level of 55 µg/cm² (Nijkamp et al., 2015).

Comparison with criteria
For unselected/consecutive dermatitis patients positive reactions range between 0 and 1.2% with 2/10 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 40% with 44 out of 74 studies reporting frequencies higher than 2%. In addition to this there are more than 900 published cases of positive patch test reactions to geraniol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that geraniol causes a *high frequency* of occurrence of skin sensitisation based on patch test data mainly from selected dermatitis patients and the number of published cases.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate classification of geraniol in sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNAs EC3 values between 5.6 (vehicle: ethanol) and 25.8% (vehicle: ethanol:diethyl phthalate 1:3) were reported for geraniol. According to the CLP Regulation an EC3 value larger than 2% indicates classification of geraniol in sub-category 1B.

In the GPMTs sensitisation was reported to be observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol, therefore, these GPMTs cannot be compared with the classification criteria.

No sensitisation was observed in the Buehler test with an induction concentration of 15% geraniol.

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies mainly with selected dermatitis patients and the total number of cases combined with the estimated relatively low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for geraniol.

Conclusions on classification and labelling
Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases, combined with the estimated relatively low exposure, a classification of geraniol as a skin sensitiser in sub-category 1A is justified.

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Appendix 11 7-Hydroxycitronellal CAS RN 107-75-5

Non-human information

Table 1 summarises relevant animal studies with 7-hydroxycitronellal i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with 7-hydroxycitronellal.

Method	Results	Remarks/Comments	Reference
LLNA: *Ex vivo* BrdU. 0.5, 1, 5 and 10% 7-hydroxycitronellal. Vehicle: 4:1 Acetone:Olive oil (AOO).	7-hydroxycitronellal was shown to have an EC3 value of 9.8%.		Ulker et al. (2014).
LLNA: 1, 3, 10, 30 and 50% 7-hydroxycitronellal. Vehicle: 1:3 ethanol:diethyl phthalate (EtOH:DEP).	7-hydroxycitronellal was shown to have an EC3 value of 19.3% (1.12 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001n) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% 7-hydroxycitronellal. Vehicle: DEP.	7-hydroxycitronellal was shown to have an EC3 value of 19.7% (1.14 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001o) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% 7-hydroxycitronellal. Vehicle: 3:1 EtOH:DEP.	7-hydroxycitronellal was shown to have an EC3 value of 22.2% (1.29 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001p) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% 7-hydroxycitronellal. Vehicle: EtOH.	7-hydroxycitronellal was shown to have an EC3 value of 26.4% (1.53 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001q) cited from SCCS (2012).
LLNA: 2.5, 5, 10, 25 and 50% 7-hydroxycitronellal. Vehicle: 4:1 AOO.	7-hydroxycitronellal was shown to have an EC3 value of 33% (1.92 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Basketter et al., 2001 cited from SCCS (2012).
LLNA: 10 and 25% 7-	7-	7-hydroxycitronellal	Smith and

Method	Results	Remarks/Comments	Reference
hydroxycitronellal. Vehicle: 4:1 AOO.	hydroxycitronellal was shown to have an EC3 value of 23% (1.34 M).	was only tested in two concentrations.	Hotchkiss 2001 cited from SCCS (2012).
GPMT: Intradermal induction 0.5% 7-hydroxycitronellal; Topical induction 100% 7-hydroxycitronellal; Challenge dose 50% 7-hydroxycitronellal. Vehicle: 70:30 acetone:polyethylene glycol 400.	60% of the animals were positive (total number of animals not reported).	According to REACH-RD (2015a) the study is reliable with restrictions (reliability 2) and performed according to Magnusson and Kligman (1970).	Basketter and Scholes 1992 cited from REACH-RD (2015a).
Buehler: Induction concentration 10 and 30% 7-hydroxycitronellal; Challenge dose 3 and 10% 7-hydroxycitronellal. Vehicle: EtOH (induction), acetone (challenge).	30% induction: 3/8 (38%) animals showed sensitising effects. 10% induction: 0/8 (0%) animals showed sensitising effects.	According to REACH-RD (2015b) the study is reliable with restrictions (reliability 2) and performed according to Buehler (1965).	Buehler 1985 cited from REACH-RD (2015b).

A total of 7 LLNAs including 1 LLNA *ex vivo* BrdU, 1 GPMT and 1 Buehler test with 7-hydroxycitronellal are summarised in table 1. The reported EC3 values for 7-hydroxycitronellal range between 9.8% and 33% both with acetone:olive oil (4:1) as vehicle. In the GPMT sensitisation in 60% of the animals (number of animals not reported) after an intradermal induction dose of 0.5% 7-hydroxycitronellal. Sensitisation was also observed in 38% of the animals in the Buehler test with an induction concentration of 30%7-hydroxycitronellal. No sensitisation was observed after 10% 7-hydroxycitronellal in the Buehler test.

No relevant *in vitro* studies on 7-hydroxycitronellal (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on 7-hydroxycitronellal involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with 7-hydroxycitronellal.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with 7-hydroxycitronellal 1% in petrolatum (pet.).	77/806 (9.6%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with 7-hydroxycitronellal in 2% pet.	20/1951 (1%, 95% CI: 0.6-1.4%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 940 selected patients tested with 7-hydroxycitronellal in pet. Concentration not reported.	24/940 (2.6%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with 7-hydroxycitronellal in pet. Concentration not reported.	8/100 (8%, 95% CI: 3.52-15.16%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1498 consecutive eczema patients patch tested with 7-hydroxycitronellal in 1% pet.	13/1498 (0.9 %) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011) and Heisterberg et al. (2012) (corrigendum).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients	Ca. 31/157 (ca. 20%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and	Turcic et al. (2011).

Method	Results	Remarks/Comments	Reference
positive to fragrance allergens) patch tested with 7-hydroxycitronellal in 1% pet.		Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	
Patch test: Retrospective study of 86 selected patients patch tested with 7-hydroxycitronellal in 5% pet.	6/86 (7%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 1214 consecutive patients and 4359 selected patients patch tested with 7-hydroxycitronellal in 1%.	14/1214 (1.17%, 95% CI: 0.48-1.85%) and 129/4359 (2.95%, 95% CI: 2.43-3.47%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Prospective study of 320 selected eczema patients patch tested with 7-hydroxycitronellal in 2% pet.	7/320 (2.2%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 153 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with 7-hydroxycitronellal in 1% pet.	a) 41/153 (26.8%) and b) 49/153 (32.03%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with	0/15 (0%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).

Method	Results	Remarks/Comments	Reference
7-hydroxycitronellal in 2% pet.			
Patch test: Retrospective study of 2063 consecutive patients patch tested with 7-hydroxycitronellal in 1% pet.	27/2063 (1.3%, 95% CI: 0.7-1.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Retrospective study of a) 33 patients positive to their own deodorant and b) 204 negative to their own deodorant patch tested with 7-hydroxycitronellal in 1% pet.	a) 4/33 (12%) and b) 9/204 (4.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998-2002).	Uter et al. (2007).
Patch test: Retrospective study of a) 31 patients positive to their own shaving product/eau de toilette/perfume and b) 210 negative to their own shaving product/eau de toilette/perfume, patch tested with 7-hydroxycitronellal in 1% pet.	a) 4/31 (13%) and b) 4/210 (2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998-2002).	Uter et al. (2007).
Patch test: study of 30 selected patients with a positive patch test to their own perfumed product patch tested with hydroxycitronellal. Concentration and vehicle not reported.	11/30 (35%) patients were positive.		Vocanson et al. (2006).
Patch test: Retrospective study	127/4900 (2.6%) patients were	A retrospective study on patch test data	Schnuch et al. (2002).

Method	Results	Remarks/Comments	Reference
of 4900 unselected patients patch tested with 7-hydroxycitronellal in 1% pet.	positive. In addition, 566 patients had a + reaction to FM and 46 (8%) of them were positive to hydroxycitronellal 1 % pet. 425 patients had ++/+++ reaction to FM and 77 (18%) were positive to hydroxycitronellal 1 % pet.	from multicentre project IVDK (1996-1999).	
Patch test: Retrospective study of 160 selected patients sensitive to FM patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.	4/160 (2.5%) patients were positive.	A retrospective study on patch test data from the seven members of the Hungarian Contact Dermatitis Research Group (1998-1999).	Temesvari et al. (2002).
Patch test: prospective study of 747 selected patients with suspected fragrance allergy patch tested with 7-hydroxycitronellal 1% in pet.	11/747 (1.5%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).
Patch test: Study of 226 selected patients sensitive to FM patch tested with 7-hydroxycitronellal 1% in pet.	15/226 (6.6%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999).	Brites et al. (2000).
Patch test: Study of 1483 selected patients with suspected cosmetic dermatitis patch tested with 7-hydroxycitronellal in 5% pet.	15/1483 (1%) patients were positive.	Nagoya, Japan (year not stated).	Sugiura et al., 2000 cited from SCCS (2012).

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with 7-hydroxycitronellal 2% in 1% sorbitan sesquioleate.	10/50 (20%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with 7-hydroxycitronellal in pet. Concentration not reported.	1/40 (2.5%) patients were positive.		Katsarma and Gawkrodger (1999).
Patch test: Study of 11 patients with perfume allergy patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.	6/11 (55%) patients were positive.	The patients' cosmetic products were subjected to chemical analysis. The content of 7-hydroxycitronellal was at average 5 times higher in cosmetics from 7-hydroxycitronellal sensitive patients compared with 7-hydroxycitronellal negative patients.	Johansen et al., 1996 cited from SCCNFP (1999).
Patch test: study of 167 selected patients suspected of fragrance sensitivity patch tested with 7-hydroxycitronellal 4%. Vehicle not reported.	23/167 (13.8%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Study of 1072 patients patch tested with 7-hydroxycitronellal 1% in pet.	8/1072 (0.75%) patients were positive.	European multicentre study with 9 different centres.	Frosch et al., 1995 cited from SCCNFP (1999).
Patch test: Retrospective study of 367 selected patients patch tested with 7-	27/367 (7.4%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark	Johansen and Menne (1995).

Method	Results	Remarks/Comments	Reference
hydroxycitronellal in 1-2% pet.		(1979-1983 and 1988-1992).	
Patch test: Prospective study of 50 selected patients positive to a fragrance mix patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.	5/50 (10%) patients were positive.	Retrospective study of patch test data from Department of Dermatology and Venereology, Hungary. Year not stated.	Becker et al. (1994).
Patch test: Prospective study of 61 selected patients positive to a fragrance mix patch tested with 7-hydroxycitronellal 5% in pet.	12/61 (20%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with 7-hydroxycitronellal 5% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Prospective study of 162 selected patients positive to a fragrance mix patch tested with 7-hydroxycitronellal 1% in pet.	10/162 (6.2%) patients were positive.	Prospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1991).	Enders et al. (1989).
Patch test: Study of 78 selected patients sensitive to FM patch tested with 7-hydroxycitronellal 5%. Vehicle not reported.	7/78 (9%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Study of 156 selected patients with pure contact allergy to cosmetic products patch tested with 7-hydroxycitronellal.	6/156 (3.8%) patients were positive.		Broneck et al., 1987 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
Concentration and vehicle not reported.			
Patch test: Retrospective study of 63 selected patients with dermatitis tested positive to perfume mixture patch tested between 1983 and 1984 with 7-hydroxycitronellal in 5% pet. and 54 selected patients with dermatitis tested positive to perfume mixture patch tested between 1984 and 1985 with 7-hydroxycitronellal in 1% pet.	Between 1983 and 1984 13/63 (21%) and between 1984 and 1985 9/54 (16%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.	7/403 (1.7%) patients were positive.	The number of patients is not clearly stated in the article by Adams & Maibach 1985.	Adams and Maibach (1985).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with 7-hydroxycitronellal 10%. Vehicle not reported.	19/182 (10.5%) patients were positive.		Malten et al.,,, 1984 cited from SCCNFP (1999).
Patch test: study of 20 selected perfume allergic	9/20 (45%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
patients patch tested with 7-hydroxycitronellal in 4%.			
Patch test and ROAT[1]: 13 7-hydroxycitronellal-sensitive patients were tested with 10 and 250 mg/kg of 7-hydroxycitronellal in 10% ethanol in a patch test and a ROAT.	10 mg/kg: 1/13 (8%) positive and 250 mg/kg: 5/13 (38%) positive. Vehicle control: 4/13 (31%) positive.		Heydorn et al., 2003 cited from SCCS (2012).
Patch test and ROAT[1]: 7 7-hydroxycitronellal-sensitive patients and 7 controls were tested with a dilution series from 4 to 0.00006% (17 steps) of 7-hydroxycitronellal in a patch test and a ROAT.	Step 1: 57% positive, Step 2: 71% positive and Step 3: 100% positive reactions were observed. No positive reactions observed in patch test <0.00012% (0.036 µg/cm^2).		Svedman et al., 2003 cited from SCCNFP (1999).

[1]ROAT: Repeated Open Application Test

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with 7-hydroxycitronellal.

Table 3. HRIPT and HMT studies with 7-hydroxycitronellal.

Method	Results	Remarks/Comments	Reference
Modified HRIPT: 7-hydroxycitronellal concentration: 2.5% Vehicle: 3:1 ethanol:diethyl phthalate (EtOH:DEP).	0/65 (0%) tests were positive.		Ford et al. (1988).
Modified HRIPT: 7-hydroxycitronellal concentration: 5% Vehicle: 3:1 EtOH:DEP.	1/66 (2%) tests were positive.		Ford et al. (1988).
Modified HRIPT: 7-hydroxycitronellal concentration: 7.5% Vehicle: 3:1	1/66 (2%) tests were positive.		Ford et al. (1988).

Method	Results	Remarks/Comments	Reference
EtOH:DEP.			
Modified HRIPT: 7-hydroxycitronellal concentration: 2.5 or 5% Vehicle: 3:1 EtOH:DEP.	33/100 (33%) tests were positive.	The test was performed in 100 of the subjects that had completed the three HRIPTs described directly above.	Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 5% Vehicle: petrolatum (pet.).	0/26 (0%) tests were positive.		Unpublished data (Epstein 1976) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 5% Vehicle: pet.	0/25 (0%) tests were positive.	Males only	Unpublished data (Kligman 1973) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 10% Vehicle: pet.	2/25 (8%) tests were positive.		Unpublished data (Kligman 1976) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 10% Vehicle: pet.	0/25 (0%) tests were positive.		Unpublished data (Kligman 1976) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	4/27 (15%) tests were positive.		Unpublished data (Epstein 1978) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	0/25 (0%) tests were positive.		Unpublished data (Kligman 1978) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	7/26 (27%) tests were positive.		Unpublished data (Epstein 1979) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	6/26 (23%) tests were positive.		Unpublished data (Epstein 1979) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	0/25 (0%) tests were positive.	7-hydroxycitronellal purified from α-pinene	Unpublished data (Kligman 1979) cited from Ford et al. (1988).
HMT:	3/25 (12%)	7-hydroxycitronellal	Unpublished data

Method	Results	Remarks/Comments	Reference
7-hydroxycitronellal concentration: 12% Vehicle: pet.	tests were positive.	purified from *d*-stereoisomer	(Kligman 1979) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	0/25 (0%) tests were positive.	7-hydroxycitronellal purified from *l*-stereoisomer	Unpublished data (Kligman 1979) cited from Ford et al. (1988).
HMT: **Pseudo** 7-hydroxycitronellal concentration: 12% Vehicle: pet.	1/25 (4%) tests were positive.		Unpublished data (Kligman 1979) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: DEP.	2/22 (9%) tests were positive.	Lower boiling point fraction	Unpublished data (Epstein 1980) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: DEP.	1/26 (4%) tests were positive.	Higher boiling point fraction	Unpublished data (Epstein 1980) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: DEP.	2/21 (10%) tests were positive.	According to Ford et al 1988 7-hydroxycitronellal was "*Tested on same panel with washed cinnamic alcohol.*".	Unpublished data (Epstein 1980) cited from Ford et al. (1988).

HRIPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Test.

Case studies

Table 4 summarises case reports with ACD where 7-hydroxycitronellal has been found to be among the causative agents.

Table 4. Case studies with 7-hydroxycitronellal.

Method	Results	Remarks/Comments	Reference
Patch test: A 48-year old male metalworker with recurrent hand dermatitis was patch tested with 7-hydroxycitronellal and a long list of other allergens.	7-hydroxycitronellal and several other allergens tested positive.	Case study (Germany, 2007-2008).	Tanko et al. (2009).
Patch test: A 52-year old man with contact allergy to his after-shave was	Positive reaction was observed.	Case study, (year and country not stated).	De Groot and Liem 1983 cited from SCCNFP

Method	Results	Remarks/Comments	Reference
patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.			(1999).
Patch test: A 32-year old barber with hand eczema was patch tested with 10% 7-hydroxycitronellal in petrolatum.	Positive reactions to 7-hydroxycitronellal, methyl 2-octynoate and cinnamyl alcohol were observed.	Case study, (year and country not stated).	Van Ketel 1978 cited from SCCNFP (1999).

A total of 39 results from patch test population studies, 4 modified HRIPTs, 15 HMTs and 3 case studies with 7-hydroxycitronellal are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0 and 55% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0.9 and 2.6% (4 studies) and for selected dermatitis patients positive reactions range between 0 and 55% (35 studies). The total number of published cases is > 800. Sensitisation was reported in 3/4 modified HRIPT studies at 7-hydroxycitronellal concentrations from 2.5 to 7.5% but without dose-response. In the HMT studies, sensitisation was reported in 0/2 tests with 5% 7-hydroxycitronellal, 1/2 tests with 10% 7-hydroxycitronellal and in 8/11 tests with 12% 7-hydroxycitronellal. Based on these data the Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT[16] (induction) of 5000 µg/cm² and a LOEL-HRIPT/HMT[17] (induction) of 5906 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 5000 µg/cm² was established for 7-hydroxycitronellal by the RIFM Expert Panel (IFRA, 2013c).

According to SCCS (2012) 7-hydroxycitronellal is used in volumes less than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 10.8% of a total of 516 consumer products; 17% of a total of 300 consumer products; ca. 8% of 3000 products and 6.3% of children's cosmetics were labelled to contain 7-hydroxycitronellal (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 27.3% of 88 tested deodorants were labelled to contain 7-hydroxycitronellal and the fragrance was detected in 70% (range: 1-1746 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for 7-hydroxycitronellal in different IFRA QRA product categories reported by IFRA (2013c and 2015) are shown in table 5.

[16] NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test.

[17] LOEL-HRIPT/HMT: Lowest Observed Effect Level-Human Repeat Insult Patch Test/Human Maximisation test.

Table 5. The IFRA standard limits for 7-hydroxycitronellal in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.1%
Category 2	Deodorants/antiperspirants	0.2%
Category 3	Hydroalcoholics for shaved skin	0.8%
Category 4	Hydroalcoholics for unshaved skin	1.0%
Category 5	Hand cream	1.0%
Category 6	Mouthwash	3.6%
Category 7	Intimate wipes	0.4%
Category 8	Hair styling aids	1.0%
Category 9	Rinse-off hair conditioners	1.0%
Category 10	Hard surface cleaners	1.0%
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

7-Hydroxycitronellal is registered under the REACH regulation with an annual tonnage band of 100 - 1000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 39 results from patch test population studies, 4 modified HRIPTs, 15 HMTs and 3 case studies were identified with 7-hydroxycitronellal. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 55% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.9 and 2.6% (4 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 55% (35 studies). The total number of published cases is > 800. A LOEL-HRIPT/HMT (induction) of 5906 µg/cm² was established for 7-hydroxycitronellal by the RIFM Expert Panel.

Non-human data

A total of 7 LLNAs including 1 LLNA *ex vivo* BrdU, 1 GPMT and 1 Buehler test were identified testing skin sensitising effects of 7-hydroxycitronellal. The reported EC3 values for 7-hydroxycitronellal range between 9.8 and 33%. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the GPMT sensitisation in 60% of the animals (number of animals not reported) after an intradermal induction dose of 0.5% 7-hydroxycitronellal. Sensitisation was also observed in 38% of the animals in the Buehler test with an induction concentration of 30% 7-hydroxycitronellal.

No relevant *in vitro* studies on 7-hydroxycitronellal (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure

According to data from IFRA (2013c) the exposure of 7-hydroxycitronellal when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.9 and 2.6% with 1/4 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 55% with 29 out of 33 studies reporting frequencies equal to or higher than 2%. In addition to this there are more than 800 published cases of positive patch test reactions to 7-hydroxycitronellal. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b). The collected data described above from patch test studies show that 7-hydroxycitronellal causes a *high frequency* of occurrence of skin sensitisation based on these three types of information.
In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the seven LLNAs EC3 values between 9.8 and 33% were reported for 7-hydroxycitronellal. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, all seven studies indicate classification of 7-hydroxycitronellal in sub-category 1B.

In the GPMT sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.5% 7-hydroxycitronellal. According to the CLP criteria a positive response ≥ 60% of the animals responding at >0.1% to ≤ 1% intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of 7-hydroxycitronellal into sub-category 1A.

Sensitisation was also observed in 38% of the animals in a Buehler test with an induction concentration of 30% 7-hydroxycitronellal. According to the CLP criteria a positive response ≥ 15% of the animals responding at >20% topical induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.3.) and thus, this study indicates classification of 7-hydroxycitronellal into sub-category 1B.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. Except from the GMTP study, which supports a sub-category 1A classification, the remaining animal studies (LLNA and Buehler) indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is thus warranted for 7-hydroxycitronellal.

Conclusions on classification and labelling
Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure a classification of 7-hydroxycitronellal as a skin sensitiser in sub-category 1A is justified.

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Appendix 12 Methyl oct-2-ynate CAS RN 111-12-6

Non-human information

Table 1 summarises relevant animal studies with methyl oct-2-ynate i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization Tests (GPMTs) and Buehler tests.

Table 1. Animal studies with methyl oct-2-ynate.

Method	Results	Remarks/Comments	Reference
LLNA, according to OECD test guideline 429.	Methyl oct-2-ynate was shown to have an EC3 value of 0.45%.	The reaction mechanistic domain was reported to be the Michael receptor.	Unpublished report by RIFM 2006 cited from Kern et al. (2010).
LLNA, according to OECD test guideline 429. 0.5, 1, 2, 5 and 10% methyl oct-2-ynate. Vehicle not reported.	Methyl oct-2-ynate was shown to have an EC3 value of <0.5% (<0.032 M).	Methyl oct-2-ynate should also have been tested at lower concentrations.	Unpublished report by RIFM 2005k cited from SCCS (2012).
GPMT, intradermal induction 0.625, 5 and 10%; topical induction 1, 3 and 30%; challenge 0.3, 0.9 and 3% methyl oct-2-ynate. Vehicle not reported.	18/20 (90%) at the least severe and middle regimens and 20/20 (100%) at the most severe.	No further information was available from Hostynek and Maibach (2006).	Unpublished report by Buehler et al., 1985 cited from Hostynek and Maibach (2006).
Buehler test, induction dose 2.5% with challenge doses of 0.5, 1.5 and 5% methyl oct-2-ynate. Vehicle not reported.	Positive reactions in 9/20 (45%), 12/20 (60%) and 14/20 (70%) at challenge doses of 0.5, 1.5 and 5% methyl oct-2-ynate, respectively.	No further information was available from Hostynek and Maibach (2006).	Unpublished report by Buehler et al., 1986 cited from Hostynek and Maibach (2006).

A total of 2 LLNAs (OECD TG 429), 1 GPMT and 1 Buehler test with methyl oct-2-ynate are summarised in table 1. In both LLNA studies an EC3 value of <0.5% was reported. In the GPMT sensitisation was observed in 90 % of the animals after an intradermal induction dose of 0.625% methyl oct-2-ynate. Sensitisation was also observed in the Buehler test with positive reactions in 45-70% of the animals after an induction dose of 2.5%

According to the review by Hostynek and Maibach (2006) several other (mostly unpublished) animal tests including open and closed epicutaneous tests, Draize tests, a Maguire test, a Freund's Complete Adjuvant Test and an additional GPMT testing the sensitising potential of methyl oct-2-ynate exist. However, these tests were either not relevant for the purpose of sub-categorisation or considered by Hostynek and Maibach (2006) to be of poor quality and are not reported here.

No relevant *in vitro* studies on methyl oct-2-ynate (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human Studies

Population studies

Table 2 summarises patch test studies on methyl oct-2-ynate involving several thousand dermatitis patients from various countries in Europe and the US. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with methyl oct-2-ynate.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 1870 patients patch tested with methyl oct-2-ynate. Concentration and vehicle not reported.	3/1870 (0.16%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective and descriptive study of 1951 eczema patients patch tested with methyl oct-2-ynate 1% in petrolatum (pet.).	3/1951 (0.15%, 95% CI: 0.0-0.3%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective and descriptive study on 211 eczema patients tested with methyl oct-2-ynate 1% in pet.	1/211 (0.5%) patients were positive. Active sensitisation was observed in two patients and the testing was stopped.	Retrospective descriptive analysis of a patch test study at Gentofte Hospital, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Study of 230 consecutive eczema patients patch tested with methyl oct-2-ynate 1% in pet.	0/230 (0%) patients were positive.	Retrospective descriptive analysis of a patch test study at Gentofte Hospital, Denmark (2007-2008).	Heisterberg (2010).
Patch test: Study of 120 consecutive	2/120 (1.67%) patients were	Retrospective descriptive analysis of	Heisterberg (2010).

Method	Results	Remarks/Comments	Reference
eczema patients patch tested with methyl oct-2-ynate 2% in pet.	positive.	a patch test study at the department of Dermatologie at CHU Saint Jacques, France.	
Patch test: Study of 988 selected patients tested with methyl oct-2-ynate 1% in pet.	1/988 (0.1%, 95% CI: 0-0.2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Study of 320 selected eczema patients patch tested with methyl oct-2-ynate 0.5% in pet.	1/320 (0.3%) patients were positive.	Retrospective analysis of a patch test study at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Study of 2401 unselected patients patch tested with methyl oct-2-ynate 1% in pet.	6/2401 (0.2%, 95% CI: 0.0-0.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2003-2004).	Schnuch et al. (2007).
Patch test: Study of 182 patients suspected of contact allergy to cosmetics patch tested with 0.5% methyl oct-2-ynate. Vehicle not reported.	2/182 (1.1%) patients were positive.		Unpublished report by Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 34 patients with allergic contact dermatitis to cosmetics patch tested with 0.5% methyl oct-2-ynate. Vehicle not reported.	1/34 (2.9%) patients were positive.	Pilot study performed prior to the study described directly above.	Unpublished report by Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 278 patients patch tested with 1% methyl oct-2-	1/278 (0.4%) patients were positive.	A study performed by the North American Contact Dermatitis Research Group.	Unpublished report by Mitchell et al., 1982 cited from SCCNFP

Method	Results	Remarks/Comments	Reference
ynate. Vehicle not reported.			(1999).

Case studies

Table 3 summarises case reports with allergic contact dermatitis in different clinics in Europe where methyl oct-2-ynate has been found as a causative agent.

Table 3. Case studies with methyl oct-2-ynate.

Method	Results	Remarks/Comments	Reference
Patch test: A 42-year old woman under investigation for postsurgical [1]ACD and with a previous reaction towards deodorants was patch tested with 2% methyl oct-2-ynate in petrolatum (pet.).	Delayed positive reaction on D16 was observed.	Case study, France (year not stated).	Heisterberg (2010).
Patch test: A 28-year old woman with facial eczema was tested and repeat tested with 1% methyl oct-2-ynate in pet.	Delayed positive reaction on D20 and on D2 (1+) in the repeat test was observed.	Case study, Denmark (year not stated).	Heisterberg (2010).
Patch test: A 21-year old woman suspected of occupational hand eczema was patch tested and repeat tested with 1% methyl oct-2-ynate in pet.	Delayed positive reaction 4 weeks after first test and positive reaction on D2 (2+) in the repeat test was observed.	Case study, Denmark (year not stated).	Heisterberg (2010).
Patch test: A 19-year old woman with work-related [1]ACD was patch tested with 1% methyl oct-2-ynate in	Positive reaction on D2 was observed.	Case study, UK (1985).	English and Rycroft (1988).

Method	Results	Remarks/Comments	Reference
methyl ethyl ketone.			
Patch test: A 32-year old man with hand eczema was patch tested with 0.5% methyl oct-2-ynate in pet.	Positive reactions to methyl oct-2-ynate, 7-hydroxycitronellal and cinnamyl alcohol were observed.	Case study, (year and country not stated).	Van Ketel (1978) cited from SCCNFP (1999).

[1]ACD: Allergic Contact Dermatitis.

A total of 11 results from patch test population studies and 5 cases with methyl oct-2-ynate are summarised above (Table 2 and 3). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0 and 2.9% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0 and 1.67% (3 studies) and for selected dermatitis patients positive reactions range between 0.1 and 2.9% (8 studies). The total number of published cases is > 25. The Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT[18] (induction) of 118 µg/cm² and a LOEL-HRIPT/HMT[19] (induction) of 194 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 110 µg/cm² was established for methyl oct-2-ynate by the RIFM Expert Panel (IFRA, 2008d).

One of the case studies reports three individual cases (table 3) (Heisterberg, 2010). In these cases late patch test reactions (after 2-4 weeks) were observed. Positive repeat tests in two of these cases were reported indicating active sensitization (i.e. the subjects were sensibilized by the patch test). The third case was not repeat patch tested.

SCCS (2012) describes methyl oct-2-ynate as an extreme but rare allergen.

According to SCCS (2012) methyl oct-2-ynate is used in volumes less than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 1.1% of 88 tested deodorants (in 2007), 1% of a total of 516 consumer products; 0% of a total of 300 consumer products; ca. 0.5% of 3000 products and 0% of children's cosmetics were labelled to contain citral (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). Based on these data SCCS (2012) concluded that methyl oct-2-ynate was among the least used fragrance ingredients in cosmetics and other consumer products.

The IFRA standard limits for methyl oct-2-ynate in different IFRA QRA product categories reported by IFRA (2008d and 2015) are shown in table 4.

[18] NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test.

[19] LOEL-HRIPT/HMT: Lowest Observed Effect Level-Human Repeat Insult Patch Test/Human Maximisation test.

Table 4. The IFRA standard limits for methyl oct-2-ynate in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.003%
Category 2	Deodorants/antiperspirants	0.004%
Category 3	Hydroalcoholics for shaved skin	0.01%
Category 4	Hydroalcoholics for unshaved skin	0.01%
Category 5	Hand cream	0.01%
Category 6	Mouthwash	0.08%
Category 7	Intimate wipes	0.008%
Category 8	Hair styling aids	0.01%
Category 9	Rinse-off hair conditioners	0.01%
Category 10	Hard surface cleaners	0.01%
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Methyl oct-2-ynate is not registered under the REACH regulation.

Summary and discussion of skin sensitization

Human data

A total of 11 results from patch test population studies and 5 case studies were identified with methyl oct-2-ynate. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 2.9% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 1.67% (3 studies) and in studies with selected dermatitis patients positive reactions range between 0.1 and 2.9% (8 studies). The total number of published cases is > 25. A LOEL-HRIPT/HMT (induction) of 194 µg/cm² was established for methyl oct-2-ynate by the RIFM Expert Panel based on unpublished reports.

Non-human data

A total of 2 LLNAs (OECD TG 429), 1 GPMT and 1 Buehler test were identified testing skin sensitising effects of methyl oct-2-ynate. In both LLNA studies an EC3 value of <0.5% was reported. In the GPMT sensitisation was observed in 90 % of the animals after an intradermal induction dose of 0.625% methyl oct-2-ynate. Sensitisation was also observed in the Buehler test with positive reactions in 45-70% of the animals after an induction dose of 2.5%.

No relevant *in vitro* studies on methyl oct-2-ynate (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure

According to data from IFRA (2008d) the exposure of methyl oct-2-ynate when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria
For unselected/consecutive dermatitis patients positive reactions range between 0 and 1.67% with 1/3 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.1 and 2.9% with 1 out of 8 studies reporting frequencies higher than 2%. In addition to this there are more than 25 published cases of positive patch test reactions to methyl oct-2-ynate. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that methyl oct-2-ynate causes a *low/moderate frequency* of occurrence of skin sensitisation based on these three types of information.
In regard to HRIPT studies positive responses were observed at exposure to 194 μg/cm² methyl 2-ocytnoate. A positive response at ≤ 500 μg/cm² in a HRIPT or HMT suggests categorisation into sub-category 1A according to Annex I: 3.4.2.2.2.1 and 3.4.2.2.2.2.

In the 2 LLNAs EC3 values were <0.5%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 90% of the animals after an intradermal induction dose of 0.625%. According to the CLP criteria a positive response ≥ 60% of the animals responding at > 0.1% to ≤ 1% intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

In the Buehler test sensitisation was observed in 45-70% of the animals after an induction does of 2.5%. According to the CLP criteria a positive response ≥ 60% of the animals responding at > 0.2% to ≤ 20% topical induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

Overall, there is clear evidence for classification in sub-category 1A based on the very low EC3 values from the LLNAs. The results from GPMT and the Buehler test also supports sub-category 1A. Data from human patch test studies and the number of published cases justify classification of methyl oct-2-ynate in sub-category 1B while data from HRIPT studies justify classification of methyl oct-2-ynate in sub-category 1A. A classification as a skin sensitiser in sub-category 1A is thus warranted for methyl oct-2-ynate.

Conclusions on classification and labelling
Based on HRIPT data, the very low EC3 value from LLNAs and results from GPMT and Buehler tests a classification of methyl oct-2-ynate as a skin sensitser in sub-category 1A is justified.

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Appendix 13 *Cinnamomum cassia* leaf oil and *Cinnamomum zeylanicum*, ext. CAS RN 84961-46-6/8007-80-5 and 84649-98-9

Cinnamomum cassia leaf oil and C*innamomum zeylanicum* ext. are not specifically defined chemical substances but oils and extracts of leaves, twigs, wood, bark or the whole plant of *Chinese Cinnamom Cinnamomum cassia* (*L.*), *Lauraceae* and *Ceylon Cinnamon, Cinnamomum zeylanicum*, *Lauraceae*, respectively.

Searching for the CAS RN for *Cinnamomum cassia* leaf oil (84961-46-6 / 8007-80-5) in the European Cosmetic ingredient database CosIng yields a total of seven INCI names (Table 1).

Table 1. *Cinnamomum cassia* leaf oil.

CAS RN	EC No	INCI Name	Description
84961-46-6 / 8007-80-5	284-635-0 / -	*Cinnamomum cassia* leaf oil	"Cassia Oil"; "Cassia leaf Oil"; "Cinnamon Oil Chinense". *Cinnamomum Cassia* Leaf Oil is the volatile oil obtained by steam distillation from the **leaves and twigs** of the Chinese Cinnamom, *Cinnamomum cassia (L.), Lauraceae*.
		Cinnamomum cassia bark	*Cinnamomum Cassia* Bark is a plant material derived from the **dried bark** of the Chinese Cinnamon, *Cinnamomum cassia (L.), Lauraceae*.
		Cinnamomum cassia bark extract	*Cinnamomum Cassia* Bark Extract is an extract obtained from the **dried bark** of the Chinese Cinnamon, *Cinnamomum cassia (L.), Lauraceae*.
		Cinnamomum cassia extract	*Cinnamomum Cassia* Extract is the extract of the **whole plant**, *Cinnamomum cassia, Lauraceae*.
		Cinnamomum cassia leaf extract*	*Cinnamomum Cassia* Leaf Extract is an extract obtained from the **leaves** of the Chinese Cinnamon, *Cinnamomum cassia (L.), Lauraceae*.
		Cinnamomum cassia oil	*Cinnamomum Cassia* Oil is the volatile oil obtained from the **whole plant** of the Chinese Cinnamon, *Cinnamomum cassia (L.), Lauraceae*.
		Cinnamomum cassia wood extract*	*Cinnamomum Cassia* Wood Extract is an extract obtained from the **wood** of the Chinese Cinnamon, *Cinnamomum cassia (L.), Lauraceae*.

*According to the Cosmetic Directive not an INCI name but Perfuming Name (http://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.results).

The constituents of Cassia bark extract and Cassia oil and their concentrations are reported reported in Annex I to the IFRA Standards (48th Amendment) (http://www.ifraorg.org/en-us/search/s/84649-98-9#.Vjsc1rcveUk) and summarized in Table 2.

Table 2. Constituents of *Cinnamomum cassia* leaf oil.

***Cinnamomum cassia* leaf oil**				**Constituents**		
CAS RN	EC No	Principle Name	Botanical name	CAS RN	Principle name	Level (%)
84961-46-6 / 8007-80-5	284-635-0 / -	Cassia bark extract	*Cinnamomum cassia* Blume	1504-74-1	o-Methoxycinnamaldehyde	2
				100-52-7	Benzaldehyde	2
				120-51-4	Benzyl benzoate	0.07
				104-55-2	Cinnamaldehyde	44
				104-54-1	Cinnamyl alcohol	0.5
				91-64-5	Coumarin	0.15
				97-53-0	Eugenol	0.03
84961-46-6 / 8007-80-5	284-635-0 / -	Cassia oil	*Cinnamomum cassia* Blume	1504-74-1	o-Methoxycinnamaldehyde	4
				100-52-7	Benzaldehyde	4
				120-51-4	Benzyl benzoate	0.14
				104-55-2	Cinnamaldehyde	87
				104-54-1	Cinnamyl alcohol	1
				91-64-5	Coumarin	0.3
				97-53-0	Eugenol	0.06

Searching for the CAS RN for *Cinnamomum zeylanicum* bark oil (84649-98-9) in the European Cosmetic ingredient database CosIng yields five INCI names (Table 3).

Table 3. *Cinnamomum zeylanicum* bark.

CAS RN	EC No	INCI Name	Description
84649-98-9	283-479-0	*Cinnamomum zeylanicum* bark oil	("Cummamon Bark Oil Ceylon"; "Cinnamon Oil Ceylon". *Cinnamomum Zeylanicum* Bark Oil is the volatile oil expressed from the **bark** of the Ceylon Cinnamon, *Cinnamomum zeylanicum, Lauraceae*. It contains cinnamaldehyde (50-60%), eugenol (4-8%), phellandrene
		Cinnamomum zeylanicum bark extract	*Cinnamomum zeylanicum* Bark Extract is an extract obtained from the **dried bark** of the Ceylon Cinnamon, *Cinnamomum zeylanicum, Lauraceae*.
		Cinnamomum zeylanicum bark powder	*Cinnamomum zeylanicum* Bark Powder is the powder obtained from the **dried, ground bark** of the Ceylon Cinnamon, *Cinnamomum zeylanicum, Lauraceae*.
		Cinnamomum zeylanicum leaf extract	*Cinnamomum zeylanicum* Leaf Extract is an extract obtained from the **leaves** of the Ceylon Cinnamon, *Cinnamomum zeylanicum, Lauraceae*.
		Cinnamomum zeylanicum leaf oil	("Cinnamon Leaf Oil Ceylon". *Cinnamomum Zeylanicum* Leaf Oil is the volatile oil obtained from the **leaves** of the Ceylon Cinnamon, *Cinnamomum zeylanicum, Lauraceae*.

The constituents of Cinnamon bark extract, Cinnamon bark oil and cinnamon leaf oil and their concentrations are reported in Annex I to the IFRA Standards (48th Amendment) (http://www.ifraorg.org/en-us/search/s/84649-98-9#.Vjsc1rcveUk) and summarized in Table 4.

Table 4. Constituents of *Cinnamomum zeylanicum* ext.

***Cinnamomum zeylanicum* ext.**				**Constituents**		
CAS RN	EC No	Principle Name	Botanical name	CAS RN	Principle name	Level (%)
84649-98-9	283-479-0	Cinnamon bark extract	*Cinnamomum* spp.	100-52-7	Benzaldehyde	0.1
				120-51-4	Benzyl benzoate	0.3
				104-55-2	Cinnamaldehyde	38
				104-54-1	Cinnamyl alcohol	0.1
				91-64-5	Coumarin	0.3
				97-53-0	Eugenol	1
				97-54-1	Isoeugenol	0.01
84649-98-9	283-479-0	Cinnamon bark oil	*Cinnamomum* spp.	100-52-7	Benzaldehyde	0.26
				120-51-4	Benzyl benzoate	0.66
				104-55-2	Cinnamaldehyde	75
				104-54-1	Cinnamyl alcohol	0.26
				91-64-5	Coumarin	0.66
				97-53-0	Eugenol	2.2
				97-54-1	Isoeugenol	0.02
84649-98-9	283-479-0	Cinnamon leaf oil	*Cinnamomum zeylanicum* Blume	100-52-7	Benzaldehyde	0.16
				120-51-4	Benzyl benzoate	3.5
				104-55-2	Cinnamaldehyde	2
				91-64-5	Coumarin	0.3
				97-53-0	Eugenol	70
				4602-84-0	Farnesol	0.12

Cinnamomum zeylanicum ext.	Constituents		
	97-54-1	Isoeugenol	0.13
	93-15-2	Methyl eugenol	0.01

In conclusion the actual composition and concentration of compounds varies between *Cinnamomum cassia* leaf oil and *Cinnamomum zeylanicum* ext. and especially according to the source of the oil or extract (e.g. leaves, bark etc.). This complicates the evaluation unless the source is specified e.g. for patch test studies or LLNA. This is reflected in the few relevant studies identified and summarised below.

Non-human information

No animal studies (LLNA, GPMT or Buehler) with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified.

No relevant *in vitro* studies (OECD TG 442C and OECD 442D) with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified.

Human information

Population studies

Table 5 summarises the few patch test studies on *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. involving less than 300 dermatitis patients.

Table 5. Population studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext.

Method	Results	Remarks/Comments	Reference
Patch test: Study of 86 selected fragrance mix I positive patients patch tested with "cassia" essential oil 2% in petrolatum (pet.).	24/86 (27.9%) patients were positive.		Rudzki & Grzywa 1986 cited from SCCS (2012).
Patch test: Study of 200 patients patch tested with "cassia" essential oil 2% in pet.	2/200 (1%) patients were positive.		Rudzki et al 1976 cited from SCCS (2012).

Case studies

Table 6 shows one case report with allergic contact dermatitis in Spain where *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. has been found as causative agents.

Table 6. Case studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext.

Method	Results	Remarks/Comments	Reference
Patch test: A 32-year old man with dermatitis was patch tested with "cinnamon oil" 0.5% in petrolatum.	Positive reaction was observed.	Case study, Spain (year not stated).	Sanchez-Perez & Garcia-Diez 1999 cited from SCCS (2012).

As reflected in table 5 and 6 very few human studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified. All three studies are cited from SCCS (2012). A total of 27 positive cases and frequencies between 1 and 27.8% in selected dermatitis patients tested with "cassia" essential oil or "cinnamon oil" were observed.

SCCS (2012) considered the "essential oil" as an 'Established contact allergen in humans' considering the content of well-known allergenic compounds.

Cinnamomum zeylanicum, ext. is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 27 positive cases and frequencies between 1 and 27.8% in selected dermatitis patients tested with "cassia" essential oil or "cinnamon oil" were observed.

Non-human data

No animal studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified.

Exposure

It has not been possible to identify any data on exposure to *Cinnamomum cassia* leaf oil or *Cinnamomum zeylanicum*, ext.

Comparison with criteria

One out of two studies with "cassia" essential oil gave a frequency of positive patch tests in selected patients of 27.8% i.e. ≥ 2%, which indicate categorisation into sub-category 1A.

No animal studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified.

Conclusions on classification and labelling

Data on *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. alone is insufficient for sub-categorisation of *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. according to CLP criteria. It may be possible to sub-categorise *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. based on their constituents by read across to the major compounds such as cinnamaldehyde (for Cassia bark extract, Cassia oil, Cinnamon bark extract and Cinnamon bark oil) and eugenol (for Cinnamon leaf oil).

References

SCCS, 2012. Opinion on fragrance allergens in cosmetic products. Scientific Committee on Consumer Safety.

Appendix 14 *Evernia prunastri* ext. CAS RN 90028-68-5

The main sensitizers of *Evernia prunastri* ext. has been identified as atranol (CAS RN 526-37-4) and chloroatranol (CAS RN 57074-21-2) which are degradation products of atranorin and chloratranorin, respectively, and very potent allergens according to the SCCS (2012).

Non-human information

Table 1 summarises relevant animal studies with *Evernia prunastri* ext. i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with *Evernia prunastri* ext.

Method	Results	Remarks/Comments	Reference
LLNA: *Ex vivo* BrdU. 0.5, 1, 5 and 10% *Evernia prunastri* ext. Vehicle: 4:1 Acetone:Olive oil.	*Evernia prunastri* ext. was shown to have an EC3 value of 3.4%.		Ulker et al. (2014).
LLNA: 2.5, 5, 10, 25 and 50% *Evernia prunastri* ext. Vehicle: 1:3 ethanol:diethyl phthalate.	*Evernia prunastri* ext. was shown to have an EC3 value of 3.9%.	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2004j) cited from SCCS (2012).
GPMT: Intradermal induction 20%; Topical induction 5% and Challenge dose 0.1, 0.3 and 1% *Evernia prunastri* ext. Vehicle: not reported.	0/8 (0%), 2/8 (25%) and 5/8 (63%) animals were sensitised at challenge doses of 0.1, 0.3 and 1% *Evernia prunastri* ext., respectively.		Ehret et al., 1992 cited from SCCNFP (2000).

A total of 1 LLNA, 1 LLNA *ex vivo* BrdU and 1 GPMT with *Evernia prunastri* ext. are summarised in table 1. The reported EC3 value for *Evernia prunastri* ext. was 3.9% in the LLNA and 3.4% in the LLNA *ex vivo* BrdU. In the GPMT sensitisation was observed in 5/8 (63%) animals after an intradermal induction dose of 20% *Evernia prunastri* ext.

No relevant *in vitro* studies on *Evernia prunastri* ext. (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on *Evernia prunastri* ext. involving several thousand dermatitis patients from various countries mainly in Europe. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with *Evernia prunastri* ext.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with *Evernia prunastri* ext. 1% in petrolatum (pet.).	221/806 (27.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with *Evernia prunastri* ext. 2% in pet.	34/1951 (1.7%, 95% CI: 1.1-2.3%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Study of 228 selected patients with occupational dermatitis tested with *Evernia prunastri* ext. Vehicle and concentration not reported.	2/228 (0.9%) patients were positive.	Study on patch test data from Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2010-2011).	Friis et al. (2013).
Patch test: Retrospective study of 940 selected patients tested with *Evernia prunastri* ext. in petrolatum. Concentration not reported.	230/940 (24.6%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with *Evernia prunastri* ext. in petrolatum. Concentration not reported.	25/100 (25%, 95% CI: 16.88-34.66%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1503 consecutive eczema patients	37/1503 (2.5 %) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology,	Heisterberg et al. (2011, 2012).

Method	Results	Remarks/Comments	Reference
patch tested with *Evernia prunastri* ext. 1% in pet.		Copenhagen University Hospital Gentofte, Denmark (2008-2010).	
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with *Evernia prunastri* ext. 2% in pet	Ca. 39/157 (ca. 25%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).
Patch test: Retrospective study of 86 selected patients patch tested with *Evernia prunastri* ext. 2% in pet.	2/86 (2.3%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 1213 consecutive patients and 4482 selected patients patch tested with *Evernia prunastri* ext. 1% in pet.	22/1213 (1.81%, 95% CI: 1.07-2.56%) and 251/4482 (5.59%, 95% CI: 4.9-6.27%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Prospective study of 320 selected eczema patients patch tested with *Evernia prunastri* ext. 2% in pet.	6/320 (1.9%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 597 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with *Evernia prunastri* ext. 1% in pet.	a) 120/597 (20.1%) and b) 165/597 (27.6%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous	0/15 (0%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).

Method	Results	Remarks/Comments	Reference
reactions from ketoprofen-containing gels patch tested with *Evernia prunastri* ext. 2% in pet.			
Patch test: Retrospective study of 2063 consecutive patients patch tested with *Evernia prunastri* ext. 1% in pet.	46/2063 (2.2%, 95% CI: 1.4-2.6%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Retrospective study of a) 28 patients positive to their own shaving product/eau de toilette/perfume and b) 153 negative to their own shaving product/eau de toilette/perfume, patch tested with *Evernia prunastri* ext. 1% in pet.	a) 8/28 (29%) and b) 14/153 (9%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998-2002).	Uter et al. (2007).
Patch test: Study of 30 selected patients with a positive patch test to their own perfumed product patch tested with *Evernia prunastri* ext. Concentration and vehicle not specified.	9/30 (30%) patients were positive.		Vocanson et al. (2006).
Patch test: Prospective study of 422 selected patients with suspected contact allergy patch tested with *Evernia prunastri* ext. 2% in pet.	6/422 (1.4%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Study of 885 consecutive eczema patients patch tested with	28/885 (3.2%) patients were positive or had a follicular patch	Two types of *Evernia prunastri* ext. (oak moss absolute) were tested, one	Johansen et al., 2002 cited from SCCS (2012).

Method	Results	Remarks/Comments	Reference
Evernia prunastri ext. Concentration and vehicle not reported.	test response.	contaminated by resin acids and one without any detectable resin acids. There was no difference in reactivity between the two types.	
Patch test: Retrospective study of 4900 unselected patients patch tested with *Evernia prunastri* ext. 1% in pet.	333/4900 (6.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Prospective study of 747 selected patients with suspected fragrance allergy patch tested with *Evernia prunastri* ext. 1% in pet. with 1% sorbitan sesquioleate (SSO).	37/747 (5%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).
Patch test: Study of 226 selected patients sensitive to FM patch tested with *Evernia prunastri* ext. 1% in pet.	50/226 (22%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999).	Brites et al. (2000).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with *Evernia prunastri* ext. 2% in 1% SSO.	22/50 (44%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with *Evernia prunastri* ext. in pet. Concentration not reported.	12/40 (30%) patients were positive.		Katsarma and Gawkrodger (1999).
Patch test: Study of 167 selected	22/167 (13.2%) patients were	Multicentre study of patch test data from	Larsen et al. (1996).

Method	Results	Remarks/Comments	Reference
patients suspected of fragrance sensitivity patch tested with *Evernia prunastri* ext. 5% in pet.	positive.	Japan, Northern Ireland, USA, UK, Switzerland and Sweden.	
Patch test: Study of 702 unselected consecutive patients patch tested with *Evernia prunastri* ext. 1% in pet. with 1% SSO.	18/702 (2.6%) patients were positive.	European multicentre study with 7 different centres.	Frosch et al. (1995a).
Patch test: Study of 702 unselected consecutive patients patch tested with *Evernia prunastri* ext. 1% in pet.	13/702 (1.9%) patients were positive.	European multicentre study with 7 different centres.	Frosch et al. (1995a).
Patch test: Study of 1072 patients patch tested with *Evernia prunastri* ext. 1% in pet with 1% SSO.	24/1072 (2.24%) patients were positive.	European multicentre study with 9 different centres.	Frosch et al. (1995b).
Patch test: Retrospective study of 367 selected patients patch tested with *Evernia prunastri* ext. 1 or 2% in pet. with 5% SSO.	86/367 (23%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	Johansen and Menne (1995).
Patch test: Prospective study of 61 selected patients positive to a fragrance mix patch tested with *Evernia prunastri* ext. 5% in pet.	21/61 (34%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with *Evernia prunastri* ext. 5% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Prospective study of 162 selected patients positive to a fragrance mix	14/162 (8.6%) patients were positive.	Prospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1991).	Enders et al. (1989).

Method	Results	Remarks/Comments	Reference
patch tested with *Evernia prunastri* ext. 1% in pet.			
Patch test: Study of 55 selected patients sensitive to perfumes or after-shave lotions patch with *Evernia prunastri* ext. 2%. Vehicle not reported.	35/55 (64%) patients were positive.	According to SCCNFP 2000 16 of the 55 patients had a definite history of contact allergy to plants following direct contact.	Thune and Sandberg 1987 cited from SCCNFP (2000).
Patch test: Prospective study of 179 selected patients patch tested *Evernia prunastri* ext. 10% in pet.	21/179 (11.7%) patients were positive.		de Groot et al. (1985).
Patch test: Study of 20 selected perfume allergic patients patch tested with *Evernia prunastri* ext. 5% in pet.	3/20 (15%) patients were positive.		Larsen (1977).
Patch test and ROAT[1]: 30 *Evernia prunastri* ext. sensitive patients were tested with 0.1% of *Evernia prunastri* ext. in a ROAT and with a serial dilution of *Evernia prunastri* ext. 0.00003-2% in a patch test. Vehicle: diethyl phthalate:ethanol 2:98	22/30 patients were positive in the ROAT. Positive reactions were observed in 6/30 (20%) patients at a concentration of 0.0027%.		Andersen et al. (2015).

[1]ROAT: Repeated Open Application Test

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) with *Evernia prunastri* ext.

Table 3. HRIPT studies with *Evernia prunastri* ext.

Method	Results	Remarks/Comments	Reference
Modified HRIPT (Draize): *Evernia prunastri* ext. Induction concentration: 5% Vehicle: 1:1 Acetone:Ethanol.	7/53 (13%) tests were positive.		Ehret et al., 1992 cited from SCCNFP (2000).
HRIPT: *Evernia prunastri* ext. Induction concentration: 5% Vehicle: 3:1 Ethanol:diethyl phthalate (EtOH:DEP).	Sensitisation was observed.		Ford and Api 1990 cited from SCCNFP (2000).
HRIPT: *Evernia prunastri* ext. Induction concentration: 0.6% Vehicle: 3:1 EtOH:DEP.	0/103 tests were positive.		Ford and Api 1990 cited from SCCNFP (2000).
HRIPT: *Evernia prunastri* ext. Induction concentration: 1.2% Vehicle: not reported.	0/47 tests were positive.		IFRA 1988 amended in 1992 and 1998 cited from SCCNFP (2000).
HRIPT: *Evernia prunastri* ext. Induction concentration: 1.2% Vehicle: not reported.	1/48 (2%) tests were positive.		IFRA 1988 amended in 1992 and 1998 cited from SCCNFP (2000).

HRIPT: Human Repeat Insult Patch Test.

Case studies

Table 4 summarises case reports with allergic contact dermatitis where *Evernia prunastri* ext. has been found to be among the causative agents.

Table 4. Case studies with *Evernia prunastri* ext.

Method	Results	Remarks/Comments	Reference
Patch test: A 41-year old female hairdresser with occupational hand dermatitis and scalp dermatitis was patch tested with *Evernia prunastri* ext.	Positive reaction to *Evernia prunastri* ext. contained in a perming solution.		Kanerva et al., 1999 cited from SCCS (2012).
Patch test: A woman with ACD[1] was patch tested with her husband's aftershave lotion and *Evernia prunastri* ext. 5% in petrolatum.	Positive reaction to *Evernia prunastri* ext. and her husband's aftershave lotion containing 3% *Evernia prunastri* ext.		Held et al., 1988 cited from SCCNFP (2000).

[1]ACD: Allergic Contact Dermatitis.

A total of 35 results from patch test population studies, 5 HRIPTs and 2 case studies with *Evernia prunastri* ext. are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0 and 64% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 1.8 and 6.8% (7 studies) and for selected dermatitis patients positive reactions range between 0 and 64% with 21/28 studies reporting frequencies equal to or higher than 5%. The total number of published cases is > 1900. Sensitisation was reported in 3/5 HRIPT studies with no reactions at 0.6%, 0-2% at 1.2% and 13% at 5% induction concentrations of *Evernia prunastri* ext. The Research Institute for Fragrance Materials, Inc. (RIFM) has on the basis of these and other unpublished studies deducted a NOEL[20]-HRIPT (induction) of 700 µg/cm², a NOEL-HMT of 1724 µg/cm² and a LOEL-HRIPT/HMT[21] (induction) of 1417 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 700 µg/cm² was established for *Evernia prunastri* ext. by the RIFM Expert Panel (IFRA, 2008e).

According to SCCS (2012) *Evernia prunastri* ext. is used in volumes less than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 4.6% of 88 tested deodorants (in 2007), 0.8% of a total of 516 consumer products; 4% of a total of 300 consumer products; ca. 1% of 3000 products and 0% of children's cosmetics were labelled to contain *Evernia prunastri* ext. (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). Based on these data SCCS (2012) concluded that *Evernia prunastri* ext. was among the least used fragrance ingredients in cosmetics and other consumer products.

The IFRA standard limits for *Evernia prunastri* ext. in different IFRA QRA product categories reported by IFRA (2008e and 2015) are shown in table 5.

[20] NOEL: No Observed Effect Level.

[21] LOEL-HRIPT/HMT: Lowest Observed Effect Level-Human Repeat Insult Patch Test/Human Maximisation test.

Table 5.The IFRA standard limits for *Evernia prunastri* ext. in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.02%
Category 2	Deodorants/antiperspirants	0.03%
Category 3	Hydroalcoholics for shaved skin	0.1%
Category 4	Hydroalcoholics for unshaved skin	0.1%
Category 5	Hand cream	0.1%
Category 6	Mouthwash	0.5%
Category 7	Intimate wipes	0.1%
Category 8	Hair styling aids	0.1%
Category 9	Rinse-off hair conditioners	0.1%
Category 10	Hard surface cleaners	0.1%
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Evernia prunastri ext. is not registered under the REACH regulation.

Summary and discussion of skin sensitization

Human data

A total of 35 results from patch test population studies, 5 HRIPTs and 2 case studies were identified with *Evernia prunastri* ext. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 64% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 1.8 and 6.8% (7 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 64% (28 studies). The total number of published cases is > 1900. A LOEL-HRIPT/HMT (induction) of 1417 µg/cm² was established for *Evernia prunastri* ext. by the RIFM Expert Panel based on unpublished reports.

Non-human data

A total of 1 LLNA, 1 LLNA *ex vivo* BrdU and 1 GPMT were identified testing skin sensitising effects of *Evernia prunastri* ext. The reported EC3 value for *Evernia prunastri* ext. was 3.9% in the LLNA and 3.4% in the LLNA *ex vivo* BrdU. In the GPMT sensitisation was observed in 63% of the animals after an intradermal induction dose of 20% *Evernia prunastri* ext.

No relevant *in vitro* studies on *Evernia prunastri* ext. (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure

According to data from IFRA (2008e) the exposure of *Evernia prunastri* ext. when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 1.81 and 6.8% i.e. all studies are reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 64% with 23 out of 28 studies reporting frequencies higher than 2%.

In addition to this there are more than 1900 published cases of positive patch test reactions to *Evernia prunastri* ext. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that *Evernia prunastri* ext. causes a *high frequency* of occurrence of skin sensitisation based on these three types of information.
In regard to the HRIPT/HMT data the positive response reported at > 500 μg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 2 LLNAs EC3 values were 3.4 and 3.9%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, both studies indicate classification of *Evernia prunastri* ext. in sub-category 1B.

In the GPMT sensitisation was observed in 63% of the animals after an intradermal induction dose of 20%. According to the CLP criteria a positive response ≥ 30% of the animals responding at > 1% intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of *Evernia prunastri* ext. into sub-category 1B.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. The results from the animal studies indicate classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is thus warranted for *Evernia prunastri* ext.

Conclusions on classification and labelling
Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure, a classification of *Evernia prunastri* ext. as a skin sensitiser in sub-category 1A is justified.

References
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Evaluation of selected sensitising fragrance substances

Fragrances are widely used in many different types of consumer products and consumers may thus be exposed to fragrances from many different sources. The exposure can be substantial despite the fact that fragrances are typically used in relatively low concentrations in individual products. Skin sensitisation (contact allergy) is a critical effect for human health for many fragrances. The purpose of this project was to retrieve and review the available data for 42 selected sensitising fragrance substances in order to assess whether these substances are potent enough fulfil the criteria for classification as strong sensitisers in sub-category 1A according to the CLP Regulation on classification, labelling and packaging. A classification as a strong sensitizer in category 1A will impact the level of information that has to be supplied on the label on e.g. washing and cleaning products containing such substances. The more stringent labelling requirements for products containing potent sensitisers will provide better protection of users (both consumers and workers) as it will allow sensitised individuals to take precautionary measures to prevent direct skin contact with such products.

Parfume er vidt udbredt i mange forskellige typer forbrugerprodukter, og forbrugere bliver eksponeret for parfume fra mange forskellige kilder. Eksponeringen kan være betragtelig på trods af, at parfumestoffer generelt anvendes i relativt lave koncentrationer i de enkelte produkter. Hudsensibilisering (kontakt allergi) er en af de kritiske effekter for sundheden for mange parfumestoffer. Formålet med dette projekt var at søge og vurdere de tilgængelige data for 42 udvalgte sensibiliserende parfumestoffer for at vurdere, om disse stoffer er potente nok til at opfylde kriterierne for klassificering som stærkt sensibiliserende stoffer i kategori 1A i henhold til CLP forordningen om klassificering, mærkning og emballering. En klassificering som stærkt sensibiliserende i kategori 1A vil have betydning for mærkningen af f.eks. vaske- og rengøringsmidler indeholdende sådanne stoffer. De strengere mærkningskrav for produkter indeholdende potente allergener vil give en bedre beskyttelse af brugerne (både forbrugere og arbejdstagere), da det vil give sensibiliserede personer den nødvendige information i forhold til at forebygge direkte hudkontakt med sådanne produkter.

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