Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

citral; 3,7-dimethylocta-2,6-dienal

EC Number: 226-394-6
CAS Number: 5392-40-5

CLH-O-0000001412-86-225/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 September 2018

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification: Citral; 3,7-dimethylocta-2,6-dienal

EC Number: **226-394-6**

CAS Number: **5392-40-5**

Index Number: **605-019-00-3**

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CITRAL; 3,7-DIMETHYLOCTA-2,6-DIENAL

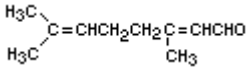
CONTENTS

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CITRAL; 3,7-DIMETHYLOCTA-2,6-DIENAL

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Citral; 3,7-dimethylocta-2,6-dienal
Other names (usual name, trade name, abbreviation)	2,6-Octadienal, 3,7-dimethyl Reaction mass of (E)-3,7-dimethylocta-2,6-dienal and (Z)-3,7-dimethylocta-2,6-dienal Reaction mass of (Z)-3,7-dimethylocta-2,6-dienal and (E)-3,7-dimethylocta-2,6-dienal Geranialdehyde, Lemonal
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	226-394-6
EC name (if available and appropriate)	Citral
CAS number (if available)	5392-40-5
Other identity code (if available)	
Molecular formula	$C_{10}H_{16}O$
Structural formula	
SMILES notation (if available)	CC(=CCC\C(=C\C=O)\C)C
Molecular weight or molecular weight range	152.233
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Citral is a reaction mass of the two cis-trans stereo-isomers: (Z)-3,7-dimethylocta-2,6-dienal (Neral) *and* (E)-3,7-dimethylocta-2,6-dienal (Geranial) (See confidential annex II regarding the ratio of the stereo-isomers).
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 95%[1] (See confidential annex II regarding purity).

[1] Information obtained from supplier webpages and from SDS of commercially available citral, sum of cis- and trans-isomers. More detailed information available in confidential annex II.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CITRAL; 3,7-DIMETHYLOCTA-2,6-DIENAL

Citral; 3,7-dimethylocta-2,6-dienal, hereafter referred to as "citral", is found in many essential oils, and is e.g. the principal constituent in lemon myrtle (Bachhousia citriodora) oil, lemongrass oil and lemon tea tree oil among others. Citral has a strong lemon like odour. Citral is commonly used as a fragrance, mainly in cosmetics but also in various cleaning and maintenance products.

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
(Z)-3,7-dimethylocta-2,6-dienal (Neral), CAS 106-26-3	See confidential annex II	None	Skin sens 1 or 1B; H317 Skin irrit. 2; H315 Eye irrit. 2; H319
(E)-3,7-dimethylocta-2,6-dienal (Geranial), CAS 141-27-5	See confidential annex II	None	Skin sens 1 or 1B; H317 Skin irrit. 2; H315 Eye irrit. 2; H319

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not applicable	See confidential annex II	-	-	-

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not applicable					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	605-019-00-3	Citral	226-394-6	5392-40-5	Skin Irrit. 2 Skin Sens. 1	H315 H317	GHS07 Wng	H315 H317			
Dossier submitters proposal	605-019-00-3	Citral	226-394-6	5392-40-5	**Modify** Skin sens **1A**	H317	GHS07 Wng	H317			
Resulting Annex VI entry if agreed by RAC and COM	605-019-00-3	Citral	226-394-6	5392-40-5	Skin Irrit. 2 Skin Sens. **1A**	H315 H317	GHS07 Wng	H315 H317			

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	**new harmonised classification proposed**	**Yes**
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

A harmonised classification of citral as sensitising and irritating to skin was adopted under Directive 67/548/EEC (R43 and R38). Under the CLP Regulation the corresponding harmonised classification of citral is Skin sens 1 (H317) and Skin irr. 2 (H315) in CLP Annex VI.

Citral is one of the 26 fragrance substances for which individual labelling is required under the Cosmetics Regulation (EC no. 1223/2009) and the Detergents Regulation (EC no 648/2004). Citral is also among the 13 allegenic fragrance substances listed in the SCCS opinion which have been frequently reported as well-recognised contact allergens in consumers and thus of most concern (SCCS 2012)..

In 2012 the Scientific Committee on Consumer Safety (SSCS) published an opinion on fragrance allergens in cosmetic products. In this opinion citral has been categorised as an established contact allergen in humans which has given rise to a significant number (>100-1000) of published cases on contact allergy (SCCS 2012).

A substance evaluation (SeV) of citral was carried out in 2015 under the REACH Regulation by the Swedish Chemicals Agency as a concern was identified due to the sensitizing properties combined with wide dispersive use, consumer use, exposure of workers and high (aggregated) tonnage. The focus of the substance evaluation was exposure and risk based concerns, and it was concluded that EU-wide measures were necessary to ensure safe use for workers and consumers. This included a revision of the DNEL and the chemical safety assessment. A specific assessment of the skin sensitising potency of citral in relation to classification was not part of the evaluation (KEMI 2015).

RAC general comment
Citral has an existing harmonised classification for the hazards skin irritation and skin sensitsation. In their proposal, the Dossier Submitter (DS) only addressed skin sensitization. No amendment to the classification for skin irritation was considered necessary.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Change in existing entry due to new data (only partly)
Change in existing entry due to changes in the criteria
Change in existing entry due to new interpretation/evaluation of existing data

Further detail on need of action at Community level

New classification criteria and new evaluation of data
With the 2nd ATP to CLP new classification criteria were introduced for skin sensitisation allowing sub-categorisation of skin sensitisers into Category 1A (strong sensitisers) and Category 1B (other sensitisers, corresponding to the existing Category 1). Substances previously classified as skin sensitisers in category 1 may in some cases fulfil the criteria for a more stringent classification in Category 1A and if data are available the classification should be updated accordingly. A classification in Cat. 1A will lead to more stringent labelling requirements for mixtures containing the substance and is currently

regarded as the most important risk management measure for such substances. Correct identification of Category 1A skin sensitisers is thus expected to increase the human protection level for strong sensitisers due to the requirement of labelling of mixtures containing Cat 1A sensitisers ≥0.01%. with EUH208: "Contains [name of of sensitising substance]. May produce an allergic reaction".

A new evaluation of the existing data for citral has been conducted and compared to the present classification criteria. Some of the data can be regarded as "new" in this context as some of the studies used for the assessment have been published after the adoption of the existing harmonised classification.

Widespread use in low concentrations

Citral is a fragrance that is manufactured in or imported to the EU in amounts of 1000-10.000 tonnes/year and is widely used in products on the EU market. The registered categories of use for consumers are mainly cosmetics and a variety of household products for cleaning and maintenance . The registered uses for professionals are cleaning agents and polishes and wax blends (see section 5 below on identified uses). As citral is widely used in a range of frequently used consumer products the general population can be exposed from many different sources.

Citral is generally present in low concentrations in individual consumer products. The International Fragrance Association (IFRA) has established maximum recommended limits of citral in specific product categories based on a quantitative risk assessment approach. The maximum limits of citral in leave-on cosmetic products are between 0.04-1.4% depending on the specific product category. The recommended limits for rinse-off cosmetic products are between 1.0-5.0% and the recommended maximum limit for non-cosmetic products with direct skin contact is 2.5% (see table 11 in section 10.8.3 on human exposure) (IFRA 2013, IFRA 2015).

The SCCS opinion refers to a number of surveys on the presence and content of various allergenic fragrances in various consumer products. Citral has i.e. been found to be present in 8-26% of the products investigated in different surveys of consumer producs. It was concluded by SCCS that taking the total exposure into account, exposure to all 26 allergenic fragrances is foreseeable in daily life (SCCS 2012). The Danish EPA has conducted surveys and assessments of a broad range of consumer products over the last decades. Citral has been identified in many different types of products, mostly in cosmetic products, followed by household products. Generally citral is found in low concentrations (>0- <0.06%) in the investigated products with some exeptions (see also section 10.8.3 on human exposure) (DK EPA database, search June 2016). Data from the Danish Product Register further show that citral is present in various products for professional use (mainly cleaning products) and mostly in low concentrations <0.1% (Danish Product Register, 2016).

Human exposure to citral seems to be low based on the IFRA recommandations and reported contents in various consumer products. However, the exposure is assessed to be frequent due to the widespread uses and the high tonnage level of citral. It is thus difficult for consumers to avoid exposure.

Human data confirm strong potency of citral

Positive patch test frequencies from 25 human patch test studies range from 0.3-16.7% and frequencies equal to or exceeding 2% for selected dermatitis and patients 1% for consecutive (unselected) dermatitis patients are reported in a number of studies. The total number of positive reactions in published cases is > 100 (more than 400). Overall the human data confirm strong the potency of citral.

5 IDENTIFIED USES

Citral is used as a fragrance mainly in cosmetics but also in cleaning and maintenance products. Registered uses for consumers include: cosmetics, personal care products, washing and cleaning products, polishes and waxes, air care products, biocidal products, coatings and paints, thinner and paint remocers, fillers, plasters, putties and modelling clay, finger paints, inks and toners. Registered uses for professionals include: washing and cleaning products and polishes and waxes.

6 DATA SOURCES

One of the primary sources of information for this CLH report is the SCCS opinion on fragrance allergens from 2012 which contains the most recent and comprehensive assessment of available information on citral as well as other fragrance allergens up to year 2011 (SCCS 2012). References on the data cited in this opinion for citral have been retrieved when possible.

A supplementary search in the open literature has been done for the period from January 2009 and until November 2016 to ensure that potentially relevant studies published after the SCCS opinion are taken into account. The searches have included literature databases such as SciFinder, PubMed and Scopus as well as searches in sources such as OECD SIDS, IPCS INCHEM. General searches via Google have also been done.

Data in the publicly available part of the REACH registration dossier for citral have been assessed as well.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	liquid	REACH registration dossier	Measured
Melting/freezing point	< -10° C at 1013 hPa < -20° C at 1013 hPa	REACH registration dossier	Measured
Boiling point	225-230° C at 1013 hPa	REACH registration dossier	Measured
Relative density	0.89-0.9 g/cm³ at 20° C	REACH registration dossier	Measured
Vapour pressure	0.071 hPa at 25° C <1.3 hPa at 100° C	REACH registration dossier	Measured
Surface tension	No data		
Water solubility	0.1-1 g/L at 18° C 0.42-0.59 g/L at 25° C 1.34 g/L at 37° C	REACH registration dossier	Measured
Partition coefficient n-octanol/water	2.76 – 2.9 at 25° C	REACH registration dossier	Measured
Flash point	91 °C - 101 °C at 1013 hPa	REACH registration dossier	Measured
Flammability	No data		
Explosive properties	No data		
Self-ignition temperature	225 °C at 1013 hPa	REACH registration dossier	Measured
Oxidising properties	No data		
Granulometry	No data/not applicable		

Property	Value	Reference	Comment (e.g. measured or estimated)
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	No data		
Viscosity (dynamic)	2.15 mPa*s at 20°C 1.46 mPa*s at 40°C	REACH registration dossier	Measured

8 EVALUATION OF PHYSICAL HAZARDS

Physical hazards have not been assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
No guideline, GLP compliance not reported. Time course of distribution of 14C-label in tissues, blood, bile, urine, feces, expired air. Rat (Fischer), male Acute study: single dose, oral (gavage) Multiple dosing study: oral pretreatment for 10 days with unlabelled citral at a dose of 5 mg/kg bw/day followed by single oral or i.v. dose of 5 mg/kg 14C-citral Conc: oral application: 5, 50, 500 mg/kg/d; i.v. application: 5 mg/kg bw/d	Citral was rapidly and completely absorbed after oral exposure (91-95%) The amounts remaining in any tissue was < 2% with the highest concentrations in liver, muscle, blood, adipose tissue (relative amounts independent of dose or route of administration). Total concentrations in tissues were 2.8-6.3% depending on dose and route of adm. Excretion profiles were independent from dose or route of administration with recoveries of 79-83% after 72h. Excretion mainly via urine (>50%, 72h), followed by exhalation of $^{14}CO_2$ and faeces Most of the citral-derived radioactivity was rapidly eliminated from the body with a whole body half-life of 8 hr after i.v. exposure. However, a small percentage tended to persist with a clearance half-life of 24 hrs.	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral (Key study)	Diliberto et al., 1988
No guideline, GLP compliance not reported. Urinary metabolites identified by reverse phase HPLC Rat (Fischer), male	Seven metabolites could be identified in sufficient purity and quantity, namely: A: 3 -hydroxy-3,7,dimethyl-6-octenedioic acid; B: 3,8-dihydroxy-3,7-dimethyl-6-	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral	Diliberto et al., 1990

Method	Results	Remarks	Reference
Single dose, oral (gavage) and i.v. application Conc: gavage: 5 and 500 mg/kg bw; i.v.: 5 mg/kg bw Sampling: 2, 7, 24 hours for urine; 5, 30, 60, 270 min for bile (only after i.v. application)	octenoic acid; C: 3,9-dihydroxy-3,7-dimethyl-6-octenoic acid; D: E-3,7-dimethyl-2,6-octadienedioic acid; E: 3,7-dimethyl-6-octenedioic acid; F: Z-3,7-dimethyl-2,6-octadienedioic acid; G: E-3,7-dimethyl-2,6-octadienoic acid. Glucuronic acid conjugates only in bile	(Key study)	
No guideline, GLP compliance not reported. Tissue distribution and time course of excretion in urine, faeces and exhaled 14CO2 measured; metabolites in urine separated by TLC (individual metabolites not identified). Rat (Wistar), male Single dose, oral (gavage) Conc.: 5, 770 and 960 mg/kg bw Sampling: tissues: 96 hours p.a. excreta: 24, 48, 72, 96 hrs $^{14}CO_2$: trapping solutions analyzed after 2, 4, 6, 7, 24, 48, 72, 96 hrs	Rapid absorption from the gastrointestinal tract Distr. in tissues: at 5 and 960 mg/kg: most 14C in gastro-intestinal tract (ca. 7 and 12.5%) and the liver (ca. 1.5 and 2%) Excretion: 5 mg/kg bw: >95% excretion within 24h; urine: 61%, exhaled CO_2: 20% and faeces:17% 960 mg/kg bw: 60-70% excretion within 24h; urine: 47%, exhaled CO_2: 7.3% and faeces: 9.5% 770 mg/kg: >95% excretion within 96h. Urinary excretion complete by 60h, CO_2 excretion complete by 48h, faecal excretion slow up to 36h and rapid from 36-72h	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral	Phillips et al., 1976
No guideline, GLP compliance not reported. Mouse (LACA strain), male Single dose, oral (gavage) Conc.: 100 mg/kg bw Sampling: 12 and 24 hrs, 2, 3, 5, 7 and 10 d Radioactivity present in the body was visualized by autoradiography.	Considerable proportion of 14C appearing throughout the tissues within 12 h. After 168 h only faint or no distribution of radioactivity could be measured in all tissues except from the liver and kidney cortex. Major route of 14C-excretion via urine detected up to day 5. Significant proportion of 14C rapidly excreted with faeces within 12 h, 14C-excretion via faeces detected up to day 3.	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral	Phillips et al., 1976
No guideline, GLP compliance not reported. Rat (Fischer), male Single dose, dermal Conc: 5, 50 mg/kg	About 1/3 of the applied dermal dose was lost due to evaporation, but the citral remaining on the skin was fairly well absorbed in rats. Approximately 24% of the initial body burden (IBB) was recovered in the dermal application caps and	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral	Diliberto et al., 1988

Method	Results	Remarks	Reference
Sampling: urine and feaces at 2, 4, 6, 8, 12, 16, 24, 32, 48, and 72 hrs, blood at 72 hrs, expired air: continuously	less than 50% of the applied dose was thus available for dermal absorption. The distribution of citral in tissues and excreta after 72h was 7-9.5% in total tisses (except dermal skin sites), 8.5-9.9% in dermal skin sites, 8.4-17.3 in urine, 3.5-3.2% in faeces, 3.4-3.8% in expired CO2 and 2.8-4.5 as expired citral (percentages depending on the dose).		
No guideline, GLP compliance not reported. Guinea pig (Hartley), female Single dose, dermal Conc: 1.88 mg/animal, ca. 63 µg/cm2 skin area Sampling: urine and faeces collected during 16 hr Analysis of organs at termination 16h p.a.: skin	The total recovery of radioactivity from the excreta urine and feces, from total skin and from unresorbed citral at the skin surface was 42.1% in a guinea pig without pre-treatment (C) and 47.7% in a guinea pig that had been subjected to an induction treatment with citral (A). The amounts absorbed into the skin within 16 hrs p.a. were 23.9% (C) and 27.5% (A).	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral	Barbier et al., 1983
No guideline, GLP compliance not reported. In-vitro test, freshly excised human skin Conc: 100% lemon myrtle oil: 20 µl/cm2 or 18 mg/cm2 1 % lemon myrtle oil product (corresponding to 1 mg citral): 0.18 mg/ cm2 Sampling: 4 skin samples per timepoint, sampling at 1, 4, 8, 12 hrs (100% oil) and 8hrs (1% oil) Analysis: GC-MS	Citral (as the main component of lemon myrtle oil) was absorbed in freshly excised full-thickness human skin at all exposure periods tested. Relative recoveries of up to approx. 2.0% was seen in epidermis/dermis (4h), 0.49% in subcutaneous fat tissue (12h) and 2.1% in receptor fluid (4h). Neral and geranial were the only detectable components of the oil in the skin discs (epidermis and dermis) and in subcutaneous fat tissue. As exposure time increased, the recovery in the fat tissue increased also. However, the recovery in epidermis/dermis showed a maximum at 4 hrs p.a.. At all timepoints, the recovery in skin layers was higher than in subcutaneous fat.	**Test material:** 100% lemon myrtle oil (Backhousia citriodora), 96.6% citral and 1% lemon myrtle oil product	Hayes et al., 2003

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The below summary of toxicokinetics is cited from the OECD SIDS assessment report for citral (OECD SIDS 2001):

"*Orally administrated citral was absorbed rapidly and almost completely from the gastro-intestinal tract in rats and mice [Pillips et al.: 1976, Diliberto: 1988]. Much of an applied dermal dose was lost due to its extreme volatility, but the citral remaining on the skin was fairly well absorbed in rats [Diliberto et al.: 1988]. After a single oral dose, citral was rapidly metabolized and excreted as metabolites, including several acids and a biliary glucuronide in male F344 rats [Diliberto et al.: 1990]. (…….).*

The disposition of [14C] citral was studied in male Fischer rats after iv, po and dermal treatments [Diliberto et al.: 1988]. At 72 hr after treatment, the amount of 14C found in any tissue was a very small percentage (< 2%) of the total dose. The relative amount of radioactivity in all tissues did not change with increasing dose or route of exposure.

Citral was excreted rapidly and most of the administered radioactivity was excreted within 72 hr by the rat and within 120 hr by the mouse after oral administration with [14C] citral [Phillips et al.: 1976]. Urine was major route of elimination, followed by feces, CO2 (via lung) and exhaled volatiles [Diliberto et al.: 1988]. The pattern of elimination was the same after iv or oral exposure in rats. However, after dermal exposure, relatively less of the material was eliminated in the urine and more in the feces, suggesting a role for first-pass metabolism through the skin.

There was no evidence for long-term retention of citral in the body, and it is sugge sted that any hazard associated with tissue accumulation after prolonged exposure will be minimal [Phillips et al.: 1976]. Repeated exposure to citral resulted in an increase in biliary elimination, without any significant change in the pattern of urinary, fecal, or exhaled excretion [Diliberto et al.: 1988]."

Supporting studies on the dermal absorption showed that relatively high amounts of dermally applied citral was absorbed in the skin of guinea pig (up to 27.5% after 16 hrs) (Barbier et al., 1983).

An in-vitro study on fresh human skin showed that citral was absorbed in the epidermis/dermis and the subcutaneous fat although in relatively low percentages. The recovery in the skin layers was higher than in subcutaneous fat at all sampling times (Hayes et al., 2003).

In conclusion citral is considered to be a substance with a relatively high capability of penetrating the skin. In dermal studies a relatively high percentage of the applied dose may be lost due to evaporation due to the high volatility of citral. An in-vitro study of fresh human skin confirms that the fraction of citral remaining on the skin is is rapidly absorbed in the epidermis/dermis and subcutaneous fat. Likewise, guinea pig studies show that relatively high amounts of dermally applied citral is absorbed in the skin.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier.

10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier.

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

10.7 Skin sensitisation

Table 9 summarises relevant animal studies with citral which include a total of 21 studies: 14 LLNAs, 6 GPMTs and 1 Buehler test. Five of the below reported studies are included in the REACH registration dossier.

Table 9: Summary table of animal studies on skin sensitisation (chronological order)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
LLNA					
LLNA, OECD 429 GLP	Mice (CBA/CaOlaHsd), female n = 6/dose	Citral (in AOO) purity 96.4%	5, 10 and 25% Exp.: 3 days, duration 6 days	EC3: 12.6%, sensitising	Basketter et al., 2012
LLNA:BrdU-FCM	Mice (Balb/c), female n = 4-6/dose	Citral (in AOO)	5, 10 and 25% Exp: 3 days, duration 6 days	EC3: 14.1%, sensitising (Compared with EC3 reference value for citral of 9.2%, reported in OECD 429)	Jung et al., 2012
LLNA, OECD 429	Mice (CBA), female n = 4/dose	Citral (in 1:3 EtOH:DEP)	2.5, 5, 10, 25 and 50% Exp: 3 days, duration 6 days	EC3: 6.3%, sensitising	Lalko and Api, 2006 and 2008 cited from REACH reg.
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 1:3 EtOH:DEP)	0.4, 2, 4, 8 and 20%	EC3: 1.2%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2004b)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 0.1% α-tocopherol in 3:1 EtOH:DEP)	0.3, 1, 3, 10 and 30%	EC3: 1.5%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results**	**Reference**
					RIFM 2003k)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 0.3% antioxidant mix* in 3:1 EtOH:DEP) *1:1:1 BHT, tocopherol and eugenol	0.3, 1, 3, 10 and 30%	EC3: 2.1%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003l)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 0.1% Trolox C in 3:1 EtOH:DEP)	0.3, 1, 3, 10 and 30%	EC3: 3.7%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003m)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 3:1 EtOH:DEP)	0.3, 1, 3, 10 and 30%	EC3: 4.6%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003n)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 0.3% antioxidant mix* in 3:1 EtOH:DEP) *1:1:1 BHT, tocopherol and eugenol	0.3, 1, 3, 10 and 30%	EC3: 4.6%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as 2003o)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 3:1 EtOH:DEP)	0.3, 1, 3, 10 and 30%	EC3: 5.3%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003p)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 0.1% Trolox C in 3:1 EtOH:DEP)	0.3, 1, 3, 10 and 30%	EC3: 5.8%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003q)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 1:3 EtOH:DEP)	2.5, 5, 10, 25 and 50%	EC3: 6.3%, sensitising *NB: This study seems to be identical to the study by Lalko and Api from 2006 cited in row no. 3 above in this table*	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
					2003r)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Citral (in 0.1% α-tocopherol in 3:1 EtOH:DEP)	0.3, 1, 3, 10 and 30%	EC3: 6.8%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003s)
LLNA (eq. or similar to OECD 429)	Mice	Citral (in AOO)	Conc. not reported Exp.: 3 days, duration 6 days	EC3: 13%, sensitising	Basketter et al., 2002a cited from Lalko and Api, 2008
LLNA, OECD 429 (duration only 4 days)	Mice (CBA), male/female	Citral (in AOO)	5, 10 and 25% Exp.: 3 days, duration 4 days	EC3: 7-15%, sensitising	Basketter and Scholes, 1992 cited from REACH reg.
GPMT					
GPMT (eq. or similar to OECD 406)	Guinea pig (Dunkin-Hartley)	Citral (vehicle not reported)	Intradermal ind.: 0.2% Topical ind.: 5% Chall. dose: 0.5% Duration: 20-22 days	Sensitisation observed, positive reactions seen in 6/10 animals	Basketter and Allenby, 1991; Basketter et al., 1991, Basketter and Scholes, 1992 cited from REACH reg.
GPMT (acc. to Magnusson and Kligman 1969)	Guinea pig	Citral (vehicle not reported)	Intradermal ind.: 10% Topical ind.: 10% Chall. dose: 10% Duration: 20-22 days	Sensitisation observed	Ishihara et al., 1986a cited from Lalko and Api, 2008
GPMT (acc. to Magnusson and Kligman 1969)	Guinea pig	Citral (vehicle not reported)	Intradermal ind.: 0.4% Topical ind.: 1% Chall. dose: 0.25% Duration: 20-22 days	Sensitisation observed, positive reactions in 4/10 animals	Goodwin and Johnson 1985 cited from Lalko and Api, 2008
GPMT (eq. or similar to	Guinea pig (Pirbright White), female	Citral (in paraffin oil DAB7 or Freunds	Intradermal ind.: 25% Topical ind.:	Sensitisation observed, 100% positive reactions	Unnamed study report 1978 cited from REACH

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD 406)		adj./dest.aqua (1:1)	25% Chall. dose: 10, 5 and 5 %		reg.
GPMT (eq. or similar to OECD 406)	Guinea pig (Pirbright White), female	Citral (in paraffin oil DAB7 or Freunds adj./dest.aqua (1:1)	Intradermal ind.: 25% Topical ind.: 25% Chall. dose: 10, 5 and 5%	Sensitisation observed, 100% positive reactions (except for after 144 hours after a 5% rechallenge where 60% positive reactions were observed).	Unnamed study report 1978 cited from REACH reg.
GPMT (acc. to Magnusson and Kligman 1969)	Guinea pig	Citral (vehicle not reported)	Intradermal ind.: 5% Topical ind.: 25% Chall. dose: subirritant	Sensitisation observed	Klecak et al., 1977 cited from Lalko and Api, 2008
Buehler test					
Buehler, modified	Guinea pig n = 5/dose	Citral (in petrolatum)	Induction conc.: 20% Challenge dose.:20% Induction: 6h closed pathc, once/week for 3 weeks. Challenge: 6h occluded patch after 10-14 days rest; readings after 24 and 48h.	Sensitisation observed in 5/5 animals	Unpublished report by RIFM 1973 cited from Lalko and Api, 2008

Table 10 summarises recent, relevant human studies with citral which include 25 patch test studies, 6 HRIPTs, 14 HMTs and 3 case studies. The studies involve thousands of dermatitis patients from different EU countries and Asia. The majority of the references cited below are not included in the REACH registration dossier.

Table 10: Summary table of human data on skin sensitisation (chronological order)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Patch tests, selected patients				
Patch test data, selected patients	Citral, 2% (in pet.)	Study of 1058 selected Fragrance mix (FM) II positive patients patch tested	**16.2%** were tested positive (n = 1058)	Geier et al., 2015

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		with citral. Data from IVDK multicentre project (IVDK: Information Network of Departments of Dermatology in Germany, Austria and Switzerland). Data obtained 2005-2013.		
PPatch test data, selected patients	Citral, 2% (in vas.)	Study of 565 selected patients patch tested with citral, data from multicenter study, Hungary. Data obtained 2009-2010.	**3.4%** were tested positive (19/565)	Ponyai et al., 2012
Patch test data, selected patients	Citral, 2% (in pet.)	Study of 205 selected patients patch tested with citral, data from Department of Dermatology, University Hospital St Rafael, Belgium. Data obtained 1990-2011.	**11.2%** were tested positive (23/205)	Nardelli et al., 2013
Patch test data, selected patients	Citral, 2% (vehicle not reported)	Study of 30 selected patients patch tested with citral. Of the 30 patients selected due to positive reactions to ascaridole (1 and 5%) two patients showed concomitant reactions to citral. Data from Department of Dermatology, University Medical Centre Groningen, The Netherlands. Data obtained 2008-2011.	**6.7%** were tested positive (2/ 30)	Bakker et al., 2011
Patch test data, selected patients	Citral, 2% (in pet.)	Study of 86 selected patients patch tested with citral, data from the Department of Dermatology, Hospital General Universitario, Alicante, Spain. Data obtained 2004-2008.	**2.3%** were tested positive (2/86)	Cuesta et al., 2010
Patch test data, selected patients	Citral, 2% (in pet.)	A study on fragrance allergy in 658 hand eczema patients from three dermatological departments in Denmark and Sweden (Gentofte, Odense, Malmö), data were obtained in 2001-2002.	**4.3%** were tested positive (28/658)	Heydorn et al., 2003
Patch test data, selected patients	Citral, 2% (in pet.)	Study of 78 selected patients patch tested with citral, multicenter study involving 6 countries	**16.7%** were tested positive (13/ 78)	Wilkinson et al., 1989 cited from Frosch et al 1989
Patch test data, selected (and non-selected?) patients dermatitis patients	Citral, 5% (vehicle not reported)	Study of 310 cosmetic dermatitis patients, 408 non-cosmetic patients and 122 control subjects patch tested with citral No further details available, but at least the cosmetic dermatitis patient group is assumed to represent selected patients	**2.6%** cosmetic dermatitis patients were tested positive (8/310) **2.2%** non-cosmetic patients were tested positive (9/ 408)	Itoh et al., 1986 and 1988 and Nishimura et al., 1984 cited from Lalko and Api 2008
Patch test data, selected (and non-selected?) patients	Citral, 2% (vehicle not reported)	Study of 310 cosmetic dermatitis patients, 408 non-cosmetic patients and 122 control subjects patch tested with citral No further details available, but at least	0.4% cosmetic dermatitis patients were tested positive (1/ 240) 0.3% non-cosmetic	Itoh et al., 1986 and 1988 and Nishimura et al., 1984 cited from Lalko and

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		the cosmetic dermatitis patient group is assumed to represent selected patients.	dermatitis patients were tested positive (2/584)	Api 2008
Patch test data, selected patients	Citral, 2% (in pet.)	Study of 182 selected patients patch tested with citral, data from 7 Dermatological University Clinics in the Netherlands. Data obtained 1977-1978.	**2.6%** were tested positive (n = 182)	Malten et al., 1984
Patch test data, selected patients	Citral, 5% (in pet.)	Patch test study of 155 cosmetic dermatitis patients and 159 other eczema/dermatitis patients tested with citral. No further details available	**2.6%** cosmetic dermatitis patients were tested positive (4/155) **3.1%** dermatitis/eczema patients were tested positive (5/159)	Ishihara et al., 1981 cited from Lalko and Api 2008
Patch tests, consecutive (unselected) patients				
Patch test data, consecutive patients	Citral, 2% (in pet.)	Study of 1951 eczema patients patch tested with citral, data from St Johns Institute of Dermatology at St Thomas Hospital, UK. Data obtained 2011-2012.	**1.0%** were tested positive (20/1951)	Mann et al., 2014
Patch test data, consecutive patients	Citral, 3.5% (in pet.) Purity: ≥98%	Study of 655 consecutive patients patch tested with citral, data from the Department of Dermatology Sahlgrenska University Hospitalm Gothenburg, Sweden. Data obtained 2010-2011.	0.92% were tested positive (6/ 655)	Hagvall and Christensson, 2014
Patch test data, consecutive patients	Citral, 1.5% (in pet.) Purity: ≥98%	Study of 1055 consecutive patients patch tested with citral, data from the Department of Dermatology Sahlgrenska University Hospitalm Gothenburg, Sweden. Data obtained 2006-2008.	0.66% were tested positive (7/1055)	Hagvall et al., 2012
Patch test data, consecutive patients	Citral, 2% (in pet.)	Study of 1502 consecutive patients patch tested with citral, data from Department of Dermato-Allergology, Copenhagen University Hospital, Gentofte. Data obtained 2008-2010.	0.3% were tested positive (4/1502)	Heisterberg et al., 2011, 2012
Patch test data, consecutive patients	Citral, 2% (in pet.)	Study of 320 consecutive eczema patients patch tested with citral, data from the University Medical Centre in Groningen, the Netherlands. Data obtained 2005-2007.	0.6% were tested positive (2/320)	Van Oosten et al., 2009
Patch test data, consecutive patients	Citral, 2% (in pet.)	Study on 2021 consecutive patients patch tested with citral, data from IVDK multicentre project (IVDK:	0.6% were tested positive (13/2021)	Schnuch et al., 2007 (also cited in REACH reg.)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		Information Network of Departments of Dermatology in Germany, Austria and Switzerland). Data obtained 2003-2004.		
Patch test data, consecutive patients	Citral, 2% (in pet.)	Study of 422 consecutive patients patch tested with citral, data from multicenter study, Korea. Data obtained 2002-2003.	**1.2%** were tested positive (5/ 422)	An et al., 2005 (also cited in REACH reg.)
Patch test data, consecutive patients	Citral, 1% (in pet.)	Study on 1701 consecutive patients attending contact dermatitis clinics at 6 dermatology departments were patch tested with citral between October 2002 and June 2003 (Dortmund, Copenhagen, Malmö, Odense, London and Leuven).	0.35% (6/1701) and were tested positive	Frosch et al., 2005a and 2005b
Patch test data, consecutive patients	Citral, 2% (in pet.)	Study on 1701 consecutive patients attending contact dermatitis clinics at 6 dermatology departments were patch tested with citral between October 2002 and June 2003 (Dortmund, Copenhagen, Malmö, Odense, London and Leuven).	0.7% (12/1701) were tested positive	Frosch et al., 2005a and 2005b
Patch test data, consecutive patients	Citral, 2% (in pet.)	Study on 1855 consecutive patients attending contact dermatitis clinics at 6 dermatology departments were patch tested with citral between October 1997 and October 1998 (Dortmund, Copenhagen, Malmö, Odense, London and Leuven).	**1.1%** were tested positive (21/1855)	Frosch et al., 2002
Patch test data, consecutive patients	Citral, 2% (in pet.)	Multicenter study on 1825 consecutive patients patch tested with citral. Data were obtained from September 1998 to April 1999.	**1.0%** were tested positive (19/ 1825)	De Groot et al., 2002
Patch test data, consecutive patients	Citral, 0.1% (in pet.)	Multicenter study on 1323 patients tested in 11 centres, 192 consecutive patients were patch tested with citral at Gentofte Hospital, Copenhagen (year of testing not stated).	0% were tested positive (0/192)	Frosch et al., 1995
Patch test data, consecutive patients	Citral, 1% (in pet.)	Multicenter study on 1323 patients tested in 11 centres, 192 consecutive patients were patch tested with citral at Gentofte Hospital, Copenhagen (year of testing not stated).	0% were tested positive (0/192)	Frosch et al., 1995
Patch test data, consecutive patients	Citral, 1% (in pet.)	Study of 228 eczema patients patch tested with citral, data from North American Contact Dermatitis Research Group. Data obtained 1973-1974.	**1.7%** were tested positive (4/228)	Michell et al., 1982
Human Repeat Insult Patch Tests (HRIPT's)				
HRIPT	Citral 1.2% (1400	No further information available in cited reference.	0% were tested positive (0/101)	Unpubl. study report from RIFM 2004b

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	µg/cm^2) Veh: 3:1 DEP:EtOH			cited in Lalko and Api 2008
HRIPT	Citral 4% (1240 µg/cm^2)[2] Veh: pet.	No further information available in cited reference.	0% were tested positive (0/50)	Unpubl. study report from RIFM 1971a cited in Lalko and Api 2008
HRIPT	Citral 1% (775 µg/cm^2) Veh: alcohol SDA39C	No further information available in cited reference.	0% were tested positive (0/40)	Unpubl. study report from RIFM 1965 cited in Lalko and Api 2008
HRIPT	Citral 5% (3876 µg/cm^2) Veh: alcohol SDA39C	No further information available in cited reference.	62.5% were tested positive (5/8)	Unpubl. study report from RIFM 1964a cited in Lalko and Api 2008
HRIPT	Citral 0.5% (388 µg/cm^2) Veh: alcohol SDA39C	No further information available in cited reference.	0% were tested positive (0/41)	Unpubl. study report from RIFM 1964b cited in Lalko and Api 2008
HRIPT	Citral, 4-8% Veh: not reported	No further information available in cited reference.	48% were tested positive (19/40)	Opdyke 1979 cited from SCCFNP 1999
Human Maximation Tests (HMT's)				
HMT	Citral 5% (3448 µg/cm^2) Veh: pet.	No further information available in cited reference.	64% tests were positive (16/25)	Unpubl. study report from RIFM 1974a cited in Lalko and Api 2008
HMT	Citral 5% (3448 µg/cm^2) Veh: pet.	No further information available in cited reference.	56% tests were positive (14/25)	Unpubl. study report from RIFM 1974c cited in Lalko and Api 2008
HMT	Citral 5% (3448 µg/cm^2) Veh: pet.	No further information available in cited reference.	48% tests were positive (12/25)	Unpubl. study report from RIFM 1974c cited in Lalko and Api 2008
HMT	Citral 5% (3448	No further information available in	32% tests were	Unpubl. study report from

[2] The concentration of 4% does not seem to correspond to a dose of 1240 µg/cm^2 when compared to the other dose calculations for the HRIPT and HMT studies. The dose is probably not reported correctly in Lalko and Api 2008.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	µg/cm^2) Veh: pet.	cited reference.	positive (8/25)	RIFM 1974c cited in Lalko and Api 2008
HMT	Citral 5% (3448 µg/cm^2) Veh: pet.	No further information available in cited reference.	45.8% tests were positive (11/24)	Unpubl. study report from RIFM 1974d cited in Lalko and Api 2008
HMT	Citral 5% (3448 µg/cm^2) Veh: butylene glycol	No further information available in cited reference.	0% tests were positive (0/25)	Unpubl. study report from RIFM 1974e cited in Lalko and Api 2008
HMT	Citral 4% (2759 µg/cm^2) Veh: pet.	No further information available in cited reference.	12% tests were positive (3/25)	Unpubl. study report from RIFM 1972b cited in Lalko and Api 2008
HMT	Citral 4% (2759 µg/cm^2) Veh: pet.	No further information available in cited reference.	12% tests were positive (3/25)	Unpubl. study report from RIFM 1972c cited in Lalko and Api 2008
HMT	Citral 4% (2759 µg/cm^2) Veh: pet.	No further information available in cited reference.	20% tests were positive (5/25)	Unpubl. study report from RIFM 1972c cited in Lalko and Api 2008
HMT	Citral 2% (1379 µg/cm^2) Veh: pet.	No further information available in cited reference.	8.3% tests were positive (2/24)	Unpubl. study report from RIFM 1972d cited in Lalko and Api 2008
HMT	Citral 8% (5517 µg/cm^2) Veh: pet.	No further information available in cited reference.	33.3% tests were positive (8/24)	Unpubl. study report from RIFM 1971b cited in Lalko and Api 2008
HMT	Citral 4% (2759 µg/cm^2) Veh: pet.	No further information available in cited reference.	36% tests were positive (9/25)	Unpubl. study report from RIFM 1971c cited in Lalko and Api 2008
HMT	Citral 4% (2759 µg/cm^2) Veh: pet.	No further information available in cited reference.	16% tests were positive (4/25)	Unpubl. study report from RIFM 1971c cited in Lalko and Api 2008
HMT	Citral 4% (2759	No further information available in cited reference.	20% tests were positive (5/25)	Unpubl. study report from

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	µg/cm^2) Veh: pet.			RIFM 1971c cited in Lalko and Api 2008
Case studies				
Patch test, 9 beauticians with bilateral hand dermatitis	Citral 2% (in pet.)	Multiple case study (UK)	Positive reactions in 5/9 of the beauticians	De Mozzi and Johnston 2014
Patch test, one patient with recurrent allergic contact cheilitis	Citral 2% (in pet.)	Case study, year not reported	Strong positive reaction to citral. The cheilitis was attributed to a lip salve containg citral.	Hindle et al., 2007
Patch test, 4 bakers with hand eczema	Citral 0.5% (in pet.)	Case study	Positive reactions in 1/4 of the bakers	Malten 1979
Patch test, other patients/studies				
Experimental study, selected patients	Citral, 2% (in pet.)	Single-centre, double-blind volunteer study of 100 selected patients diagnosed with contact allergy to FMI and/or FMII. The patients were patch tested with commercial patch test fragrances incl. citral. Data from Department of Dermatology of the VI University Medical Centre, The Netherlands. Data obtained 2005-2010.	9.0% were tested positive (9/100)	Nagtegaal et al., 2012

10.8 Short summary and overall relevance of the provided information on skin sensitisation

The sensitising properties of citral have been intensively studied in both animals and humans. Citral already has a harmonised classification as a Category 1 skin sensitiser and is one of the established reference skin sensitisers listed in the guidance document of the OECD TG 429 (LLNA). Numerous animal studies confirming the sensitising properties of citral are available. The animal studies reported in table 9 represent guideline studies as well as older studies based on testing principles, that are equivalent to current test guidelines for skin sensitisation. According to the CLP criteria the results of LLNA (OECD 429), GPMT and Buehler tests (OECD 406) are directly applicable for classification and sub-categorisation of skin sensitisation.

Furthermore, a large number of publications are available on the sensitising properties of citral seen in human patch tests. For diagnostic testing of contact allergy to fragrances in humans, standardised fragrance mixtures (FMI and FMII) are used in the European baseline series used for standardised patch testing in dermatological clinics. Citral is a component of FM II, which has routinely been used for diagnostic patch testing in Europe (and elsewhere) since 2005. FMII contains 1% citral and a total of 14% fragrance allergens (SCCS 2012). When tested individually the recommended concentration for citral in pet. is 2% (Recommendation of the European Society of Contact Dermatitis). Follow-up testing of the single fragrance substances showing positive reactions in patch tests with FMI and FMII is routinely done in many dermatological clinics and the sensitising properties of citral are well documented in humans. Patch test studies with citral involving several thousand dermatitis patients from dermatological clinics in various countries in Europe and Asia are thus available. Diagnostic patch test data are generally seen as the primary source of clinical information on the occurrence of skin sensitisation and are considered to represent the most important human data in relation to this classification proposal. Results of human volunteer studies (which

are no longer performed due to ethical reasons) are also available for citral and may according to the guideline of the application of the CLP criteria be used as weight of evidence for sub-categorisation (ECHA 2015).

10.8.1 Animal data

A total of 14 LLNAs, 6 GPMTs and 1 Buehler test were identified for citral (table 9).

The reported EC3 values in the LLNAs range between 1.2% and 15% in different vehicles, most studies reporting EC3 values > 2% (Basketter et al. 2012, Jung et al. 2012, SCCS 2012, Lalko and Api 2008, Basketter and Scholes 1992). Except for the study by Jung et al., all LLNA studies were reported as being conducted according to or as being equivalent to OECD 429. The Jung study was performed according to a non-radioisotopic assay (the LLNA:BrdU-FDM). The lowest EC3 values were generally seen in studies where EtOH:DEP was used as a vehicle (EC3 range 1.2%-6.8%) whereas studies using AOO as vehicle generally report higher EC3 values (EC3 range 7-15%). This could indicate a potential influence of the vehicle used on the results.

Lymphocyte proliferation may be influenced by choice of vehicle as some vehicles may either suppress or enhance the proliferative response of certain chemicals. This may especially be important for weak sensitisers with high EC3 values (Anderson et al., 2011). AOO (4:1) is among the recommended vehicles in OECD 429 test guideline. Other vehicles than those recommended may be used if sufficient scientific rationale is provided. Ethanol (EtOH) containing vehicle systems are apparently frequently used for assessing dermal effects of fragrance materials in both human and experimental studies, and the use of EtOH:DEP as an alternative vehicle to AOO has been investigated in a comparative study. EtOH:DEP induces a background proliferative lymph node response similar to that of AOO, and it was concluded that EtOH:DEP is a suitable alternative to AOO in the LLNA (Betts et al. 2007). Provided that the vehicle is suitable and does not elicit unwanted increases in background proliferative lymph node response, the choice of vehicle would not be expected to have a marked impact on the magnitude of the stimulation index (SI) as it is measured as the increase in lymphocyte proliferation upon exposure to a test substances relative to that of the vehicle control (Anderson et al., 2011).

In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 2/6 studies (with intradermal induction doses of 5 and 10% citral, respectively, vehicle not reported) (Lalko and Api 2008). In a GPMT with an intradermal induction dose of 0.2% positive responses were seen in 60% of the animals (vehicle not reported) (Basketter and Allenby 1991, Basketter et al., 1991 and Basketter and Scholes 1992). In a GPMT with an intradermal induction dose of 0.4% positive responses were seen in 40% of the animals (vehicle not reported) (Lalko and Api 2008). In two of the GMPT studies 60-100% of the animals responded after intradermal induction doses of 25% citral (vehicle: paraffin pol or Freunds adjuvant/dest. Aqua) (study reports cited from REACH reg.).

Sensitisation was also observed in 100% of the animals in the Buehler test with an induction concentration of 20% citral (vehicle: petrolatum) (Lalko and Api 2008).

The above reported animal studies identified are relevant in terms of classification and confirm the sensitising properties of citral. For most of the studies robust information is not available and the results are cited from reviews (SCCS 2012 and Lalko and Api, 2008). Although the quality and reliability cannot be assessed in detail the results of the animal studies are, however, relatively consistent.

Other (and older) animal studies on the skin sensitising properties of citral are also identified but not included in table 9. Such studies include Draize tests, modified Maguire delayed hypersensitivity tests, Open Epicutaneous Tests (OET), Single Injection Adjuvant Tests (SIAT). These studies all confirm the sensitising properties of citral (Lalko and Api, 2008). However, as such studies are not directly applicable for sub-categorisation of skin sensitisers according to the CLP criteria and guidance, these studies have not been included in the current CLH report as plenty of currently accepted guideline studies are available.

10.8.2 Human data

A total of 25 diagnostic patch tests, 6 HRIPTs, 14 HMTs and 4 case studies were identified for citral (table 10).

Diagnostic patch testing is conducted in order to diagnose contact allergy to a substance and is performed according to international standards by dermatologists (Johansen et al. 2015). The results of such patch tests are usually reported as number of patients/subjects having positive reactions in relation to the total number tested, i.e. the frequency of positive patch tests. An important factor when assessing the prevalence of positive reactions in diagnostic patch tests is how the group of patients are defined, i.e. selected patients versus consecutive (unselected) patients. Selected patients can be i.e. patients with eczema suspected of being contact allergy to fragrances or cosmetics or other patients with a history of skin symptoms provoked by e.g. scented products (aimed testing). Consecutive (unselected) patients are groups of patients for whom allergic contact dermatitis (ACD) is generally suspected.

The positive patch test frequencies from the 25 reported diagnostic patch tests vary between 0.3 and 16.7% in all dermatitis patients and the highest frequencies of positive patch test reactions with citral were generally seen in patch tests with selected patients. In patch tests with selected dermatitis patients positive reactions range between 0.3 and 16.7% and high frequencies of positive reactions (≥2.0%) were seen in 10 out of the 11 tests. Complete absence of positive reactions was not observed in any of the patch tests with selected patients. The patient groups were mostly larger than 100 patients. In patch tests with consecutive (unselected) dermatitis patients positive reactions range between 0.3 and 1.7%. Complete absence of positive reactions was observed in 2 of the 14 patch tests with unselected patients whereas relatively high frequencies of positive reactions (≥1.0%) were seen in 5 of the 14 tests. The patient groups were mostly larger than 500 patients. Citral was typically tested in concentrations of 2% (in petrolatum) in the diagnostic patch tests, which is the concentration recommended by the European Society of Contact Dermatitis. The total number of positive reactions in the published cases is > 400. The results of the many patch tests confirm that positive reactions to citral are commonly observed in dermatitis patients and with relatively high frequencies observed in a number of tests. The patch test data collectively cover information from the last 3-4 decades and from many different dermatological clinics in different countries. Although it is not possible to directly compare these findings and draw conclusions on any tendencies in the sensitisation rates, it is obvious that high sensitisation frequencies have been observed for citral in recent years and that patients in many countries are affected.

Induction of sensitisation was also reported in 2 of 6 HRIPT studies after exposures to between 4-8% (>500 µg/cm^2) citral (difference vehicles or vehicle not reported). Sensitisation was observed in 13 of 14 HMT studies after exposure to between 2-8% (>500 µg/cm^2) citral (vehicle in all studies: petrolatum, except one HMP where butylene glycol was used). The number of volunteers tested ranged from 8-101 in the HRIPT studies and 24-25 in the HMT studies. Concentrations lower than 500 µg/cm^2 citral were generally not tested in these studies except for one HRIPT study (conc. 0.5% / 388 µg/cm^2 citral) where no sensitisation was seen in the 41 tested subjects. Robust study information is not available for these studies which are all cited from reviews (Lalko and Api, 2008 and SCCFNP, 1999).

A few case studies are reported. One study from UK reports positive reactions to citral (2.0% in pet.) in 5 out of 9 beauticians with bilateral hand dermatitis. The beauticians were patch tested with FMI and FMII (9 patients), additional fragrance series (7 patients), own products (5 patients) and a cosmetic series (4 patients) (De Mozzi and Johnston, 2014). One study reports strong positive reactions to citral (and FMII) in a female patient with recurrent allergic contact cheilitis (inflammation of the lips). The cheilitis was attributed to a lip salve containing citral (Hindle et al., 2007). A third study investigating 4 bakers with hand eczema showed that 1 out of 4 were tested positive when patch tested with 0.5% citral (in pet.) (Malten 1979). The case studies confirm the general picture observed in the other patch tests with dermatitis patients described above.

In an experimental study the possible role of skin irritation response in relation to polysensitisation to fragrances was investigated in 100 volunteer patients with confirmed fragrance contact allergy. All patients were patch tested (on the back) with 27 fragrance chemicals including citral. Furthermore a simultaneous patch test was done with sodium lauryl sulphate (a known skin irritant) on the upper arm of the patients. The study was not a clinical diagnostic patch test but the tests were nevertheless performed according to the guidelines of the International Contact Dermatitis Research Group. In this study 9.0% of the patients had

positive reactions to citral (in 2% petrolatum). This result thus confirms the high frequencies of positive reactions to citral found in routine diagnostic patch testing with selected patients (Nagtegaal et al. 2012).

The human studies identified are all relevant in terms of classification and confirm the sensitising properties of citral. The comprehensive set of diagnostic patch test data covering the last 3-4 decades with several of the studies being published very recently are seen as the key information for this classification proposal. For the HMT and HRIPT studies (older volunteer studies) robust study information is not available and the results are cited from reviews (SCCFNP 1999 and Lalko and Api, 2008). These data are seen as supporting evidence.

10.8.3 Human exposure

Citral is a fragrance that is manufactured in or imported to the EU in amounts of 1000-10.000 tonnes/year and is widely used in products on the EU market. The registered categories of use for consumers are cosmetics and a variety of household and professional cleaning and maintenance products. Data from the fragrance industry (cited in SCCS 2012) indicate that 80% of the total fragrance chemical volume is used in cosmetics and 20% in household products. Although cosmetics are assessed to be the main use category for citral, the use in other products (household and other products) may thus account for a substantial volume. As citral is widely used in many different types of consumer products the general population can be exposed from many different sources.

Citral is generally present in low concentrations in individual consumer products. The International Fragrance Association (IFRA) recommends maximum limits of citral in leave-on cosmetic products between 0.04-1.4% depending on the product category and 1.0-5.0% in rinse-off cosmetic products and other consumer products as shown in Table 11 (IFRA 2013, IFRA 2015). (Note that other product types than those specifically mentioned in the table driving the category consumer exposure level are also covered under the different categories).

Table 11: The IFRA standard limits for citral in IFRA QRA (Quantitative Risk Assessment) product categories (IFRA 2013, IFRA 2015):

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

*Maximum pragmatic level

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CITRAL; 3,7-DIMETHYLOCTA-2,6-DIENAL

The SCCS opinion refers to a number of surveys on the presence and content of the 26 fragrances subject to labelling requirements (for cosmetics and detergents) in various consumer products. The reported occurrence of the fragrances is mostly based on labelling information alone, i.e. whether the substances are mentioned on the label of the product. In one survey the content was verified by chemical analysis. Table 12 summarises the results of the surveys with respect to the occurrence of citral in various consumer products.

Table 12: Occurrence of citral in consumer products, different surveys (cited from SCCS 2012):

Product type	**Number of products investigated**	**% products labelled to contain citral**	**Reference in SCCS 2012**
Children's cosmetics	n.a	8.2%	Table 10.1, p. 72
Deodorants	88	26.1% (44% products found to contain citral; measured conc. from 39-554 ppm)	Table 10.2, p. 75
Consumer products (cosmetics, household products)	300	25%	Table 10.3, p. 77
Consumer products	516	11.6%	Table 10.4, p. 78
Consumer products	3000	Approx. 12%	Figure 10.1, p 78

Citral was found to be present in 8-26% of the products covered in the different surveys based on labelling information alone. One study of deodorants showed that the occurrence of citral was even more frequent than expected based on subsequent chemical analysis. It was concluded by SCCS that taking the total exposure into account, exposure to all 26 allergenic fragrances is foreseeable in daily life.

The Danish EPA has conducted surveys and assessments of a broad range of consumer products on the Danish market over the last decades. Citral has been identified in many different types of products but mostly in cosmetic products, including day-to-day cosmetic products such as deodorants, soaps, shampoo/conditioner, lotions and creams as well as e.g. eterical oils, scented oils and massage oils. Citral has also been found in household products such as cleaning agents, stain removers and air care products and in articles such as erasers and pens. Generally citral is found in low concentrations (>0- <0.06%) in the investigated products but with some exceptions. High concentrations have thus been identified in massage oils (up to 3.25%); eterical oils/scented oils (up to 78%) and air fresheners (up to 26%) (DK EPA database, search June 2016).

The Danish Product Register contains information of hazardous substances in mixtures for professional use. Data from the Register confirm that citral is used in a wide range of products on the market, especially cleaning products. The concentrations are generally lower than 0.1% in the majority of the products. However, concentrations above 1% are found in fragrance mixtures and scented oils (Danish Product Register, 2016).

The substance evaluation (SeV) performed for citral in 2015 (under REACH) refers to estimated exposure values for citral (dermal long-term route) from the registration dossier. The exposure values for workers are estimated to be between 47-100 µg/cm^2 depending on the exposure scenario (50-75 µg/cm^2 for the use in cleaning agents). Exposure values for consumers are estimated to be 47-50 µg/cm^2 for the use in cleaning agents. The exposure values for use of cleaning agents are based on the highest concentrations of citral reported by the Registrant(s) in the exposure scenarios for the use in cleaning agents. These concentrations correspond to <1.5% for workers and <0.5% for consumers. However, it is noted in the SeV conclusion report that products with higher concentrations of citral are found on the Swedish market (KEMI 2015).

Human exposure to citral generally seems to be low based on the above information. The exposure is, however, assessed to be frequent due to the widespread uses, primarily as a fragrance in consumer products, and the high tonnage level of citral. It is thus difficult for consumers to avoid exposure. According to the data from IFRA the exposure of citral when used as a fragrance in cosmetics is low with standard limits for citral in most leave-on products being below 1% (except for IFRA QRA Product Category 8). For rinse-off cosmetics and for non-cosmetic products with direct skin contact (IFRA QRA Product Category 10) cleaning agents higher standard limits are allowed (≥1%), but a relatively low exposure is expected due to the intermedient nature of the exposure and shorter duration of exposure compared to leave-on products.

10.9 Comparison with the CLP criteria

Citral is a widely used fragrance and a well known skin sensitizer as reflected by the existing harmonized classification as Skin sens 1. A new assessment of the skin sensitizing properties of citral has been conducted according to the current classification criteria as the data are considered sufficient for assessing the appropriate sub-category for this hazard class.

According to the classification criteria sub-category 1A represent "*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*" (CLP table 3.4.2).

According to the classification criteria sub-category 1B represent "*Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered*" (CLP table 3.4.2).

10.9.1 Animal data

According to the classification criteria evidence from animal studies for sub-category 1A and 1B, respectively, can include the following types of data and results (CLP tables 3.4.3 and 3.4.4):

	Animal data	
Sub-category 1A	LLNA	EC3 value ≤ 2 %
	GPMT	≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose
	Buehler	≥ 15 % responding at ≤ 0,2 % topical induction dose or ≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose
Sub-category 1B	LLNA	EC3 value > 2 %
	GPMT	≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
	Buehler	≥ 15 % to < 60 % responding at > 0,2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

Test results from the LLNA, GPMT and Buehler tests can be used directly for classification and potency assessment. In two out of 14 reported LLNAs a high potency of citral was demonstrated with EC3 values < 2% (1.2 and 1.5%, respectively), i.e. equivalent to Category 1A. In the other 12 LLNAs a moderate potency of citral was demonstrated with EC3 values ranging from 2.1-15%, i.e. equivalent to Category 1B. One EC3 value of 2.1% was, however, borderline to the cut-off criteria for sub-categorisation. The lowest EC3 values were generally obtained in studies using EtOH:DEP as vehicle. This vehicle has been demonstrated to induce a similar background proliferative lymph node response in the LLNA compared to AOO (one of the

preferred vehicles in the LLNA) and was considered to be equally suitable. The results from LLNAs using EtOH:DEP are thus considered to be of equal reliability to those using AOO as a vehicle.

Six GPMTs are available. In one GPMT using an intradermal induction dose of 0.2% positive responses were seen in 60% of the animals, indicating a high potency (i.e. Cat 1A). In a GPMT with an intradermal induction dose of 0.4% positive responses were seen in 40% of the animals, indicating a moderate potency (i.e. Cat 1B). In two GPMTs with intradermal induction doses of 5 and 10% citral, respectively, sensitisation was observed but not quantified (i.e. number of animals affected). A decision on sub-categorisation is thus not possible for these studies. In other two GMPTs 100% of the animals responded after intradermal induction doses of 25% citral (with the exception that in one study 60% positive reactions were seen after 144h after a 5% rechallenge). Although these two studies would indicate a moderate sensitising potency due to the high intradermal doses used it cannot be ruled out that a high response would have been observed if lower intradermal induction doses had been used. These two studies are thus not suitable for drawing conclusions on sub-categorisation either.

In the Buehler test sensitisation was observed in 100% of the animals with an induction concentration of 20% citral, indicating a high potency of citral (i.e. Cat 1A, but borderline to Cat 1B).

The LLNA (OECD 429) is generally regarded as being better suited for potency assessment compared to the guinea pig guideline studies (Basketter et al., 2005, ECHA 2015). The LLNA only targets the induction phase of sensitisation, provides dose-response information and has a quantitative and unambiguous endpoint. Assessment of the potency based on GPMT and Buehler tests may be associated with some uncertainty as these tests give no information on dose-response relationships (only one induction dose is used) and the endpoints measured are related to elicitation and are of a qualitative nature. As the guinea pig tests should be conducted at the highest induction dose causing mild-to-moderate sensitisation the concentrations used are often not in the low range that triggers a sub-category 1A classification. The sensitisation potency may thus be underestimated. Only for strong sensitisers tested at low induction doses in the guinea pig guideline tests a relatively certain conclusion can be drawn with relation to potency.

In summary 4 of the 21 animal studies – including 2 LLNAs, one GPMT and one Buehler test - indicate a high sensitizing potency of citral. The remaining studies either indicate that citral is a skin sensitizer of moderate potency or do not allow conclusions on potency due to the design of the studies (doses used, lack of quantification of response). For most of the studies robust study information is not available to assess the quality more precisely. Caution should thus be exerted in drawing firm conclusions on sub-categorisation based on the animal data alone. Collectively, the results of the animal studies confirm the sensitizing properties of citral in a relatively consistent manner with a potency ranging from moderate to strong.

10.9.2 Human data

According to the classification criteria human evidence for sub-category 1A and 1B, respectively, can include the following types of data (CLP section 3.4.2.2.2):

	Human data
Sub-category 1A	(a) positive responses at ≤ 500 μg/cm^2 (HRIPT, HMT — induction threshold); (b) 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; (c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.
Sub-category 1B	(a) positive responses at > 500 μg/cm^2 (HRIPT, HMT — induction threshold); (b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure; (c) other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

The guidance on the application of the CLP criteria further outlines how high or low frequency of occurrence of skin sensitization shall be assessed. The exposure level is determined according to table 3.4.2-b in the guidance as shown below (ECHA 2015).

Table 3.4.2-b Relatively high or low exposure* (copied from ECHA 2015)

Human diagnostic patch test data	**High frequency**	**Low 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

* Only one or two types of information may be sufficient for sub-categorisation.

The key evidence for the assessment of the potency of citral in this classification proposal is the human data from diagnostic patch tests. Patch test data are available from several dermatological clinics in many different countries in and outside EU. In the patch test studies summarized in table 10 relatively high frequencies of positive reactions are seen upon exposure to citral. For selected dermatitis patients positive reactions range between 0.3 and 16.7% with frequencies ≥2% in 10 of 11 studies. For consecutive (unselected) dermatitis patients positive reactions range between 0.3 and 1.7% are observed with 5 of 14 studies reporting frequencies ≥1%. These studies represent more than 400 published cases of positive patch test reactions to citral.

The collected data from patch test studies thus show that

- a high frequency (≥1%) of occurrence of skin sensitization is observed in a relevant part (5 of 14) of the patch tests with consecutive (unselected) dermatitis patients
- a high frequency (≥2%) of occurrence of skin sensitization is observed in the majority (10 of 11) of the patch tests with selected dermatitis patients
- the number of tested dermatitis patients showing positive reactions to citral is well above 100 (>400 cases)

These findings show a high frequency of occurrence of sensitization for citral in humans. For deciding on the appropriate sub-category the data from patch test studies need to be seen in conjunction with the estimated exposure (see chapter 10.9.1.3 below).

Furthermore, three case studies of ACD are available including two studies related to occupational exposure. Citral was found to be among the causative agents of the ACD. The quality and relevance of these studies for the purpose of classification are questionable and they are only seen as supportive evidence for the findings of the patch test studies.

The positive responses reported at relatively high concentrations > 500 µg/cm^2 in two older HRIPT studies and in 13 older HMT studies indicate a moderate sensitisation potential of citral. The HRIPT and HMT studies are non-clinical studies based on healthy volunteers representing the general population (and are no longer conducted due to ethical reasons).Robust study information is not available for the HRIPT and HMT studies. The estimated induction concentrations (>500 µg/cm^2) are calculated by fragrance industry and the original data have not been published. They are considered of lower relevance for this classification proposal.

In an experimental volunteer study sensitisation to citral was reported in 9% of the fragrance allergy patients patch tested with 27 fragrance chemicals.

10.9.3 Exposure considerations

The occurrence of skin sensitization in human studies needs to be seen in conjunction with the level of exposure in order to make a decision on sub-categorisation of skin sensitisers. As described in chapter 10.8.3 the exposure to citral is generally considered to be low based on the current IFRA standard limits and supported by information of the actual concentration of citral in various consumer products reported in different surveys.

According to the guidance on the application of the CLP criteria an additive exposure index shall be set in order to decide on the appropriate sub-category for skin sensitisers (when based on human data). An additive exposure index of 1-4 equates to relatively low exposure, whereas 5-6 reflects relatively high exposure. The exposure index is determined according to table 3.4.2-c in the guidance as shown below (ECHA 2015).

Table 3.4.2-c Relatively high or low exposure (adapted from ECHA 2015)

Exposure data	**Relatively low exposure (weighting)**	**Relatively high exposure (weighting)**	**Score for citral**
Concentration / dose	< 1.0% < 500µg/cm^2 (score 0)	≥ 1.0% ≥ 500µg/cm^2 (score 2)	0
Repeated exposure	< once/daily (score 1)	≥ once/daily (score 2)	2
Number of exposures (irrespective of concentration of sensitizer)	<100 exposures (score 0)	≥ 100 exposures (score 2)	2

To achieve the exposure index a response in each row in table 3.4.2-c above is necessary. The exposure index of citral is estimated based on the following assumptions:

- **Score 0** for concentration/dose: based on expected and observed concentrations < 1.0% of citral in relevant (consumer) products on the market. Exposure estimates in the range 47-100 µg/cm^2 (workers and consumers) for dermal long-term exposure as referred in the Substance Evaluation for citral (KEMI 2015) are also indicative of low exposure.

- **Score 2** for repeated exposure: based on the frequent occurrence of citral in consumer products with estimated daily use.

- **Score 2** for number of exposures: based on an anticipated exposure of sensitised individuals to citral at least more than 100 times.

An additive exposure index of maximum 4 (0+2+2) is thus estimated indicating a relatively low exposure. A decision on the appropriate sub-category for skin sensitisers based on human data is done according to table 3.4.2-d in the guidance:

Table 3.4.2-d Sub-categorisation decision table (from ECHA 2015)

Exposure data	**Relatively low frequency of occurrence of skin sensitisation**	**Relatively high frequency of occurrence of skin sensitisation**
Relatively high exposure (score 5-6)	Sub-category 1B	Category 1 or case by case evaluation
Relatively low exposure (score 1-4)	Category 1 or case by case evaluation	**Sub-category 1A**

10.9.4 Weight of Evidence

Both animal and human data are available documenting the skin sensitizing properties of citral. These data are considered in a total weight of evidence assessment (WoE) according to the CLP criteria and guidance.

The animal data provide some evidence of strong sensitising effects of citral as reflected in 4 out of 21 guideline studies fulfilling the criteria for a sub-category 1A classification. Among the standardized animal tests for skin sensitisation the LLNA is considered best suited for potency assessment (Basketter et al., 2005, ECHA 2015). Two LLNAs have EC3 values < 2% fulfilling the criteria for sub-category 1A classification. Furthermore one LLNA shows an EC3 value of 2.1% that is borderline for classification in category 1A or 1B. One GPMT and one Buehler assay confirm the strong sensitisation potential of citral whereas the remaining part of the animal studies either indicate moderate sensitisation (Cat 1B) or do not justify sub-categorization. For most of the animal studies robust study information is not available to assess the quality more precisely. It is noted that the expert group assessing classification criteria for skin sensitising potency by use of existing (animal) methods stated that if EC3 values are available from several studies then the lowest value should normally be used. The expert group further concluded that if a variety of animal data leads to different categorisation of the same substance the higher potency category should apply (Basketter et al., 2005). Although these considerations are not fully reflected in the guidance this speaks in favour of a sub-category 1A classification.

The human data available provide substantial evidence of strong sensitising effects of citral especially based on the results of patch tests with selected patients. Diagnostic patch test data obtained from eczema patients attending individual dermatology clinics or collected clinic data is the primary source of clinical information on the occurrence of skin sensitisation (ECHA 2015) and diagnostic patch tests are generally performed under internationally standardised conditions. Human patch test studies with citral show a high frequency of occurrence of skin sensitisation of citral according to the classification criteria. According to the guidance the following three types of human information confirm the high frequency of occurrence of skin sensitisation: Data from unselected and selected dermatitis patients as well as a high number of published cases (>100). The comprehensive set of patch test data include thousands of dermatitis patients tested in dermatological clinics in different countries, mostly in EU. Older volunteer studies in humans (HRIPT and HMT studies) generally confirm the sensitising properties of citral and indicate a moderate potency. Original study information is generally not available for these non-clinical experimental studies.

Although frequent/daily exposure to citral is anticipated the overall exposure to citral is estimated to be relatively low based on information on the use in consumer products such as cosmetics and cleaning products and also in professional cleaning products.

Based on the high frequencies of skin sensitisation observed in human patch tests with citral(≥2.0% in 10 of 11 patch tests with selected dermatitis patients and ≥1.0% in 5 of 14 patch tests with unselected dermatitis patients) and the high number of published cases combined with the estimated low exposure, a classification of citral as a strong skin sensitiser in sub-category 1A is justified.

10.10 Conclusion on classification and labelling for skin sensitisation

The available animal and human studies confirm the sensitising properties of citral in accordance with the existing harmonised classification as a skin sensitiser in Category 1. The focus of the current CLH proposal for citral is the sensitising sensitising potency of citral, which is most clearly reflected from the human patch test data.

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

While the animal data are not uniform in their results with respect to a potency assessment, four guideline studies are available confirming a strong sensitising potency of citral. Collectively, the available data fulfil the criteria for classification of citral as a strong skin sensitiser in sub-category 1A.

Specific concentration limits can be set for skin sensitisers when reliable and adequate information is available to support that the specific hazard is evident below (or above) the GCL. The setting of an SCL for

sensitisers is based on potency. For skin sensitisers the guidance clearly describes how an SCL can be set based on the results of certain animal studies (i.e. when a high response level is observed below a certain low dose). Further, relevant information e.g. from workplaces with known exposure levels can be used to justify a different SCL than those recommended based on the results of the animal studies.

The guidance does not provide any information on how an SCL may be set based on human data alone. Whereas the human patch test data support that citral is a strong sensitizer fulfilling the criteria for Category 1A these data do not provide clear dose-response information or specific information on the previous exposure regime for these patients. These data alone are thus not considered to support the establishment of an SCL. Furthermore, those animal studies that support a strong sensitizing potential of citral do not indicate extreme potency. Collectively the data do not justify the setting of an SCL.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Proposal before public consultation

The sensitising properties of citral have been intensively studied in both animals and humans. Citral already has a harmonised classification as Skin Sens. 1, and it is one of the established reference skin sensitisers listed in the guidance document of OECD TG 429 (local lymph node assay). Both guideline and non-guideline studies in animals are available; the positive results of numerous local lymph node assays, Guinea pig maximisation tests and a Buehler test are directly applicable for classification and sub-categorisation. A large number of human patch tests are also available. Citral is a component of one of the standardised fragrance mixtures used in the European baseline series used for diagnostic patch testing in dermatological clinics. Follow-up testing with the single fragrance substances is done routinely in many clinics; the sensitising properties of citral are well documented. Results of historical human volunteer studies are also available for citral and provide supporting evidence for sub-categorisation.

All of the available animal studies on citral are positive for sensitisation. Four studies (two local lymph node assays, one Guinea pig maximisation test and the Buehler test) indicate a strong potency. The remaining studies either indicate that citral is a skin sensitiser of moderate potency, or do not allow conclusions on potency due to the design of the studies (doses used, lack of quantification of response). Robust study information is not available for most of the animal studies, so conclusions on sub-categorisation cannot be made on the animal data alone.

The human data provide substantial evidence of the strong sensitising effects of citral, especially based on the results of patch tests with selected patients. Data are available from thousands of selected and unselected patients, with well over 400 published cases of positive reactions. Although robust study information is not available for some of the older volunteer studies in humans (human repeat insult patch tests and maximisation tests: HRIPTs and HMTs), the studies generally confirm the sensitising properties of citral and indicate a moderate potency.

There is wideapread use of citral as a fragrance in cosmetics and other consumer products,

and a high tonnage is placed on the market (1000 – 10000 tonnes/year). Although frequent or daily exposure to citral is anticipated, the overall exposure to citral is estimated to be relatively low based on information on how citral is used in these products.

Overall, there is a high frequency of skin sensitisation in human patch tests (≥ 2.0% in 10 of 11 patch tests with selected dermatitis patients and ≥ 1.0% in 5 of 14 patch tests with unselected dermatitis patients) and a high number of published cases, set against an estimated low exposure. This justifies classification in sub-category 1A. The animal data are not uniform in their results with respect to a potency assessment, however four guideline studies are available which confirm a strong sensitising potency of citral. Collectively, the available data fulfil the criteria for classification of citral in sub-category 1A.

Comments received during public consultation

Comments were received from three Member State Competent Authorities (MSCAs), three non-governmental groups of dermatologists, an expert individual, a manufacturer and a trade association.

Differing views on sub-categorisation were expressed. Some contributors supported the proposal to classify in sub-category 1A, however the expert individual and manufacturer concluded that the data supported classification in sub-category 1B. The trade association criticised the approach taken and did not support classification in category 1A; however, it was not clear from their comments whether they felt category 1 or 1B was appropriate. One MSCA commented that both the human and animal data were borderline between the two sub-categories.

Two non-governmental groups provided a short statement supporting the proposal to classify in sub-category 1A. The remaining contributors provided comprehensive comments, covering the animal data, human data and exposure considerations.

Animal data

All three MSCA agreed with the DS that due to the lack of detail/information about most of the animal studies, conclusions on the potency could not be made based on the animal data alone; one noted that it was difficult to assess the reliability of the studies in the absence of reliability scores. One MSCA, the expert individual and the manufacturer commented that the animal data supported classification in sub-category 1B.

A MSCA, a non-governmental group and the manufacturer noted the effect of vehicle on the results of the LLNAs. Four studies used acetone:olive oil (AOO), which is the standard and most commonly used vehicle in the LLNA, whereas ten assays used mixtures of ethanol:diethyl phthalate (EtOH:DEP). An increase in the sensitising potency for citral was seen in the LLNAs using EtOH:DEP; two of the LLNAs with EtOH:DEP gave EC values < 2, which supports classification in sub-category 1A. The non-governmental group noted that the vehicle had an influence on the skin absorption of a substance and therefore its sensitising potency, but pointed to an experimental study which concluded that EtOH:DEP provided a suitable vehicle for use in the LLNA (Betts *et al.*, 2007). The group concluded that it was acceptable to use this solvent and noted that all experiments using EtOH:DEP were performed by the fragrance industry since this vehicle is considered more appropriate with regard to the exposure from fragranced consumer products.

Generally, the manufacturer noted that there was a significant range of EC3 values for citral (1.2 – 15%), and that EC3 values varied even when the studies were conducted by the same laboratory with the same solvent. Specifically, the manufacturer mentioned that the 3 LLNAs with EC3 values below or close to the cut-off of 2% had been repeated by the same laboratory with a comparable protocol, all giving different EC values (i.e., > 2%). Furthermore, 2 of these studies used tocopherol or BHT/tocopherol/eugenol mixes, which did not represent standard vehicles for LLNAs. Therefore, the reliability of these studies for classification purposes could not be confirmed. The manufacturer commented that the variability, validity and reproducibility of the results had to be taken into account. Given that most studies gave EC values > 2% the manufacturer concluded that the animal data supported classification in sub-category 1B.

The manufacturer and one MSCA highlighted limitations regarding the Bühler test (i.e., dosing regime and animal numbers).

Human data

Two MSCAs provided an analysis of the data and agreed with the DS that the human patch test data provided the key evidence for the assessment of potency. One authority agreed that "high frequency" could be assigned to the selected patients, however disagreed that it could also be assigned to unselected patients. They also questioned the inclusion of data from North American and Korean studies, given that the available exposure data refered to the European situation only. The second MSCA noted that the HRIPT studies were performed over a range of concentrations, but resulted in few cases of sensitisation. In order to clarify the outcome of the HRIPTs, it was suggested that these studies were evaluated and discussed further in the CLH report, if possible. The remaining MSCA fully supported the statement that there was a high frequency of sensitisation for citral in humans, however questioned why different concentrations (0.1-5%) of citral were used in the patch tests. They noted that citral was a skin irritant, and as such some reported reactions could be due to irritation rather than sensitisation.

The expert individual, a clinician based in Germany, concluded that the animal data supported classification in sub-category 1B, and that the human data were insufficient to overrule the animal data. He would not have used the data from selected patients for hazard or risk assessment given the heterogeneous nature of the selection process. Of the fragrances in the standard series used for patch testing, citral was not a substance that had given an especially high frequency of responses in non-selected patients; several substances had given higher response rates. When sensitising frequencies (clinical data) and exposure frequencies (volumes in consumer products) were compared for the standard series, as an indicator of risk, citral appeared not to be of high concern.

The manufacturer and the industry association disagreed with the DS's assessment of the human patch test data. They argued that it is impossible to know the induction exposure levels and the conditions of the patients in the studies showing a high frequency of reactions to Citral. Due to the clearly defined induction exposure conditions used in the HRIPTs and the HMTs, they considered these studies to be a more useful source for the assessment of potency. The industry association disagreed that the HRIPT and HMT studies, which did not indicate a high sensitisation potency of citral, were supporting information only, and argued that the absence of robust study information could not be used to prove lower relevance of

this information in the classification decision. The manufacturer had provided the DS with further details of these studies, and stated that all of the tests in human volunteers supported classification in sub-category 1B.

Although the manufacturer accepted that the cumulative data on selected dermatitis patients met the criteria of a "high frequency" of cases according to the CLP criteria, a meta-analysis of all other data for unselected dermatitis patients (including two new studies published in 2017 – see Additional Key Elements) met the criteria for low frequency. According to this analysis, the positive response rate was 0.89% (192/21692 patients tested).

Exposure

One MSCA accepted that some consumer products contained high levels of citral, but noted that these exceptions mainly refered to products that were not intended for long skin contact. They, and another MSCA, thus agreed that exposure to citral could generally be regarded as "low" due to an relative exposure index of 4 that was calculated when considering the frequency of exposure to citral (the score '0' was given for concentration/dose in the meaning of the CLP guidance, table 3.3). The MSCA who thought that the classification was a borderline argued that the overall score for the exposure data could be 5 (rather than 4, as proposed by the DS), which would have led to the category "relatively high exposure" (rather than "relatively low exposure").

The manufacturer and the industry association disagreed with the DS's assessment that exposure to citral was 'low', and suggested that the content of the substance in consumer products leading to the induction of sensitisation had been underestimated. In addition, they noted that exposure to citral occurs also from natural food sources, e.g. citrus fruits. It was not possible to know if those patients who had responded positively on patch testing with citral had mostly been induced by low concentrations.

Although the International Fragrance Association (IFRA) had in 2006 issued a limit of 1% on the content of citral in many consumer products, the manufacturer and industry association disagreed that this was additional evidence for a 'low exposure'. The limit would not have translated through to many of the products actually being used for some years later, and potentially as late as 2013. Furthermore, the manufacturer argued that the DS had not provided adequate justification for excluding from their analysis products that were exempt from the IFRA limit, and historical exposures to other products containing > 1% citral. The manufacturer noted that most publications reporting a high frequency of reactions in unselected patients and selected patients covered clinical patch test studies that were carried out in periods including up to 2013. The manufacturer and industry association both commented that actual and historic exposures to concentrations > 1% citral should have given a dose or concentration score of 2, which would have led to an additive exposure index of 2+2+2=6 and would have defined exposure as relatively high.

The manufacturer concluded that the low frequency of positive patch test results in unselected dermatitis patients combined with a strong potential for high estimated exposure both from a historical and current perspective provided a justification for a classification in sub-category 1B.

Analysis of further information received during the public consultation

During the public consultation, the manufacturer provided information about two additional studies.

The aim of the first of these was to report the prevalence of sensitisation to the 26 EU-labelled fragrance allergens (one of which is citral) from 2010 to 2015, using data from a single university clinic (University Hospotal Herlev-Genofte, Denmark) on consecutive, unselected patients (Bennike *et al*., 2017). The study reported a positive reaction rate to citral of 0.39% from 2010 to 2015. The publication also reported a clear decreasing prevalence trend from 2010 to 2015.

The aim of the second study (Mowitx *et al.*, 2017) was to investigate the frequency of allergic reactions to fragrance mix I (FM I),fragrance mix II (FM II) and their ingredients in consecutive patients. The data showed 1.1% positive reactions to citral (22/2248) during the period 2009 - 2012, and 1.3% (30/2248) positive reactions during 2013-2015.

In their response to the public consultation, one MSCA indicated that further evaluation and discussion of the HRIPT studies would have assisted in the assessment of citral. During the public consultation, the manufacturer provided further information about these tests, including full study reports for 3 of the 6 studies. This additional information is summarised in the following table.

Unless otherwise stated, the tests involved nine 24 h occluded induction applications (3 times a week over 3 weeks), followed approximately 2 weeks later by a 24 hour occluded challenge application to a virgin site. Reactions were read at patch removal and again at 24 and 72 hours after patch removal. Similarly, unless stated, no information was provided in the study report on the sex, age, ethnicity or health condition of the volunteers.

Additional information on the Human Repeat Insult Patch Tests			
Study details and Reference	**Participants**	**Results**	**RAC observations**
Reactions read at 24, 48 and 72 hours after patch removal. 1.2% citral in 3:1 DEP:ethanol (1400 µg/cm^2) RIFM (2004b)	101 volunteers (30 male and 71 female, age range 18-69). Subjects did not exhibit any dermatological or other medical condition which would preclude topical application of the test material.	No reactions (0/101)	No evidence of sensitising potential was observed.
Fifteen 24 hours occluded induction patches (3 times a week) followed 14 days	50 volunteers.	No reactions (0/50)	No evidence of sensitising potential was observed. Relatively small group size limits statistical power of

later by a 24 hour occluded challenge patch. Reactions were read at patch removal and 24 and 48 hours later. Induction and challenge: 4% citral in petrolatum (444 µg/cm^2) RIFM (1971a)			the study.
Induction and challenge: 1% citral in alcohol SDA 39C (775µg/cm^2) RIFM (1965)	40 volunteers (11 males and 29 females).	No reactions (0/40)	No evidence of sensitising potential was observed. Relatively small group size limits statistical power of the study.
Induction and challenge: 5% citral in alcohol SDA 39C (3875 µg/cm^2) RIFM (1964a)	8 volunteers (all female). No information on the age, ethnicity or health condition of the volunteers is available.	5/8 reactions	A high number of volunteers (62.5%) reacted to a high induction dose. This would support classification in category 1B (although category 1A cannot be excluded, as doses < 500µg/cm were not tested).
Induction and challenge: 0.5% citral in ethanol (388 µg/cm^2) RIFM (1964b)	41 volunteers (12 males and 29 females).	0/41 reactions	No evidence of sensitising potential was observed. Relatively small group size limits statistical power of the study.
Patches were semi-occluded after the 6th patch. Induction: 8% citral in petrolatum (applications 1-2), 4% citral in petrolatum (applications 3-9). Concentration was lowered to reduce the occurrence of irritation. Challenge: 4% Opdyke (1979)	40 volunteers (5 male and 35 females, aged between 16 and 60).	19/40 reactions	A high number of volunteers (48%) showed reactions during this study. The precise dose (per unit area of skin) is not clear from the study report, but according to information received during the public consultation it was > 3000 µg/cm^2. This study supports classification in Category 1B (although Category 1A cannot be excluded, as doses < 500µg/cm were

			not tested).

The manufacturer also provided study reports for the HMT. Sensitisation was observed in all but one of these studies, however as they were all conducted at high induction doses (≥ 1379µg/cm^2), they could not be used to support sub-categorisation. Therefore additional information about these studies is not presented here.

Assessment and comparison with the classification criteria

Animal data

The sensitising potential of citral has been tested comprehensively in both Guinea pigs and mice. As shown in the following tables, there was limited reporting of the results from some of the Guinea pig studies, but overall there is sufficient, reproducible evidence from both species to demonstrate that citral should be classified as a skin sensitiser.

A total of 14 LLNAs, 6 GPMTs and 1 Buehler test are documented in the CLH report.

All of the LLNAs were well conducted. Thirteen of the studies were conducted according to OECD TG 429. A range of EC3 values was reported (1.2 – 15%). In 2 of the 14 LLNA, a high potency of citral was demonstrated (EC3 values < 2%), i.e., which would support classification in sub-category 1A. In 1 LLNA, the result was borderline between category 1A and 1B (EC3 = 2.1%). In the remaining LLNAs, a moderate potency was demonstrated (EC3 > 2%). Comments received during the public consultation suggested that the variability could be due to the different vehicles used in the studies. However, RAC notes that even when the same vehicle was used, significantly different EC3 values were obtained in separate studies. For example, when 1:3 Ethanol:DEP was used as the vehicle, EC3 values of 1.2% and 6.3% were obtained by the same laboratory (using the same strain and sex of mice).

Summary of the available Local Lymph Node Assays*		
Number of studies	**Result (EC3 values)**	**Assessment by RAC against CLP criteria**
2 studies (both reported in 2009)	1.2%, 1.5%	Skin Sens. Cat. 1A

12 studies (2002-2012)	2.1%, 3.7%, 4.6%, 4.6%, 5.3%, 5.8%, 6.3%, 6.3%, 6.8%, 12.6%, 13%, 14.1%	Skin Sens. Cat. 1B

**In addition, an early study was conducted in 1992. Limited information is available about this study, which reported a range of EC values (7-15%). At this time, the LLNA was still under development.*

In one of the GPMT, 60% of animals responded at an intradermal induction dose of 0.2%, which would support classification in sub-category 1A. Three of the studies suggest that citral is a moderate skin sensitiser (40% responding at a 0.4% intradermal dose in one study; 100% responding at a 25% intradermal dose in the other two studies) however it cannot be excluded that a high response would also have been seen if lower induction doses were used; these studies therefore cannot be used for sub-categorisation. In the remaining two GPMT, sensitisation was observed but not quantified; therefore these studies cannot be used for sub-categorisation either.

In the Buehler test, sensitisation was observed in 100% of animals with an induction concentration of 20% citral. Although fewer animals were used in this study than required by the guideline, the study supports classification in sub-category 1A.

Summary of the available Guinea pig studies		
Method (study date)	**Result**	**Assessment by RAC against CLP criteria**
Maximisation (1991) Induction: 0.2% (intradermal) Challenge: 0.5%	Positive reactions observed in 6/10 animals (60%)	Sub-category 1A
Maximisation (1986) Induction: 10% (intradermal) Challenge: 10%	Sensitisation observed	Not possible
Maximisation (1985) Induction: 0.4% (intradermal) Challenge: 0.25%	Positive reactions observed in 4/10 animals (40%)	Moderate potency (sub-category 1B), however lacking data to exclude sub-category 1A.
Maximisation (1978) Induction: 25% (intradermal) Challenge: 10, 5 and 5%	Positive reactions observed in 100% of animals	Moderate potency (sub-category 1B), however lacking data to exclude sub-category 1A.
Maximisation (1978) Induction: 25% (intradermal) Challenge: 10, 5 and 5%	Positive reactions observed in 100% of animals (except after 144 hours after a 5% challenge, where 60% positive reactions were observed).	Moderate potency (sub-category 1B), however lacking data to exclude sub-category 1A.
Maximisation (1977) Induction: 5% (intradermal)	Sensitisation observed	Not possible

Challenge: subirritant		
Buehler, modified (1973) Induction: 20%	Positive reactions observed in 5/5 animals (100%)	Sub-category 1A

To summarise, all of the available animal studies indicate that citral is a skin sensitiser. However, the studies gave varying indications of potency. Four of the studies (two LLNAs, one GPMT and a Buehler test) indicate a strong potency for citral. Thirteen studies (all LLNAs) suggest that citral is a moderate sensitiser, and support classification in sub-category 1B. The remaining studies (all GPMT) do not provide clear support for sub-categorisation.

Overall, although it is clear that citral is a skin sensitiser, a reliable estimate of potency cannot be derived from the animal data. The reason for the variability in the animal data is not known. Given this profile, Skin Sens. 1 (H317) without sub-categorisation is considered by RAC the most appropriate classification based on the animal data alone.

Human data

The available human data consists of case studies, HRIPT, HMT and diagnostic patch tests.

Case studies

Three case studies are summarised in the CLH report. One study from the UK reported positive reactions to citral (2.0% in petrolatum (pet.)) in 5 out of 9 beauticians with bilateral hand dermatitis. Another study reported strong positive reactions to citral in a patient with recurrent allergic contact cheilitis (inflammation of the lips). In a third study, four bakers with hand eczema were patch tested with 0.5% citral (in pet.); one of the four tested positive. These case studies are consistent with the results of the human patch tests discussed below.

HRIPT data

A number of volunteer HRIPTs have assessed the skin sensitisation potential of citral. Although the conduct of such studies is not permitted for compliance with CLP for ethical reasons, it is possible to take account of such data as part of a weight of evidence analysis if it is available historically. According to the ECHA guidance, positive responses at induction doses ≤ 500 μg/cm^2 support classification in sub-category 1A, and positive responses at induction doses > 500 μg/cm^2 support classification in sub-category 1B.

The HRIPTs documented in the CLH report used induction doses ranging from 388 to > 3000 μg/cm^2. In four of the studies, no skin reactions were observed. The highest dose tested in these four studies was 1400 μg/cm^2; this study also used the highest number of volunteers and therefore has the greatest statistical power. This study used male and female volunteers covering a wide age range, although information on the skin type or ethnicity of the volunteers was not provided in the study report.

Skin reactions were observed in the remaining two HRIPT studies. One of these used a very high induction dose (3875 μg/cm^2) and a low number of volunteers (8). The induction dose used in the other study is not clear, but is understood to be > 3000 μg/cm^2. These two studies suggest that citral is a moderate sensitiser, and support classification in sub-category 1B. It is not known whether the volunteers in these two studies would have responded to a

lower induction dose (i.e., category 1A cannot be excluded), although RAC notes that no reactions were seen in the other HRIPT studies conducted at lower doses. The absence of detailed information about the volunteers precludes further analysis. Overall, the HRIPT data confirm the sensitising properties of citral, but do not provide sufficient support for sub-categorisation.

HMT studies

The historic HMT studies were all conducted using high induction doses (> 500 µg/cm^2), and sensitisation was observed in 13 of the 14 studies. As it is not known whether the individuals in these studies would have responded to lower induction doses, the data from the HMT studies confirm the sensitising properties of citral, but cannot be used to support sub-categorisation.

Overall, the HRIPT and HMT studies support classification of citral in Skin Sens. Cat. 1. However, the data do not provide sufficient support for sub-categorisation.

Human Diagnostic Patch Tests

The diagnostic patch tests provide supporting information to the classification assessment. They were conducted according to standardised guidelines and with well defined challenge conditions. A total of 25 patch tests were documented in the CLH report, covering both selected (11 studies) and unselected (14 studies) patients (see tabulated information, below). Selected patients are those who have a known skin condition and who are suspected of having a contact allergy to fragrances/cosmetics, or other patients with a history of skin symptoms provoked by scented products (aimed testing). Unselected patients are groups of patients for whom allergic contact dermatitis is generally suspected.

Summary of the human diagnostic patch tests

Test substance[a]	**Study details**	**% of patients testing positive**	**Frequency[b]**
Selected patients			
Citral, 2%	Multicentre project (Germany, Austria, Switzerland).	16.2% (n = 1058)	High
Citral, 2%	Multicentre study, Hungary	3.4% (19/565)	High
Citral, 2%	Belgium	11.2% (23/205	High
Citral, 2% (vehicle not reported)	The Netherlands	6.7% (2/30)	High
Citral, 2%	Spain	2.3% (2/86)	High
Citral, 2%	Denmark & Sweden	4.3% (28/658)	High
Citral, 2%	Multicentre project, 6 countries, not specified	16.7% (13/78)	High
Citral, 5% (vehicle not	310 cosmetic dermatitis patients, 408 non-cosmetic patients and 122 control subjects. Country not	2.6% cosmetic dermatitis patients	High

reported)	stated.	(8/310) 2.2% non-cosmetic patients (9/408)	High
Citral, 2% (vehicle not reported)	310 cosmetic dermatitis patients, 408 non-cosmetic patients and 122 control subjects. Country not stated.	0.4% cosmetic dermatitis patients (1/240) 0.3% non-cosmetic dermatitis patients	Low/moderate Low/moderate
Citral, 2%	The Netherlands	2.6% (n =182)	High
Citral, 5%	155 cosmetic dermatitis patients and 159 other eczema/dermatitis patients	2.6% cosmetic dermatitis patients (4/155) 3.1% dermatitis/eczema patients	High High
Unselected patients			
Citral, 2%	UK	1.0% (20/1951)	High
Citral, 3.5%	Sweden	0.92% (6/655)	Low/moderate
Citral, 1.5%	Sweden	0.66% (7/1055)	Low/moderate
Citral, 2%	Denmark	0.3% (4/1502)	Low/moderate
Citral, 2%	The Netherlands	0.6% (2/320)	Low/moderate
Citral, 2%	Multicentre study: Germany, Austria, Switzerland	0.6% (13/2021)	Low/moderate
Citral, 1%	Multicentre study: Germany, Denmark, Sweden, UK, Belgium	0.35% (6/1701)	Low/moderate
Citral, 2%	Multicentre study: Germany, Denmark, Sweden, UK, Belgium	0.7% (12/1701)	Low/moderate
Citral, 2%	Multicentre study: Germany, Denmark, Sweden, UK, Belgium	1.1% (21/1855)	High
Citral, 2%	Multicentre study; country not known	1.0% (19/1825)	High
Citral, 0.1%	Multicentre study, Denmark	0% (0/192)	Low/moderate
Citral, 1%	Multicentre study, Denmark	0% (0/192)	Low/moderate
Citral, 1%	North America	1.7% (4/228)	High
Citral, 2%	Multicentre study, Korea	1.2% (5/422)	High

[a]*in petrolatum, unless otherwise stated.*

[b]Relatively high or low frequency of occurrence of skin sensitisation, according to Table 3.4.2-b in the Guidance on the Application of the CLP criteria:

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)	≥ 2.0%	< 2.0%

In patch tests on selected dermatitis patients, positive reactions ranged between 0.3 and 16.7%, and "high frequencies" (≥ 2.0%) were seen in 10 out of 11 tests.

In unselected dermatitis patients, positive reactions ranged between 0 and 1.7%. Complete absence of positive reactions was observed in 2 of the 14 tests, which both employed 192 test subjects (these two tests were conducted using 0.1%, and 1.0% citral in pet.).

Relatively high frequencies of positive reactions (≥ 1.0%) were seen in 5 of the 14 studies, however two of these studies were conducted outside of the EU (i.e., in North America and Korea). Given that the classification criteria require the frequency of responses to be compared with the exposure data, and exposure data is only available for the EU, these non-EU studies are excluded from the analysis. This leaves 3 out of 12 studies on unselected patients showing sensitisation rates equal to or higher than 1% (1.0%, 1.0% and 1.1%), 7 of the studies showing a low/moderate sensitisation rate (i.e. < 1.0%) and 2 of the studies showing no sensitisation at all. The largest study tested 2021 patients, and found a low/moderate frequency of sensitisation.

In addition to the patch tests, an experimental study is reported in the CLH proposal which investigated the possible role of the skin irritation response in relation to polysensitisation to fragrances. 100 volunteer patients with confirmed fragrance contact allergy (i.e., selected patients) were patch tested with 27 fragrance chemicals; 9.0% of patients tested positive to citral (2.0% in pet.). The results of this study are consistent with the results of the human patch tests on selected patients discussed above.

For classification purposes, the major limitation of the diagnostic patch tests is that the induction doses are not known. To account for this, the CLP guidance describes principles for deriving an exposure index leading to an assessment of relatively high or low exposure that can be matched against the patch test data to inform on potency and sub-categorisation.

Citral is widely used as fragrance ingredient in cosmetic and household cleaning products. In 2006, IFRA recommended maximum levels of citral in leave-on cosmetic products between 0.04-1.4%, and 1.0-5.0% in rinse-off cosmetic products. However, it is not clear how quickly or completely products on the market came to adhere to these recommendations.

In 2012, the Scientific Committee on Consumer Safety (SCCS) considered a number of surveys on the presence and content of certain fragrances in consumer products, based mostly on labelling information. Citral was present in 8.2 – 44% of the products covered; the SCCS concluded that exposure to citral is foreseeable in daily life. Further surveys (conducted

by the Danish EPA on the Danish Market) have found citral to be present in day-to-day cosmetic products such as deodorants, soaps, shampoo/conditioner, lotions and creams, and household cleaning products such as cleaning agents, stain removers and air care products. The surveys suggest that citral is generally found in low concentrations (< 0.06%) in cosmetic products, however high concentrations were found in other products; massage oils (up to 3.25%), eterical oils/scented oils (up to 78%) and air fresheners (up to 26%). Data from the Danish Product Register (which contains information on hazardous substances in mixtures for professional use) confirm that citral is used in a wide range of products on the market, especially cleaning products. The concentrations are generally lower than 0.1%, however concentration above 1% are found in fragrance mixtures and scented oils.

The REACH Substance Evaluation (SEv) dossier on citral refers to the estimated exposure values in the REACH registration dossier. These are 47-100µg/cm^2 for workers (depending on the exposure scenario) and 47-50 µg/cm^2 for consumers. The exposure values are based on the highest concentrations of citral reported by the registrants in the exposure scenarios for the use in cleaning agents, which correspond to < 1.5% for workers and < 0.5% for consumers. However, it is noted in the SEv conclusion that products with higher concentrations of citral are found on the Swedish market.

In characterising the nature of the exposure of EU citizens to citral in order to make a comparison with the numbers of positive patch tested individuals, RAC is mindful that there is much uncertainty about the nature of the products that may have induced the sensitisation, the periods during which the induction occurred, and the concentrations encountered by those being induced. Although according to the DS the IFRA limits have helped to reduce exposure, it is possible that patients may have been exposed to consumer products containing unrestricted concentrations of citral as late as 2013 (according to comments received from industry during the public consultation).

Exposure data	Indicator of relatively low exposure	Indicator of relatively high exposure	Assessment by RAC
Concentration/dose at induction	< 1.0% < 500 µg/cm^2	≥ 1.0% ≥ 500 µg/cm^2	The content of citral in many consumer and professional products appears to have decreased significantly in recent years; surveys suggest that current levels may be very low. However, it also appears that higher content levels (≥ 1.0%) will have prevailed during the periods when most of the contact allergy patients were induced to citral. *Conclusion: relatively high exposure*
Repeated exposure	< once/daily	≥ once/daily	Given the wide range of consumer products shown to contain citral, repeated exposure every day seems very likely.

			Conclusion: relatively high exposure
Number of exposures (irrespective of the concentration of the sensitiser)	< 100 exposures	≥ 100 exposure	Given the types of consumer and professional products shown to contain citral, it is highly likely that individuals will have been exposed 100s of times. *Conclusion: relatively high exposure*

This assessment contrasts with the view of the DS, who concluded that concentration/dose levels at induction were relatively low.

In accordance with the CLP criteria, this assessment of relatively high skin exposure indicates that citral should not be regarded as a high potency skin sensitiser in spite of the high number of positive patch test results reported.

Conclusion

Citral already has the harmonised classification Skin Sens. 1; H317. Both the animal and human data presented by the DS confirm that citral is a skin sensitiser.

Under the CLP Regulation, classification into sub-categories is permitted when the data are sufficient. The DS noted especially that the high number of positive patch tests seen in patients attending dermatitis clinics over the last 20-30 years may justify sub-categorisation, accounting for high potency.

However, the results of the available animal studies are not consistent, and a reliable estimate of potency cannot be derived from them. The results from HRIPTs clearly support classification. However, these data cannot be used to support sub-categorisation because information about the sensitising potential of sufficiently low doses of citral to assess potency is lacking. Similarly, the HMT studies were all conducted at high induction doses. The results of these studies support classification as Skin Sens. 1, but cannot be used for sub-categorisation.

RAC agrees with the DS that high frequencies of sensitisation were observed in some of the diagnostic patch tests (in selected and unselected patients, and in the high number of published cases), however, the exposures responsible for inducing sensitisation in these individuals may have been relatively high, but it is not entirely clear. Given this uncertainty, the diagnostic patch tests cannot be used to support sub-categorisation.

Therefore, RAC concludes that the sensitising properties of citral have been confirmed, however the available data are not sufficient for sub-categorisation. Therefore, RAC concludes that classification as **Skin Sens. 1; H317 (May cause an allergic skin reaction)** is warranted for citral.

The available data are not sufficient to the establishment of a specific concentration limit. Furthermore, the data do not suggest that citral has an extreme potency.

10.11 Germ cell mutagenicity

Hazard class not assessed in this dossier.

10.12 Carcinogenicity

Hazard class not assessed in this dossier.

10.13 Reproductive toxicity

Hazard class not assessed in this dossier.

10.14 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

10.15 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier.

10.16 Aspiration hazard

Hazard class not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Environmental hazards have not been assessed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Additional hazards have not been assessed in this dossier.

13 ADDITIONAL LABELLING

Given that citral is classified as a skin sensitiser in Category 1A, labelling with EUH 208 will apply when citral is present in mixtures in concentrations ≥ 0.01%.

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CITRAL; 3,7-DIMETHYLOCTA-2,6-DIENAL

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15 ANNEXES

Annex I: detailed study summaries

Annex II: confidential information on substance identity

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification: Citral; 3,7-dimethylocta-2,6-dienal

EC Number: **226-394-6**

CAS Number: **5392-40-5**

Index Number: **605-019-00-3**

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CONTENTS

1 PHYSICAL HAZARDS

Classification for physical hazards is not a part of the CLH proposal for citral.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The information below on toxicokinetics have largely been copied from the public part of the registration dossier (only with minor editorial changes).

2.1.1 STUDY 1

Reference:

Diliberto JJ, Usha G, Birnbaum LS: Disposition of citral in male Fischer rats. Drug Metab. Dispos. 16, 721-727, 1988

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: non-guideline study.

Method: Time course of distribution of 14C-label in tissues, blood, bile, urine, feces, expired air measured by liquid scintillation counting after single and repeated application; separation of unchanged and metabolized citral in blood and bile by HPLC (metabolites not identified).

Test substance:

Citral and ^{14}C citral, purity >= 98%. No data on impurities.

Composition of test material (isomer ratio): 74% geranial, 26% neral

Radiolabelling, specific activity: 10.7 mCi/mmol (labelled at C1 and C2)

Test animals:

Rat (Fischer 344), male

- Source: Charles River Breeding Laboratories, Portage, MI, USA
- Age at study initiation: 2-3 month
- Weight at study initiation: 200-250 g
- Fasting period before study: no data

- Housing: individually
- Individual metabolism cages: yes
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 +/-2
- Humidity (%): 50 +/- 5
- Air changes (per hr): no data, air flow rate through the cages 0.3-0.4 L/min
- Photoperiod (hrs dark / hrs light): 12/12

Dosing:

Acute study: single dose, oral (gavage).

Multiple dosing study: oral pretreatment for 10 days with unlabelled citral at a dose of 5 mg/kg bw/day followed by single oral or i.v. dose of 5 mg/kg 14C-citral.

Concentrations: oral application: 5, 50, 500 mg/kg/d; i.v. application: 5 mg/kg bw/d

No. of animals per dose: not specified

Sampling:

Tissues and body fluids sampled: urine, faeces, expired air, blood, liver, kidneys, adrenals, thymus, spleen, brain, heart, lungs, testes, skin, adipose tissue, muscle, stomach contents, small intestine contents, large intestine contents, tail site (for i.v. application), bile

Time and frequency of sampling:

- excreta samples at 2, 4, 6, 8, 12, 16, 24, 32, 48, and 72 hrs;
- tissue samples at sacrifice at 72 hrs p.a.
- bile samples: at 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270 min after dosing by cannulation of the common bile duct
- blood samples: at 1, 2, 5, 10, 15, 20, 430, 45, 60, 90, 120, 150, 180, 210 and 240 min p.a. by cannulation of the jugular vein
- air samples: the total air flow through the metabolism cages was continuously passed through two consecutive traps (charcoal trap and bubbler trap, see below)

Detailed study summary and results:

Citral was rapidly and completely absorbed from from the gastrointestinal tract (91 - 95%) after oral exposure. The amount remaining in any tissue was < 2%; the highest concentrations in liver, muscle, blood,

adipose tissue. The relative amount in tissue independent of dose or route of administration. The excretion profiles were independent from the dose or route of administration:

Recovery after single 5 mg/kg oral dose:

- 24 hours: 67% with 45% in urine, 16% as exhaled 14CO2, 6% in feces, <1% as exhaled 14C-citral; production of 14CO2 essentially ceased by 12 hrs;
- 72 hours: 83% (+- 10 %) with 51% in urine, 17% as exhaled 14CO2, 12% in feces, 3% in tissues, <1% as exhaled 14C-citral

Recovery after single 5 mg/kg i.v. dose:

- 12 hours: 57% with 47% in urine, 7% as 14CO2, 2% in feces, <1% as exhaled 14C-citral; elimination essentially completed within 24 hrs
- 72 hours: 79% (+- 18 %) with 58% in urine, 8% as 14CO2, 7% via the feces, 6% tissues, <1% as exhaled 14C-citral

Elimination via bile after a single 5 mg/kg i.v. dose: 20% of the dose appeared in bile within 1 hr, with another 7% appearing by 4.5 hrs. The amount excreted in the bile was 4 times higher than that excreted in the feces within 3 days. HPLC of bile, even as early 5 min after treatment, demonstrated the complete absence of any unmetabolized citral.

Effect of multiple dosing: In rats pretreated for 10 days (5 mg/kg bw/d orally), biliary excretion was increased to 34%, while excretion via urine, feces or expired CO2 was not affected. Therefore, repeated exposure did not alter the overall pattern of disposition.

In summary, most of the citral-derived radioactivity was rapidly eliminated from the body with a whole body half-life of 8 hr after i.v. exposure. However, a small percentage tended to persist with a clearance half-life of 24 hrs.

2.1.2 STUDY 2

Reference:

Diliberto JJ, Srinivas P, Overstreet D, Usha G, Burka LT, Birnbaum LS: Metabolism of citral, an a,ß-unsaturated aldehyde, in male F344 rats. Drug Metab Dispos, 18, 866-875, 1990

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics (metabolism).

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Metabolism

Test guideline: non-guideline study.

Method: Urinary metabolites identified by reverse phase HPLC

Test substance:

Citral and ^{14}C citral, purity >= 98%. No data on impurities.

Composition of test material (isomer ratio): 74% geranial, 26% neral

Radiolabelling, specific activity: 10.7 mCi/mmol (labelled at C1 and C2)

Test animals:

Rat (Fischer 344), male

- Source: Charles River Breeding Laboratories
- Age at study initiation: 2-3 months
- Weight at study initiation: 200-250 g
- Housing: individual
- Individual metabolism cages: yes
- Diet: pelleted NIH 31 lab chow ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 +/-2
- Humidity (%): 50 +/- 5
- Photoperiod (hrs dark / hrs light): 12/12

Dosing:

Single dose, oral (gavage) and i.v. application.

Concentrations: gavage: 5 and 500 mg/kg bw; i.v.: 5 mg/kg bw

No. of animals per dose: N ≥ 3

Sampling - metabolite characterisation studies:

- Tissues and body fluids sampled: urine, bile (only after i.v. application)
- Time and frequency of sampling: 2, 7, 24 hours for urine; 5, 30, 60, 270 min for bile
- From how many animals: no data

- Method type(s) for identification: HPLC, GC, Liquid scintillation counting, UV absorption, 1H-NMR spectra, mass spectrometry

Treatment for cleavage of conjugates:

Enzymatic hydrolysis of pooled samples of urine or bile by treatment with ß-glucuronidase or sulfatase, or sequentially with ß-glucuronidase followed with sulfatase; subsequently the relative amounts of metabolites and parent citral were analyzed by HPLC and compared to synthetic standards.

Detailed study summary and results:

Seven metabolites could be identified in sufficient purity and quantity, namely:

A: 3 -hydroxy-3,7,dimethyl-6-octenedioic acid;

B: 3,8-dihydroxy-3,7-dimethyl-6-octenoic acid;

C: 3,9-dihydroxy-3,7-dimethyl-6-octenoic acid;

D: E-3,7-dimethyl-2,6-octadienedioic acid;

E: 3,7-dimethyl-6-octenedioic acid;

F: Z-3,7-dimethyl-2,6-octadienedioic acid;

G: E-3,7-dimethyl-2,6-octadienoic acid.

Glucuronic acid conjugates only in bile

Pathways of biotransformation:

The biotransformation of citral includes reduction or hydration of the 2,3-double bond, oxidation of the aldehyde function, and allylic oxidation at C-8, and, possibly, C-9. Enzymes involved in the formation of these metabolites can be aldehyde dehydrogenase, ß-oxidation by aldehyde oxidase, oxygenation at C-8 or C-9 by cytochrome P-450, and alcohol dehydrogenase for oxidation of intermediate alcohols. The molecules formed by these processes are more hydrophilic, since they contain COOH and other polar groups; conjugation reactions, such as with glucuronic acid, may form even more polar metabolites. Based on the structure of the isolated metabolites, it would appear that nucleophilic 1,4-addition reactions of the a,b-unsaturated aldehyde structure, e.g. with glutathione, are not a major function in the metabolism of citral.

Enzymatic hydrolysis did not appear to affect the chromatographic profile of urinary radioactivity. However, the biliary profile changed after glucuronidase treatment. Sulfatase treatment appeared to have no effect.

2.1.3 STUDY 3

Reference:

Phillips JC, Kingsnorth J, Gangolli SD, Gaunt IF: Studies on the adsorption, distribution and excretion of citral in the rat and mouse. Fd. Cosmet. Toxicol. 14, 537-540, 1976.

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: non-guideline study

Method: Tissue distribution and time course of excretion in urine, faeces and exhaled 14CO2 measured; metabolites in urine separated by TLC (individual metabolites not identified)

Test substance:

Citral supplemented with [1,2-14C]-citral, purity 99%.

Radiolabelling, specific activity: 0.305 mCi/mmol (labelled at C1 and C2)

Test animals:

Rat (Wistar), male

- Source: Scientific Agribusiness Consultants
- Weight at study initiation: 150 g
- Housing: all-glass metabolism cages
- Diet: ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20 +/- 1
- Air changes (per hr): air drawn through the system at a constant rate of 250 mL/min

Dosing:

Single dose, oral (gavage)

Concentrations: 5, 770 and 960 mg/kg bw

No. of animals per dose: 3

Sampling:

PHARMACOKINETIC STUDY (Absorption, distribution, excretion)

- Tissues and body fluids sampled: liver, lung, kidney, heart, spleen, stomach (wall + content), intestine (wall + content), brain

- Excreta: urine, faeces, exhaled 14CO2: trapped in ethanolamine-2-ethoxyethanol (1:4, v/v)
- Time and frequency of sampling: tissues: 96 hours p.a., excreta: 24, 48, 72, 96 hrs, 14CO2: trapping solutions were analyzed after 2, 4, 6, 7, 24, 48, 72, 96 hrs

METABOLITE CHARACTERISATION STUDIES
- Tissues and body fluids sampled: urine
- Time and frequency of sampling: 24, 48, 72, 96 hrs
- From how many animals: 3
- Method type(s) for identification: urine was extracted with hexane; extracts and aqueous residue were subjected to TLC. The distribution of radioacitivity along the chromatograms was determined by removing bands 0.5 cm wide and counting (individual metabolites were not identified).

Detailed study summary and results:

Distribution in tissue: at doses of 5 and 960 mg/kg: at 24h [*but tissue sampling reported to be only at 96h*] most 14C in gastro-intestinal tract (ca. 7 and 12.5%) and the liver (ca. 1.5 and 2%).

Excretion:

5 mg/kg: 24 hours: >95% with 61% in urine, 20% as 14CO2 and 17% in faeces; < 0.5% of 14C in urine as unchanged citral; exhalation of 14CO2: constant rate up to 6 h (ca. 16% of the dose applied), substantially completed by 10 h (ca. 20% of the dose applied).

770 mg/kg: 96 hours: >95%; urinary excretion substantially complete by 60 h; faecal excretion slow phase up to 36 hrs, rapid phase between 36 and 72 hrs; excretion of 14CO2 complete by 48 hrs.

960 mg/kg: 24 hours: 60 -70% with 47% in urine, 7.3% as 14CO2 with constant rate up to 24 h after an initial lag phase of 2 h, 9.5% in faeces.

Metabolites:

Most of 14C in the urine as polar hexane-insoluble unsatured compounds (not identified).

2.1.4 STUDY 4

Reference:

Phillips JC, Kingsnorth J, Gangolli SD, Gaunt IF: Studies on the adsorption, distribution and excretion of citral in the rat and mouse. Fd. Cosmet. Toxicol. 14, 537-540, 1976.

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: non-guideline study

Method: Tissue distribution and time course of excretion in urine, faeces and exhaled 14CO2 measured; metabolites in urine separated by TLC (individual metabolites not identified)

Test substance:

Citral supplemented with [1,2-14C]-citral, purity 99%.

Radiolabelling, specific activity: 0.305 mCi/mmol (labelled at C1 and C2)

Test animals:

Mouse (LACA strain), male

- Source: Scientific Agribusiness Consultants
- Weight at study initiation: 10-15g
- Housing: SPF conditions
- Individual metabolism cages: yes
- Diet: ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20 +/- 1

Dosing:

Single dose, oral (gavage)

Concentrations: 100 mg/kg bw

No. of animals per dose: 3

Sampling:

PHARMACOKINETIC STUDY (Absorption, distribution, excretion)

- Tissues and body fluids: radioactivity present in the body was visualized by autoradiography; data available for muscle, tongue, heart, lung, skin, hair follicles, eye lens, adipose tissue, testes, thymus, spleen, brain,

salivary glands, stomach (wall and content), intestine (wall and content), caecum (wall and content), urinary bladder, liver, kidney (cortex and medulla), bone, spinal cord, urine, faeces

- Time and frequency of sampling: 12 and 24 hrs, 2, 3, 5, 7 and 10 d

Detailed study summary and results:

Distribution in tissue: Considerable proportion of 14C appearing throughout the tissues within 12 h but with a declining trend over 72 hours. After 168 h only faint or no distribution of radioactivity could be measured in all tissees except from the liver and kidney cortex. After 240 h the 14C was generally only detected at low levels in all tissues except from the liver.

Excretion: Major route of 14C-excretion via urine detected up to day 5. Significant proportion of 14C rapidly excreted with faeces within 12 h, 14C-excretion via faeces detected up to day 3.

2.1.5 STUDY 5 – dermal absorption (same reference as STUDY 1)

Reference:

Diliberto JJ, Usha G, Birnbaum LS: Disposition of citral in male Fischer rats. Drug Metab. Dispos. 16, 721-727, 1988

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics (dermal absorption).

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics (dermal absorption)

Test guideline: non-guideline study.

Method: Time course of distribution of 14C-label in tissues, blood, bile, urine, feces, expired air measured by liquid scintillation counting after single and repeated application; separation of unchanged and metabolized citral in blood and bile by HPLC (metabolites not identified).

Test substance:

Citral and ^{14}C citral, purity >= 98%. No data on impurities.

Composition of test material (isomer ratio): 74% geranial, 26% neral

Radiolabelling, specific activity: 10.7 mCi/mmol (labelled at C1 and C2)

Test animals:

Rat (Fischer 344), male

- Source: Charles River Breeding Laboratories, Portage, MI, USA
- Age at study initiation: 2-3 month
- Weight at study initiation: 200-250g
- Housing: individually
- Individual metabolism cages: yes
- Diet: NIH 31 rat chow ad libitum
- Water: ad libitum
- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 +/-2
- Humidity (%): 50 +/- 5
- Air changes (per hr): no data, air flow rate through the cages 0.3-0.4 L/min
- Photoperiod (hrs dark / hrs light): 12/12
- Photoperiod (hrs dark / hrs light): 12/12

Dosing:

Single dose, dermal (perforated tissue capsule held over the treated area with cyanoacrylate adhesive)

Concentrations: 5, 50 mg/kg

No. of animals per dose: 3

Sampling:

Duration of exposure: 72 h. Inital body burden: immediately after application rats were sacrificed and analyzed for total radioactivity present in the carcass, dosing site and dermal caps at zero time.

- Collection of blood: 72 hrs p.a.
- Collection of urine and faeces: 2, 4, 6, 8, 12, 16, 24, 32, 48, and 72 hrs
- Collection of expired air: the total air flow through the metabolism cages was continuously passed through two consecutive traps (charcoal trap and bubbler trap)

Sacrifice at 72 hrs p.a. Analysis of application site: dermal skin sites and dermal metallic caps. Analysis of organs: liver, kidneys, adrenals, thymus, spleen, brain, heart, lungs, testes, skin, adipose tissue, muscle, stomach contents, small intestine contents, large intestine contents.

Detailed study summary and results:

The total recovery after 72 hrs in tissues, excreta, skin test site and non-occlusive cover at doses of 5 and 50 mg/kg was 61.50% and 68.21% of the Initial body burden (IBB), respectivelt. About 2/3 of the applied dose

was present in the carcass, dosing site and non-occlusive cover at 0h due to evaporation during administration of the dermal dose (loss of about 1/3 of the dose). Less than 50% of the applied dose was available for dermal absorption as the IBB was reduced by adsorption to the dermal caps (24% of IBB).

The distribution of citral (i.e. citral derived radioactivity) in tissues and excreta after 72h was 7-9.5% in total tisses (except dermal skin sites), 8.5-9.9% in dermal skin sites, 8.4-17.3% in urine, 3.5-3.2% in faeces, 3.4-3.8% in expired CO2 and 2.8-4.5% as expired citral (percentages depending on the dose).

2.1.6 STUDY 6 – dermal absorption

Reference:

Barbier P, Benezra C: The influence of limonene on induced delayed hypersensitivity to citral in Guinea Pigs. II. Label distribution in the skin of 14C-labelled citral. Acta Dermatovener (Stockholm) 63, 93-96, 1983.

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics (dermal absorption).

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: non-guideline study.

Method: Distribution of 14C in different layers of skin and in urine and faeces investigated by liquid scintillation counting.

Test substance:

Citral and [1,2-14C]-citral, no information on purity.

Radiolabelling, specific activity: 83 µCi/mg (labelled at C1 and C2)

Test animals:

Guinea pig (Hartley), female

- Source: Versault, Luisetaines, France
- Weight at study initiation: 300-350 g

PRE-TREATMENT:

As the study was designed to investigate the role of dermal absorption in elicitation of hypersensitivity, groups of guinea pigs were used that had been subjected to different induction procedures by applying the Freund Complete Adjuvant (FCA)Test: Group A - citral treatment: 0.5 mL citral was dissolved in 4.75 mL FCA and then emulsioned with 4.75 mL saline, using a syringe. Each animal received intradermally 5 injections of 0.1 mL each, on alternate days. Group B - FCA-treated control: 4.75 mL FCA was emulsioned with 4.75 mL saline, using a syringe. Each animal received intradermally 5 injections of 0.1 mL each, on alternate days. Group C - untreated control: no pretreatment procedure. All groups: This induction procedure was followed by a 2-week rest, before elicitation (see below) was performed.

Dosing:

Single dose, dermal

Concentrations: 1.88 mg/animal, ca. 63 µg/cm2 skin area. Dose volume: 188 µl of a 0.5% solution per area, 2 areas in total.

No. of animals per dose: 1

Sampling:

- Collection of urine and faeces: during 16 hr via a metabolism cage
- Terminal procedure: 16 hr p.a.
- Analysis of organs: skin

Detailed study summary and results:

The total recovery of radioactivity from the excreta urine and feces, from total skin and from unresorbed citral at the skin surface was 42.1% in a guinea pig without pre-treatment (C) and 47.7% in a guinea pig that had been subjected to an induction treatment with citral (A). Amounts evaporated from the site of application, excreted via exhalation of 14CO2, or deposited in body tissues were not recorded in this study. The amounts absorbed into the skin within 16 hrs p.a. were 23.9% (C) and 27.5% (A). The amount of 14C in the stratum corneum was comparable in all groups (10.0-12.2 %). There was a greater variation in the penetration to deeper skin layers between the single animals (6.4-10.5%). However, the 14C-recovery after stripping the stratum corneum from the deeper skin layers was incomplete compared to 14C-activity in total skin. Additionally, the values were recorded from single animals only, so that the values may represent individual variation.

2.1.7 STUDY 7 – dermal absorption

Reference:

Hayes AJ, Markovic B: Toxicity of Australian essential oil Backhousia citriodora (lemon myrtle). Part 2: Absorption and histopathology following application to human skin. Food Chem Toxicol 41: 1409-1416, 2003.

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics (dermal absorption).

Material and methods

Test guideline:

Type of method: In vitro

Objective of study: Toxicokinetics (dermal absorption)

Test guideline: non-guideline study.

Method: In vitro test system with full thickness human skin mounted in a Franz cell diffusion system: absorption into the skin and the subcutaneous fat layer monitored by GC-MS up to 12 hrs post application.

Test substance:

Lemon myrtle oil (Backhousia citriodora) from Australia.

Purity: 96.6% citral (no information on impurities)

Composition of isomers: 51.4% geranial, 40.9% neral, 2.6% trans-isocitral, 1.7% cis-isocitral

Radiolabelling: no

Test system:

Human skin (full thickness human skin mounted in a Franz cell diffusion system)

Duration of exposure: 100% essential oil: 1, 4, 8, 12 hrs; 1% essential oil product: 8 hrs

SKIN PREPARATION

- Source of skin: human
- Ethical approval if human skin: yes
- Type of skin: abdominal skin
- Preparative technique: majority of subcutaneous fat layer removed by gross dissection
- Thickness of skin (in mm): 2
- Membrane integrity check: yes
- Storage conditions: immediate use

PRINCIPLES OF ASSAY

- Diffusion cell: Franz cell

- Receptor fluid: DMEM
- Solubility of test substance in receptor fluid: not analyzed in separate pretests, not detectable in the receptor fluid during the skin absorption experiments
- Static system: yes
- Test temperature: 21 +- 1 °C
- Humidity: not applicable
- Occlusion: watch glass
- Reference substance(s): no data

Dosing:

- 100% lemon myrtle oil: 100 µl or 89.5 mg applied to 4.9 cm2, corresponding to 20 µl/cm2 or 18 mg/cm2
- 1 % lemon myrtle oil product: 100 mg product (corresponding to 1 mg citral) applied to 4.9 cm2, corresponding to 0.18 mg/cm2

Sampling:

4 skin samples per timepoint

Detailed study summary and results:

Citral (neral and geranial) found in all layers of full thickness skin.

Relative recovery of citral (% of administered dose) after 1, 4, 8 and 12 hrs of exposure:

- 100% essential oil: in epidermis/dermis 1.25%, 1.97%, 1.52% and 1.08%, maximum after 4 h; in subcutaneous fat tissue 0.04%, 0.11%, 0.17%, 0.49%, continuous increase up to 12 h; not detectable in receptor fluid; total recovery 1.29%, 2.08%, 1.69%, 1.57%; unresorbed fraction not determined
- 1% essential oil formulation (only data for 8 h exposure): epidermis/dermis 0.22%; in subcutaneous fat tissue 0.061%; not detectable in receptor fluid; total recovery 0.281%

Citral (as main component of lemon myrtle oil) was absorbed in freshly excised full-thickness human skin at all exposure periods tested. Neral and geranial were the only detectable components of the oil in the skin discs (epidermis and dermis) and in subcutaneous fat tissue. As exposure time increased, the recovery in the fat tissue increased also. However, the recovery in epidermis/dermis showed a maximum at 4 hrs p.a.. At all timepoints, the recovery in skin layers was higher than in subcutaneous fat.

Absorption through full-thickness skin (epidermis + dermis + fat) per skin area after application of neat essential oil:

- at 1 hr: 1.16 mg, corresponding to 0.24 mg/cm2

- at 12 hrs: 1.41 mg, corresponding to 0.29 mg/cm2

Receptor media of diffusion cells: no components of lemon myrtle oil detectable, assumed to be caused by low water solubility

3 HEALTH HAZARDS

3.1 Skin sensitisation

3.1.1 Animal data

3.1.1.1 STUDY 1 (LLNA)

Study reference:

Basketter D, Kolle SN, Schrage A, Honarvar N, Gamer AO, van Ravenzwaayb B and Landsiedelb R: Experience with local lymph node assay performance standards using standard radioactivity and nonradioactive cell count measurements. Journal of Applied Toxicology; 32 (2012): 590–596.

Detailed study summary and results:

Test type

LLNA (OECD 429), GLP compliant.

Test substance

Citral, purity 96.4% (Sigma–Aldrich, Taufkirchen, Germany)

Test animals

Mice (CBA/CaOlaHsd), female

6 animals per dose

Age: 6-12 weeks old

The animals were kept in fully air-conditioned rooms at a temperature of 20–24 °C and a relative humidity of 30–70%, with a 12 h light-dark cycle (lights on 6 a.m. to 6 p.m.). Tap water and food (Provimi Kliba, Kliba-Labordiät, Maus/Ratte Haltung 'GLP', SA, Kaiseraugst, Basel, Switzerland) were given ad libitum.

Administration/exposure

Three groups of mice (n=6 per dose) were treated with 5, 10 and 25% citral. Vehicle: acetone-olive oil (AOO) 4:1. One group was treated with vehicle alone (vehicle control). The test substance or vehicle alone was applied epicutaneously to the dorsal part of both ears (25 µl per ear for three consecutive days at the

same site). About 66–72 h after the last application of test substance to the ears, the mice were injected intravenously into a tail vein with 20 μCi of 3H-thymidine in 250 μl of sterile saline. Mice were sacrificed 5 h after the 3H-thymidine injection.

Results and discussion

The responses to test substances exposure were characterized by 3H-thymidine incorporation into the lymph node cells (dpm) and ear weight (EW) and the stimulation index at each dose was calculated as the ratio of the test group mean values divided by those of the vehicle control group. Citral was shown to be sensitising with an EC3 value of 12.6%. Irritation was not observed for citral (determined by whether a greater than 20% increase in ear weight compared to the pre-test value occurred or not). Detailed information of the responses of each animal per test group are not presented in the article.

Calclated stimulation index, citral

Applied concentration	5%	10%	25%
3H-Thymidine SI	1.29	1.70	9.09

3.1.1.2 STUDY 2 (LLNA)

Study reference:

Jung KM, Jang WH, Lee YK, Yum YN, Sohn S, Kim BH, Chung JH, Park YH, Lim KM: B cell increases and ex vivo IL-2 production as secondary endpoints for the detection of sensitizers in non-radioisotopic local lymph node assay using flow cytometry. Toxicology Letters 209 (2012), 255– 263

Detailed study summary and results:

Test type

LLNA:BrdU-FMC, GLP: not reported.

Test substance

Citral, no information on purity

Test animals

Mice (Balb/c), female

4-6 animals per dose (several substances tested, specific information on number of animals per dose for citral not stated)

Age at study initiation: 8-9 weeks

Weight at purchase: 18-22 (7-8 weeks old)

The animals were kept under controlled conditions of temperature (23±3 °C) and relative humidity (50±10 %) with alternating 12h light and dark. The animals has ad libitum access to tab water and were kept on solid laboratory diet (Purina Co., Korea).

Administration/exposure

Groups of mice (N=4 or 6) were treated daily with 25µl citral in vehicle or vehicle alone on the back of both ears for three consecutive days (day 1-3). The concentrations were 5, 10 and 25% citral and the vehicle was acetone-olive oil (AOO). On day 5 mice were interperitoneally injected with BrdU and were sacrificed after a day. After sacrifice, auricular lymph nodes were isolated, weighed and undergone lymphocyte preparation. After bilateral auricular lymph nodes were pooled on individual basis, lymph node cells were prepared by disaggregation through 70µm mesh in 1 ml PBS. The lymph node cells (LNCs) were counted using a hemacytometer after staining with tryphan blue.

Results and discussion

The SI in the LLNA:BrdU-FCM is the ratio of the mean number of LNCs with incorporated BrdU from mic in each of the test substance dose groups to the mean number of LNCs with incorporated BrdU from mice in the vehicle control group. Citral was shown to be sensitising with an EC3 value of 14.1% in the LLNA:BrdU-FCM assay. No information on irritation was reported but it was stated that the test concentrations were selected to include the known LLNA EC3 value for sensitizers that were free from systemic toxicity and/or excess local skin irritation. Detailed information of the responses of each animal per test group are not presented in the article. The obtained EC3 value was compared to the reference EC3 value of 9.2% for citral given in the OECD 429 guideline for the “traditional” LLNA assay (pooled result based on 6 studies). The study as a whole included LLNA:BrdU-FCM assays of the 22 reference substances listed in the OECD TG 429 and EC3 values were compared for these two assays. It was concluded that using BrdU incorporation with flow cytometry can provide a good non-radioisotopic alternative to the traditional radioisotope LLNA.

3.1.1.3 STUDY 3 (LLNA) (cited from REACH registration dossier)

Study reference:

Lalko J and Api AM: Investigation of the dermal sensitization potential of various essential oils in the local lymphnode assay. Food Chem Toxicol 44 (2006): 739-746

Detailed study summary and results:

Test type

LLNA, OECD 429.

Test substance

Citral; 2,6-octadienal, 3,7-dimethyl.

Purity: 99.5%

Test animals

Mice (CBA), female

4 animals per dose

Age at study initiation: 8-12 weeks

Weight at study initiation: 17-21 g

The animals were kept at 19-25 °C and relative humidity 30-70 % with alternating 12h light and dark. The animals has ad libitum access to water and diet.

Administration/exposure

Each test group received one of the five test concentrations in 1:3 EtOH:DEP or the vehicle at a test volume of 25 uL dosed on the dorsum of both ears on three consecutive days. After a two days rest, on the 6th day after the first treatment, all mice were injected i.v. by the tail vein with 20 µCi of [3H]methyl thymidine. Five hours later the mice were euthanized and the draining auricular lymph nodes were excised and pooled for each test group. After separation of the cellular fraction, the incorporation of [3H]TdR in lymph node cells was measured by ß-scintillation counting and expressed as dpm (disintegrations per minute) per lymph node for each test group.

Results and discussion

Citral was shown to be sensitising with an EC3 value of 6.3%. No information on irritation is reported in the registration dossier.

Calclated stimulation index, citral

Applied concentration	2.5%	5%	10%	25%	50%
SI	2.8	2.3	5.1	11.4	22.1

3.1.1.4 STUDY 4-13 (LLNA, 10 studies cited in SCCS 2012, only limited information)

Study reference:

Unpublished summary reports by the Research Institute for Fragrance Materials (RIFM), cited in:

Scientific Committee on Consumer Safety SCCS OPINION on Fragrance allergens in cosmetic products. June 2012 (SCCS 2012). RIFM references: 2004b, 2003k, 2003l, 2003m, 2003n, 2003o, 2003p, 2003q, 2003r, 2003s.

Detailed study summary and results:

Test type

LLNA with no reported deviations from OECD 429 according to SCCS 2012.

Test substance

Citral, no information on purity.

Test animals

Mice, n= 4 animals per dose.

No further information available in SCCS 2012.

Administration/exposure

Citral was tested in concentrations of either 0.4-2-4-8-20%, 0.3-1-3-10-30% or 2.5-5-10-25-50%. Vehicles used were either:

- 1:3 ethanol:diethyl phthalate (EtOH:DEP) (2 studies)
- 0.1% α-tocopherol om 3:1 EtOH:DEP (2 studies)
- 0.3% antioxidant mix (1:1:1 butylated hydroxytoluene [BHT], tocopherol and eugenol) in 3:1 EtOH:DEP (2 studies)
- 0.1% Trolox C in 3:1 EtOH:DEP (2 studies)
- 3:1 EtOH-DEP (2 studies)

No further information available in SCCS 2012.

Results and discussion

Although detailed information is not available for the studies conducted by RIFM the results generally confirm the sensitising properties identified for citral in other LLNA studies. The EC3 values reported by RIFM are in the range 1.2%-6.8%.

3.1.1.5 STUDY 14 (LLNA)

Study reference:

Basketter DA, Wright Z, Gilmour NJ, Ryan CA, Gerberick GF, Robinson MK, Dearman RJ, Kimber I: Prediction of human sensitization potency using local lymph node assay EC3 values. Toxicologist 66 (2002) (1-S), 240, cited in:

Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the stydy and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

LLNA

Test substance

Citral, no information on purity in cited reference. Vehicle: AOO

Results and discussion

An EC3 value of 13% was reported.

3.1.1.6 STUDY 15 (LLNA) (cited from REACH registration dossier)

Study references:

Basketter DA and Scholes EW: Comparison of the local lymph node assay with the guinea pig maximization test for the detection of a range of contact allergens. Food Chem Toxicol 30 (1992): 65-69;

Basketter DA, Scholes EW, Kimber I, Botham PA, Hilton J, Miller K, Robbins MC, Harrison PTC, Waite SJ: Interlaboratory evaluation of the local lymph node assay with 25 chemicals and comparison with guinea pig data. Toxicol Meth 1 (1991): 30-43

Basketter DA, Scholes EW, Kimber I: The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. Food Chem Toxicol 32 (1994): 543-547

Detailed study summary and results:

Test type:

LLNA, equivalent or similar to OECD 429. The study was terminated on day 4 following three days of exposure.

Test substance

Citral; 2,6-octadienal, 3,7-dimethyl.

Purity: > 98%

Test animals

Mice (CBA), male/female

4 animals per dose

Age at study initiation: 8-12 weeks

Administration/exposure

Each test group received one of the 3 test concentrations in acetone:olive oil 4: 1 (v/v) or the vehicle alone at a test volume of 25 uL dosed on the dorsum of both ears on three consecutive days. On the 4th day after the first treatment, all mice were injected i.v. by the tail vein with 20 µCi of [3H]methyl thymidine. Five hours later the mice were euthanized and the draining auricular lymph nodes were excised and pooled for each test group. After separation of the cellular fraction, the incorporation of [3H]TdR in lymph node cells was measured by ß-scintillation counting and expressed as dpm (disintegrations per minute) per lymph node for each test group.

Results and discussion

Citral was shown to be sensitising with EC3 values reported from 4 different laboratories ranging from ca. 7-15%. No information on irritation is reported in the registration dossier.

Calclated stimulation index, citral

Applied concentration	Calculated SI			
	Lab. A	Lab. B	Lab. C	Lab. D
2.5%	2.1	0.9	2.2	0.9
5%	5.0	2.2	5.1	2.4
10%	9.3	6.2	20.5	4.7
Data published in	Basketter et al. 1991 Basketter and Scholes 1992	Basketter et al. 1991	Basketter et al. 1991	Basketter et al. 1991

3.1.1.7 STUDY 16 (GPMT) (cited from REACH registration dossier)

Study reference:

Basketter DA, Allenby CF: Studies of the quenching phenomenon in delayed contact hypersensitivity reactions. Contact Dermatitis, 25 (1991), 160-171

Basketter DA, Scholes EW, Kimber I, Botham PA, Hilton J, Miller K, Robbins MC, Harrison PTC, Waite SJ: Interlaboratory evaluation of the local lymph node assay with 25 chemicals and comparison with guinea pig data. Toxicol Meth 1 (1991): 30-43

Basketter DA and Scholes EW: Comparison of the local lymph node assay with the guinea pig maximization test for the detection of a range of contact allergens. Food Chem Toxicol 30 (1992): 65-69;

Detailed study summary and results:

Test type:

Similar or equivalent to OECD 406 (Guinea pig maximation test) but with less detailed documentation of test conditions and test results.

Test substance

Citral, perfume industry quality. Purity: > 98%. Vehicle: not reported.

Test animals

Guinea pig (Dunkin-Hartley)

10 animals per dose (test group), 4 per control group

Administration/exposure:

10 guinea pigs were treated in the shoulder region with a series of six intradermal injections of test material at a slightly irritant concentration in combination with Freund 's complete adjuvant to induce sensitization. 6-8 days later, sensitization was boosted by an occluded 48 h patch of test material at a mildly irritating concentration placed over the injection sites. 12-14 days later, the animals were challenged on one clipped and razored flank by an occluded 24 h patch containing test material at the maximum non-irritant concentration. A group of 4 animals treated as above but without the test material served as controls. Reactions were scored 24 and 48 h after patch removal for oedema and erythema on a scale of 0-3. The intradermal induction dose was 0.2%, the topical induction dose was 5% and the challenge dose 0.5%.

Results and discussion

Sensitisation was observed in 6/10 animals with a mean erythema score of 1.2.

3.1.1.8 STUDY 17 (GPMT)

Study reference:

Ishihara M, Itoh M, Nishimura M, Kinoshita M, Kantoh H, Nogami T, Yamada K: Closed epicutaneous test. Skin Res.28 (Suppl 2)(1986a), 230–240, cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the stydy and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

Guinea pig maximation test according to the Magnusson and Kligman 1969 method.

Test substance

Citral, no information on purity in cited reference. Vehicle: not reported.

Test animals

Guinea pig

No. of animals per dose not specifically stated

Administration/exposure (cited from Lalko and Api, 2008)**:**

Guinea pig maximization tests according to the Magnusson and Kligman (1969) method were conducted on citral. Induction consisted of a series of 6 intradermal injections with and without FCA of the test material followed 6–8 days later by a 48-h occluded patch. The animals were challenged 12–14 days later by an occluded 24 h patch application. Reactions were read 24 and 48 h after patch removal.

Doses were 10% citral throughout the induction and challenge period.

Results and discussion

Sensitisation was observed (no further details were provided).

3.1.1.9 STUDY 18 (GPMT)

Study reference:

Goodwin BFJ, Johnson AW:Single injection adjuvant test.Curr. Probl. Dermatol. 14 (1985), 201–207, cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the stydy and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

Guinea pig maximation test according to the Magnusson and Kligman 1969 method.

Test substance

Citral, no information on purity in cited reference. Vehicle: not reported.

Test animals

Guinea pig

No. of animals per dose not specifically stated

Administration/exposure (cited from Lalko and Api, 2008)**:**

Guinea pig maximization tests according to the Magnusson and Kligman (1969) method were conducted on citral. Induction consisted of a series of 6 intradermal injections with and without FCA of the test material followed 6–8 days later by a 48-h occluded patch. The animals were challenged 12–14 days later by an occluded 24 h patch application. Reactions were read 24 and 48 h after patch removal.

Induction was conducted with 0.4% for the intradermal injection and 1% for the occluded patch. Challenge application was conducted with 0.25%.

Results and discussion

Sensitization reactions were observed in 40% (4/10) of the animals.

3.1.1.10 STUDY 19 (GPMT) (cited from REACH registration dossier)

Study reference:

Unnamed study report, 1978

Detailed study summary and results:

Test type:

Similar or equivalent to OECD 406 (Guinea pig maximation test).

Test substance

Citral synthetic; 2,6-Dimethyl-2,6-octadien-8-al; Substance No. 77/711, no further data

Test animals

Guinea pig (Pirbright White)

10 per test group (10 for 1st challenge, 5 for rechallenges), 5 per control group (animals with challenge treatment only)

Weight at study initiation (injection): 507.7 g

Administration/exposure:

1st application = intradermal induction:

- Freund´s adjuvant/aqua dest (1:1) without test substance
- 25 % test substance in paraffin oil DAB7
- 25 % test substance in paraffin oil DAB7 and Freund´s adjuvant/aqua dest. (1:1)

2nd application = Percutaneous induction:

- with 25 % test substance in paraffin oil DAB7

1st challenge: 10 % test substance in paraffin oil DAB7

1st rechallenge: 5 % test substance in paraffin oil DAB7

2nd rechallenge: 5 % test substance in paraffin oil DAB7

A. INDUCTION EXPOSURE

- 1x6 intradermal injections and one week later, treatment with SLS, followed by one percutaneous induction according to OECD 406
- Readings: 24 hours after the intradermal application and 48 hours after the percutaneous induction
- Control group: not treated during induction phase
- Site: shoulder, respective on the same area

B. CHALLENGE EXPOSURE

- No. of exposures: 3 challenges
- Day(s) of challenge: 1st challenge: 19 days after percutaneous induction, 1st rechallenge: 28 days after percutaneous induction, 2nd rechallenge: 33 days after percutaneous induction
- Site: 1st challenge: right flank, 1st rechallenge: left flank, 2nd rechallenge: right flank
- Concentrations: 1st challenge: 10 % test substance in paraffin oil DAB7, 1st and 2nd rechallenge: 5 % test substance in paraffin oil DAB7

- Evaluation (hr after challenge): 1st challenge: treated and control animals 48 and 72 hours; 1st rechallenge: treated animals only 24 h, control animals 24 and 72 h; 2nd rechallenge: treated and control animals 24, 48 and 72 h.

Results and discussion

Positive reactions were observed in 100% of the animals in the test groups (10/10 for first challenge and 5/5 for rechallenges). The erythema scores were 2 or 3 at all readings except for after the third challenge/third reading, where a score of 1 was observed for 4/5 animals.

3.1.1.11 STUDY 20 (GPMT) (cited from REACH registration dossier)

Study reference:

Unnamed study report, 1978

Detailed study summary and results:

Test type:

Similar or equivalent to OECD 406 (Guinea pig maximation test).

Test substance

Citral synthetic; 2,6-Dimethyl-2,6-octadien-8-al; Substance No. 77/712, no further data

Test animals

Guinea pig (Pirbright White)

10 per treated group (10 for 1st challenge; 5 for rechallenges); 5 per control group without induction treatment

Mean weight at study initiation: 551.3 g

Administration/exposure:

1st application = intradermal induction:

- 25 % test substance in paraffin oil DAB7
- Freund's adjuvant/aqua dest (1:1) without test substance
- 25 % test substance in Freund's adjuvant/aqua dest (1:1)

2nd application = percutaneous induction:

- 25 % test substance in paraffin oil DAB7

1st challenge: 10 % test substance in paraffin oil DAB7;

1st rechallenge: 5 % test substance in paraffin oil DAB7

2nd rechallenge: 5 % test substance in paraffin oil DAB7

A. INDUCTION EXPOSURE

- 1x6 intradermal injections and one week later one percutaneous induction according to OECD 406
- Readings: 24 hours after the intradermal application and 48 hours after the percutaneous induction
- Control group: not treated during induction
- Site: shoulder, respective on the same area

B. CHALLENGE EXPOSURE

- No. of exposures: 3 challenges
- Day(s) of challenge: 1st challenge: 19 days after percutaneous induction, 1st rechallenge: 28 days after percutaneous induction, 2nd rechallenge: 33 days after percutaneous induction
- Site: 1st challenge: right flank, 1st rechallenge: left flank, 2nd rechallenge: right flank
- Concentrations: 1st challenge: 10 % test substance in paraffin oil DAB7, 1st and 2nd rechallenge: 5 % test substance in paraffin oil DAB7
- Evaluation (hr after challenge): 1st challenge: 48 and 72 hours; 1st rechallenge: 24 h and 6 days; 2nd rechallenge: 24, 48 and 72 h.

Results and discussion

Positive reactions were observed in 100% of the animals in the test groups (10/10 for first challenge and 5/5 for rechallenges) except for after 114 hours after a 5% rechallenge hcere 60% positive reactions were observed. The erythema scores were 2 or 3 at most readings except for after the 2nd challenge/1st reading (144h) and after the 3rd challenge/3rd reading (72h), where scores of 0 and 1 were observed in some of the animals.

3.1.1.12 STUDY 21 (GPMT)

Study reference:

Klecak G, Geleick H, Frey JR: Screening of fragrance materials for allergenicity in the guinea pig. I. Comparison of four testing methods. J. Soc. Cosmet. Sci. 28 (1977), 53–64, cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the stydy and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

Guinea pig maximation test according to the Magnusson and Kligman 1969 method.

Test substance

Citral, no information on purity in cited reference. Vehicle: not reported.

Test animals

Guinea pig

No. of animals per dose not specifically stated

Administration/exposure (cited from Lalko and Api, 2008)**:**

Guinea pig maximization tests according to the Magnusson and Kligman (1969) method were conducted on citral. Induction consisted of a series of 6 intradermal injections with and without FCA of the test material followed 6–8 days later by a 48-h occluded patch. The animals were challenged 12–14 days later by an occluded 24 h patch application. Reactions were read 24 and 48 h after patch removal.

Induction was conducted with 5% for the intradermal injection and 25% in petrolatum for the occluded patch. Challenge application was conducted with a subirritant concentration in petrolatum.

Results and discussion

Sensitization reactions were observed (No further details were provided).

3.1.1.13 STUDY 22 (Buehler)

Study reference:

Unpublished report by RIFM, 1973: The determination of citral in cosmetic formulations. Unpublished report from Rodia. RIFM Report Number 12471. RIFM, Woodcliff Lake, NJ, USA., cited in:

Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the stydy and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

Modified Buehler test

Test substance

Citral, no information on purity in cited reference. Vehicle: petrolatum

Test animals

Guinea pigs

No. of animals per dose not specifically stated, but results expressed as reactions in 5/5 animals

Administration/exposure (cited from Lalko and Api, 2008)**:**

"Citral was tested in a Modified Buehler Delayed Hypersensitivity Test in guinea pigs (Buehler, 1965; Ritz and Buehler, 1980). Induction consisted of three 6-h closed patch applications to the same clipped site on the dorsal surface with 20% citral in petrolatum. Induction applications were made once a week for 3 weeks. Following a 10–14 day rest, the guinea pigs were challenged with 20% citral in petrolatum. Challenge application was a 6-h occluded patch at a naive skin site. Control animals were challenged at the same time in an identical manner. Reactions were read 24 and 48 h after patch removal. Sensitization was observed in 5/5 animals (RIFM, 1973). Under the same conditions, samples of citral were tested to determine if changes in sample storage conditions would affect the sensitization potential. Sensitization reactions (5/5 animals per test) were observed to samples of citral that had been stored under nitrogen, stored with the addition of (butylated hydroxyanisole) BHA and after oxygen saturation (RIFM, 1973)."

Results and discussion

Sensitisation was observed in 5/5 animals, but no further information is available from Lalko and Api, 2008.

3.1.2 Human data

3.1.2.1 STUDY 1 (Patch test, selected)

Study reference:

Geier J, Uter W, Lessmann H, Schnuch A: Fragrance mix I and II: results of breakdown tests. Flavour Fragr. J. 2015, 30, 264–247.

Detailed study summary and results:

Test type

The IVDK (a network of departments of Dermatology in Germany, Austria and Switzerland) has performed a retrospective study of patch test data on the standardised fragrance mixtures Fragrance Mix I and II (FMI and FMII) obtained in the period from 1998-2013 and 2005-2013, respectively. Citral is a component of

FMII (1% citral). In cases where positive reactions were observed for FMII, testing of the full mix breakdown (and other fragrance allergens) have been done. FMII was patch tested in 84,724 patients in 2005–2013. Of these 4265 patients (5.0%) had a positive reaction. Time trends were analysed by dividing the time span into four 2-year periods and one 1-year period (i.e. 2013). The FM II full mix breakdown was tested in 1058 patients with a positive reaction to FM II. The results obtained with citral alone are based on patch tests with 2% citral in petrolatum.

Description of test method as cited from Geier et al. 2015: "*Diagnosing contact sensitization is done by patch testing. Briefly, during this procedure, the incriminated allergen, incorporated in a vehicle (usually petrolatum or water) in a standardized concentration, is filled into a test chamber which is applied occlusively on the patient's upper back for 1 or 2 days. After removal of the patches, reactions in the test areas are observed at least until 3 days after the application. In case of an allergen-specific sensitization, a positive reaction with erythema, infiltration and possibly papules (+), additionally vesicles (++), or even coalescing vesicles (+++) occurs, depending on the degree of sensitization. Patients, who are not sensitized, usually show no reaction at all; however, in some cases, irritant or doubtful reactions can occur, which are coded as 'ir' and '?', respectively. Within the IVDK, patch tests are performed according to international and DKG guidelines [ref]. All patch test preparations were obtained from Almirall Hermal, Reinbek, Germany*."

Patch test results at day three were evaluated (except in a few cases where no reading could be done at day 3, a day 4 reading was chosen instead). Statistical analysis and data management were done using SAS software (SAS 9.3, SAS Institute, Cary, NC, USA).

The results for citral showed that during the period 2005-2013 16.2% of the 1058 selected (FMII postitive) patients were tested positive for citral. The results divided into time spans are listed in the table below (note that the patient counts of the single time periods to not sum up to 1058 as FMII and its single components were tested in different time periods in 26 patients):

IVDK results of retrospective analysis of patch tests with citral 2% in petrolatum:

Year, patient count	2005-2006 n = 170	2007-2008 n = 250	2009-2010 n = 300	2011-2012 n = 222	2013 n = 90	2005-2013 n = 1058
Percent positive reactions (95% conf. intervals)	18.2% (12.7-24.9)	14.4% (10.3-19.4)	14.7% (10.9-19.2)	18.0% (13.2-23.7)	18.9% (11.4-28.5)	**16.2%** **(14.0-18.5)**

3.1.2.2 STUDY 2 (Patch test, selected)

Study reference:

Pónyai G, Németh I, Altmayer A, Nagy G, Irinyi B, Battyáni Z, Temesvári E: Patch Tests With Fragrance Mix II and Its Components. Dermatitis, Vol 23 no. 2 (2012), 71-74.

Detailed study summary and results:

Test type

A prospective study of data from 6 centres participating in a multicentre study in Hungary from 2009-2010 has been performed on behalf of the Hungarian Contact Dermatitis Group. A total of 565 patients with a history of skin symptoms provoked by scented products were included in the study. Clinical diagnoses of the patients were: contact dermatitis, 388 (allergic 208, irritative 180); atopic dermatitis, 44; dyshidrosis, 22; seborrhoeic dermatitis, 23; rosacea, 9; perioral dermatitis, 3; nummular eczema, 4; stasis dermatitis, 17; psoriasis, 16; and others, 39 (urticaria, 24; prurigo nodularis, 4; Morbus Hailey-Hailey, 4; discoid lupus erythematodes, 2; alopecia areata, 2; lichen simplex, 1; systemic lupus erythematodes, 1; and purpura pigmentosa, 1).

Description of test method as cited from Pónyai et al.: "*All the tests were performed with Brial GmbH D-Greven allergens;the skin reactions were evaluated (with 48-hour occlusion) at 48, 72, and 96 hours and also on the seventh day. The following fragrances were tested in the environmental contact patch test series: FM II (14% in vaseline [vas]), FM I (8% in vas), Myroxylon pereirae (balsam of Peru; 25% in vas), colophonium (20% in vas), wood-tar mix (12% in vas), propolis (10% in vas), and sesquiterpene lactone mix (0.1% in vas.). Apart from the environmental contact patch test series, tests with the FM II components were also carried out - citral 2%, farnesol 5%, coumarin 5%, citronellol 1%, >-hexyl-cinnamaldehyde (AHCA) 10%, and hydroxy-isohexyl-3-cyclohexene-carboxaldehyde (HICC; Lyral) 5% (all of them in vaseline).*
The test results were analyzed by using items of the MOAHLFA index."

The results showed that 3.4% (19/565) of the selected patients had positive reactions (contact hypersensivity) for citral when tested in 2% vaseline.

3.1.2.3 STUDY 3 (Patch test, selected)

Study reference:

Nardelli A, Carbonez A, Drieghe J, Goossens A: Results of patch testing with fragrance mix 1, fragrance mix 2, and their ingredients, and Myroxylon pereirae and colophonium, over a 21-year period. Contact Dermatitis, 68 (2013), 307–313.

Detailed study summary and results:

Test type

The Department of Dermatology at University Hospital St Rafael, Belgium, has performed a retrospective study of patch test data for 13332 patients who had been patch tested in the period from 1990-2011. A total of 3416 patients were tested with FMII (starting from 2005). The number of patients reacting to FMII (which includes 1% citral) was 205. Subsequent patch testing was in done with the individual ingredients of the fragrance mixture.

Description of test method as cited from Nardelli et al.: *All subjects had been tested with the European baseline series (Trolab, Hermal, Reinbeck, Germany) containing FM 1, M. pereirae (balsam of Peru), and colophonium. Since 2002, 3927 have been tested with HICC 5% pet., and from 2005, 3416 have been tested with FM 2. The patients reacting to FM 1 and FM 2 were, in most cases, tested with the individual ingredients, and some of the subjectswereoccasionally also testedwithother fragrance components. The patch tests were administered with Van der Bend patch test chambers (Van der Bend, Brielle, The Netherlands) applied on the back with Micropore™ (3M Health Care, Borken, Germany), and fixed with Fixomull (Beiersdorf, Hamburg, Germany), and later with Mefix (Mölnlycke Health Care, Göteborg, Sweden).*
The patch test readings were performed according to the international guidelines of the International Contact Dermatitis Research Group (12) after 2 days, 3 days (exceptionally), and 4 days, and sometimes later.

Statistical analysis of the patch data were performed with SAS™ version 9.2 (SAS Institute, Cary, NC, USA).

The results showed that 11.2% of the selected patients (23/ 205) had positive reactions for citral when tested at 2% in petrolatum.

3.1.2.4 STUDY 4 (Patch test, selected)

Study reference:

Bakker CV, Blömeke B, Coenraads P-J, Schuttelaar M-L: Ascaridole, a sensitizing component of tea tree oil, patch tested at 1% and 5% in two series of patients. Contact Dermatitis, 65 (2011), 239–248

Detailed study summary and results:

Test type

The Department of Dermatology, University Medical Centre Groningen, The Netherlands has performed a study investigating the sensitising properties of ascaridole, a component of tee trea oil. In this study patch tests were performed with the European baseline series, a cosmetic and/or perfume series and ascaridole (1%

or 5%) in two series of consecutive patients (602 and 144 patients, respectively). In the patients with positive reactions to ascaridole concomitant positive patch reactions were registered.

Description of test method as cited from Bakker et al., 2011: "*From March 2008 until August 2010, 602 consecutive patients who were suspected of having allergy related to cosmetic or perfume use underwent patch tests with the European baseline series, a cosmetic and/or perfume series, and ascaridole 1% pet. From August 2010 to February 2011, we consecutively tested a similar series of 144 patients with ascaridole 5% pet. instead of 1% pet. Patch tests were applied and read according to the International Contact Dermatitis Research Group guidelines. Ascaridole was provided by the Institute of Pharmacology, University of Bonn, Germany."*

The results showed that among the 30 selected patients with positive reactions to ascaridole (1 or 5%), 7% (2 of 30 patients) had concomitant positive reactions to citral.

3.1.2.5 STUDY 5 (Patch test, selected)

Study reference:

Cuesta L, Silvestre JF, Toledo F, Lucas A, Pérez-Crespo M , Ballester I: Fragrance contact allergy: a 4-year retrospective study. Contact Dermatitis 63 (2010): 77–84.

Detailed study summary and results:

Test type

The Department of Dermatology, Hospital General Universitario in Alicante, Spain performed a 4-year retrospective study of patients tested with the Spanish baseline series and/or fragrance series. A total of 1253 patients were patch tested with the baseline Spanish Group series. Positive reactions to a fragrance marker in were observed in 9.3% (n = 117) of these patients. A total of 86 patients were further tested with the Chemotechnique® fragrance series either because they were positive to the baseline series or because of a clinical suspicion. The objective of the study was to define the characteristics of the population allergic to perfumes, to determine the usefulness of markers of fragrance allergy in the baseline GEIDAC series, and to describe the contribution made by the fragrance series to the data obtained with the baseline series.

Description of test method as cited from Cuesta et al., 2010:

"The allergens used both in the standard series and in the fragrance series were supplied by Chemotechnique Diagnostics®. The markers of the baseline Spanish Group series used in our study to detect fragrance allergic contact dermatitis were: the 'traditional' markers (M. pereirae and FM I), hydroxyisohexyl 3-cyclohexene carboxaldehyde (included as of October 2005), and FM II (included as of January 2007)."

"The patches were prepared using Finn Chambers® fixed with Scanpor® adhesive and removed after 2D in contact with the skin. Readings were taken at D2 and D4, with the evaluation criteria (+, ++, and +++) recommended by the ICDRG. If the result was doubtful, a late reading was taken at D7. The relevance was considered current if the clinical picture could be attributed totally or partially to the fragrance obtained, past if this positivity explained only previous dermatitis, and unknown if the clinical picture could not be attributed to the use of these fragrances. Patients who were positive to any fragrance marker in the GEIDAC baseline series (M. pereirae,FM I, hydroxyisohexyl 3-cyclohexene carboxaldehyde,or FM II) were identified, and the percentage of patients positive to each of the markers was determined."

The results showed that among the patients tested with the Chemotechnique® fragrance series 2.3% of the selected patients (2/86) had positive reactions to citral when tested at 2% in petrolatum. It was concluded that the fragrance markers detect the majority of cases of fragrance contact allergy. Furthermore it was recommended to include FM II in the Spanish baseline series, as in the European baseline series, and to use a specific fragrance series to study patients allergic to a fragrance marker.

3.1.2.6 STUDY 6 (Patch test, selected)

Study reference:

Heydorn S, Johansen JD, Andersen KE, Bruze M, Svedman C, White IR, Basketter DA, Menné T: Fragrance allergy in patients with hand eczema – a clinical study. Contact Dermatitis 2003: 48: 317–323.

Detailed study summary and results:

Test type

A study of fragrance allergy in hand eczema patients from three dermatological departments in Denmark and Sweden (Gentofte, Odense, Malmö) was done in 2001-2002. 658 consecutive patients presenting with hand eczema were patch tested with the European standard series and the developed selection of fragrances. The aim of the study was to investigate patients referred with hand eczema concerning their frequency of positive patch tests to allergens in a selection of fragrances and to the European standard series. Citral (95%) was obtained from Dr. D. Basketter, Unilever Research (Sharnbrook, UK).

Description of patch test as cited from Heydorn et al., 2003: "*The patch tests were applied to the skin of the upper back for 2 D, using Finn Chambers1(Epitest,Helsinki,Finland)onScanpor1 tape (Norgesplaster A/S, Vennesla, Norway). Readings were taken on D2 and/or D3–4 and on D7. ICDRG recommendations were followed (10). A patch test was considered positive when the reading was +, ++ or +++. A + patch-test reaction was defined as homogeneous erythema and infiltration, whereas only erythema was not. The standard series used in Gentofte was from Hermal1 (Reinbek, Germany) apart from sesquiterpene lactone mix, which became unavailable from Hermal1 and was therefore obtained from Chemotechnique1 (Malmo¨, Sweden). In Odense, the standard series was TRUE TestTM (Chemotechnique1), supplemented by test*

substances from Hermal1. In Malmö , the standard series was from Chemotechnique1. In Odense, they tested 229, in Gentofte 220 and in Malmö 209 patients with hand eczema. As seen in tables 2 and 3, patch-test results from Hermal1, Chemotechnique1 and TRUE TestTM were combined for each allergen in the standard series.

Statistical analysis of the data was performed using the SAS® system for Windows® release 8.02 TS level 02MO© 1999–2001 by SAS Institute Inc. (Cary, NC, USA)

The results showed that 4.3% (28/658) of the patients were tested positive for citral at 2% (in petrolatum). Although the patients are described as consecutive patients in the publication they are considered to represent selected patients in this context (selected based on hand eczema).

3.1.2.7 STUDY 7 (Patch test, selected)

Study reference:

Wilkinson JD, Andersen KE, Camarasa JG, Ducombs G, Frosch P, Lahti A, Menné T, Rycroft RJG and White I: Preliminary results of the effictiveness of two forms of fragrance mix as screening agents for fragrance sensitivity. In Frosch PJ et al. (eds): Current Topics in contact dermatitis. Heidelberg: Springer-Verlag,1989:127-131.

Detailed study summary and results:

Test type

A total of 2455 consecutive patients attending dermatological clinics in England, Denmark, Spain, France, Germany and Finland were tested with two fragrance mixes: the Hermal ("Larsen") standard fragrance mix and a new experimental fragrance mix ("Hausen mix") containing citral. 78 selected patients positive to either of the mixes were patch tested with the individual ingredients.

Description of patch test as cited from Frosch et al., 1989: *"The two fragrance mixes studied were (a) the Hermal (Larsen) 8% fragrance mix and (b) a 9.5% (Hausen) fragrance mix. The Hermal 8% Mix consists of cinnamul alcohol 1%, cinnamaldehyde 1%, eugenol 1%, amyl cinnamaldehude 1%, hydroxycitronnellal 1¤, geraniol 1%, isoeugenol 1% and oak moss absolute 1% with sorbitan sesquioleate as emulsifier. The Hausen 9.5% Mix contained cinnamyl alcohol 0.5%, cinnamaldehyde 0.5%, isoeugenol 1%, eugenol 1%, dihydrocoumarin 2.5%, hydroxycitronellal 2.5%, geraniol 0.5% and citral 1%. 2455 consecutive patients attenting patch test climics in England, Denmark, Spain, France, Federal Republic of Germany and Finland were, wherever possible, tested to both fragrance mixes and, when one or other of these was positive, to all the individual fragrance compounds contained in both mixes. Patch test technique and readings were as*

recommented by the International Contact Dermatitis Research roup and, for positive results, an assessment of clinical relevance was also made.

The results of the study showed that 16.7% of the selected patients (13/78) were tested positive for citral at 2% in petrolatum.

3.1.2.8 STUDY 8-10 (Patch test, selected, 3 studies)

Study reference:

Ishihara et al. (1981), Itoh et al. (1986, 1988), Nishimura et al. (1984) cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the stydy and results is not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type

Diagnostic patch tests with citral

Description of studies as cited from Lalko and Api 2008: *"Ishihara et al. (1981) reported on the results of patch tests with 5% citral. Reactions were observed in cosmetic dermatitis patients (4/155) and eczema/dermatitis patients (5/159). No reactions were observed in control subjects (0/48). Patch tests were conducted between the years 1978–1986 in dermatologic patients (Itoh et al., 1986, 1988; Nishimura et al., 1984). When citral was tested at 5% (vehicle not reported), reactions were observed in cosmetic dermatitis patients (8/310), non-cosmetic dermatitis patients (9/408) and in one control subject (1/122). When citral was tested at 2% (vehicle not reported), reactions were observed in cosmetic dermatitis patients (1/240) and non-cosmetic dermatitis patients (2/584). No reactions were observed in control subjects (0/105).*

The results of the studies showed that

-when patch tested with 5% citral: 2.6% of cosmetic dermatitis patients (8/310), 2.2% non-cosmetic dermatitis patients and 0.8% control subjects (1/122) were stested positive for citral

-when patch tested with 2% citral: 0.4% of cosmetic dermatitis patients (1/240), 0.3% non-cosmetic dermatitis patients (2/584) and 0 control subjects (0/105) were stested positive for citral

-when patch tested with 5% citral: 2.6% cosmetic dermatitis patients (4/155) and 3.1% eczema/dermatitis patients (5/159) were tested positive for citral.

Higher frequencies of postitive reactions were thus observed with increasing dose of citral.

3.1.2.9 STUDY 11 (Patch test, selected)

Study reference:

Malten KE, van Ketel WG, Nater JP, Liem DH: Reactions in selected patients to 22 fragrance materials. Contact Dermatitis 1984:11:1-10.

Detailed study summary and results:

Test type

The working group on Occupational Dermatoses of the Dutch Society for Dermatology and Venereology and the (Dutch) Governmental Food Inspection Service carred out an investigation of allergic reactions to gragrance raw materials with the aim of composing a fragrance patch test screening series for patients with suspected cosmetic dermatitis. 182 patients suspected of suffering from contact sensitization to cosmetics were patch tested with a series of 22 fragrance and flavour raw materials including citral. The patients were suspected of suffering from cosmetic dermatitis based on either 1) history between severity of reaction and contact with cosmetics, 2) dermatitis localised on body regions where cosmetics are commonly applied, 3) generalised pruritus, redness and slight scaling without any other apparent causes, 4) patients with frequent occupational contact with cosmetics and related materials, 5) positive patch test reactions to specific indicator substances such as woodtars, colophony, oil of turpentine and/or balsam of Peru and 6) positive reactions after tests with dilutions of patients own cosmetics.

Description of the patch test as cited from Malten et al., 1984: *"The following investigations were performed. (i) The history and clinical patterns were recorded, guided by a questionnaire which also contained data about atopy. The patients where asked whether they had suffered from asthma (chronic bronchitis), hay fever, allergic rhinitis, or atopic dermatitis, and whether such atopic conditions were or had been present in members of their family: parents, brothers, sisters or children. (ii) Primary site and distribution of the eruption. (iii) Patch tests with: (a) 20 standard ICDRG allergens (Table 1); (b) 22 fragrance raw materials selected as possible contact allergens (Tables 2 and 3). The patch test reactions were read ad 48 and 72 h; the last reading was recorede as definitive. (iv) Analysis was performed on the presence of the 22 substances in 79 cosmetics which were sent in by the patients or their physicians because they were suspected of causing actual complaints (Table 3). 1 year after their manufacture, the stability of the 22 room-stored solutions was controlled (Table 3)".*

Citral was tested in a concentration of 2.0% in pet. The results showed that 2.6% of the selected patients (n = 182) were tested positive for citral. Citral was found to be present in 4 out of 79 cosmetics used (i.e. citral appeared in approx. 5% of the products analysed).

3.1.2.10 STUDY 12 (Patch test, consecutive)

Study reference:

Mann J, McFadden JP, White JML, White IR, Banerjee P: Baseline series fragrance markers fail to predict contact allergy. Contact Dermatitis, 70 (2014), 276–281.

Detailed study summary and results:

Test type

The St Johns' Institute of Dermatology at St Thomas' Hospital, UK has performed a retrospective study of patch test data by reviewing the records of 1951 eczema patients, routinely tested with the 26 fragrance substances requiring labelling and with an extended European baseline series (FMI and FMII) in 2011 and 2012. The objective was to determine the frequencies of positive test reactions to the 26 fragrance substances for which labelling is mandatory in the EU, and how effectively reactions to fragrance markers in the baseline series (FMI and FMII) predict positive reactions to the fragrance substances that are labelled. The study thus explored whether routine patch testing with all individual fragrance substances that are labelled above a threshold identified cases of fragrance contact allergy that would have remained undetected when using the baseline series.

Description of test method as cited from Mann et al.: *The patch test records of all eczema patients who underwent routine testing with the fragrance series and the European baseline series during 2011 and 2012 were retrieved from the database at St John's Institute of Dermatology at St Thomas' Hospital, London. The data recorded at the time of consultation included the age, sex and occupation of patients, the primary site affected by eczema, and the duration of eczema. Positive reactions, on or after day 4 of testing, to fragrance markers in the European baseline series (FM I, FMII, Myroxylon pereirae, and HICC) or allergens from the fragrance series (the26labelled fragrances and trimethylbenzenepropanol, but excluding HICC) were tabulated with spss™ version 12. Data were also collected for patientswhoreacted tocolophoniumandepoxyresin. The concentrations and constituents of the fragrance markers are shown in Table 1, and those of the allergens used in the fragrance series are shown in Table 2. Limonene and linalool were used in their unoxidized forms throughout the study. Patch testing was performed with aluminium Finn Chambers® provided by Bio-Diagnostics® (Upton-Upon-Severn, United Kingdom) and allergens provided by Bio-Diagnostics®, Trolab® (Hermal Almirall, Reinbeck, Germany) and Chemotechnique® (Vellinge, Sweden). Allergens were in petrolatum. Reactions were read on days 2 and 4, according to the*

recommendations of the International Contact Dermatitis Research Group. Reactions documented as questionable or irritant were considered to be negative.

The results showed that 1.03% (20/ 1951) (95% CI: 0.6-1.4%) of the consecutive eczema patients had positive reactions for citral when tested at 2% in petrolatum.

Overall the study showed that >40% of those patients reacting to a substance in the fragrance series would have been missed if evidence of fragrance allergy had been investigated exclusively with the European baseline series, and that a similar proportion of those reacting to FM I or FM II constituents did not react to the mixes themselves. In general the study indicates a very high rate of fragrance allergy as >14% of the patients reacted to either a fragrance marker or a substance in the fragrance series.

3.1.2.11 STUDY 13 (Patch test, consecutive)

Study reference:

Hagvall L, Christensson LB: Cross-reactivity between citral and geraniol – can it be attributed to oxidized geraniol? Contact Dermatitis 71 (2014), 280–288.

Detailed study summary and results:

Test type

The Department of Dermatology at Sahlgrenska University Hospital, Gothenburg, Sweden has performed a prospective study of patch test data for 655 patients who were patch tested with citral and its constituents neral and geranial as well as pure and oxidised geraniol. Data were obtained in the period from 2010-2011. Citral (66% geranial, purity: 98% and 34% neral, purity >99%) was obtained from Bedoukian Research Inc. (Danbury, CT, USA) and was distilled prior to use.

Prior to the patch test study an irritancy study was conducted. 22 patients were thus treated with 2.5 and 5% citral in petrolatum. The test substances were applied together with the ordinary patch test, and irritant reactions were evaluated on D3–D4. The reactions were evaluated visually (with a scale from 0-9 described by Basketter et al.). For citral the irritancy was low at 2.5% (mean score: 0.09) and increased at 5.0% (mean score: 0.91). A concentration of 3.5% pet. was chosen for further separate testing (of citral) on the basis of the results from the irritancy study.

Description of patch test as cited from Hagvall and Christensson 2014: "*Consecutive patients patch tested with the Swedish baseline series at the Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden, during the period September 2010 up to and including December 2011, were included in the study. Six hundred and fifty-five patients participated in the study (200 men, 455 women, mean age*

45.2 years, SD ±17.8). Patch test preparations of ~20mg (30) were applied in small Finn Chambers® (diameter 8 mm, inner area of 0.5 cm2; Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Vennesla, Norway) to the back of the patient, left under occlusion for 2 days, and then removed by the patient. Readings were performed according to the ICDRG recommendations (31) on D3–D4 and D7."

Statistical analysis was carried out with R version 3.0.3:A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria). Wilcoxons's signed rank test ($p<0.05$) was used to compare the visual readings. McNemar's test was used to evaluate differences in frequencies of positive patch test reactions to the study materials.

The results showed that 0.92% of the consecutive patients (n = 655) were tested positive for citral.

The results further suggest that geranial is the main sensitizer in the mixture citral, and that there is little cross-reactivity between pure geraniol and citral (results regarding cross-reactivity not described in detail in this annex).

3.1.2.12 STUDY 14 (Patch test, consecutive)

Study reference:

Hagvall L, Karlberg A-T, Christensson JB: Contact allergy to air-exposed geraniol: clinical observations and report of 14 cases. Contact Dermatitis, 67 (2012), 20–27.

Detailed study summary and results:

Test type

The Department of Dermatology at Sahlgrenska University Hospital, Gothenburg, Sweden has performed a prospective study of patch test data for 1055 patients who were patch tested with citral and its constituents neral and geranial as well as pure and oxidised geraniol. Data for citral were obtained in the period from 2006-2008. Citral (66% geranial, purity: 98% and 34% neral, purity >99%) was obtained from Bedoukian Research Inc. (Danbury, CT, USA) and was distilled prior to use.

Description of patch test as cited from Hagvall et al., 2012: "*Consecutive patients patch tested with the Swedish baseline series at the Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden, during the period January 2006 to August 2010, were included in the study. Patch test preparations of approximately 20 mg were applied in small Finn Chambers® (diameter 8 mm, inner area of 0.5 cm2; Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Vennsela, Norway) to the back of the patient, left under occlusion for 2 days, and then removed by the patient. Readings were performed according to the International Contact Dermatitis Research Group recommendations (28) on D3–4 and D7.*"

The results showed that 0.66% of the consecutive patients (n = 1055) were tested positive for citral. (In addition, further 0.28% of the patients showed doubtful reactions to citral).

3.1.2.13 STUDY 15 (Patch test, consecutive)

Study reference:

Heisterberg MV, Menné T, Johansen JD: Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. Contact Dermatitis, 65 (2011), 266–275 and corrigendum in: Contact Dermatitis, 67 (2012), 58.

Detailed study summary and results:

Test type

The Department of Dermato-Allergology, Copenhagen University Hospital, Gentofte has performed a retrospective study on consecutive eczema patients patch tested with citral. The objective of the study was to investigate frequencies of sensitization to the 26 individual fragrances and evaluate the sensitivity of the standard fragrance screening markers (FMI and FMII), i.e. would testing with the invididual substances reveal fragrance allergy that is not detected when using the standard fragrance markers. Patients (n = 1508) were patch tested with at least one of the 26 fragrance ingredients in the period from January 2008 to July 2010 were included in the study. 1502 patients were patch tested with citral.

Description of patch test as cited from Heisterberg et al., 2011: "*The patch testswere performed according to international guidelines (9), with Finn Chambers applied on the back with Scanpor tape (Vitalfo Scandinavia, AB, Allerød, Denmark) for a period of 2 days. Readings were performed on days 2, 3 or 4, and 7, according to the recommendations of the International ContactDermatitis Research Group (10). Not all subjects were patch tested with limonene and linalool, as the patch test material during the study period changed from being the pure compounds (Hermal) to oxidizedmaterials (G¨oteborg),because several studies have shown that it is the oxidized products that cause allergy (11–17). In this study, we report the results of patch testing with the pure compounds. Methyl 2-octyonate 1% was not patch tested in all of the subjects routinely patch tested, because active sensitization was observed in two patients, and we then stopped patch testingwith it; thus only 211 patientswere tested (18). Data management and statistical analysis were performed using SPSS™ version 15. Percentages of positive patch test reactions and confidence intervals were calculated with www.openepi.com. Chi-square tests and Fisher's exact tests for characteristic differences were performed, and p < 0.05 was considered to be significant.*"

The results showed that 0.3% of the consecutive patients (5/ 1502) were tested positive for citral. It was furthermore concluded that 11.7% of fragrance allergy subjects would be undetected with a fragrance allergy

if they had not been patch tested with the fragrance series, which underlines the value of patch testing all subjects with a fragrance series.

3.1.2.14 STUDY 16 (Patch test, consecutive)

Study reference:

Van Oosten EJ, Schuttelaar M-L A, Coenraads PJ: Clinical relevance of positive patch test reactions to the 26 EU-labelled fragrances. Contact Dermatitis 2009: 61: 217–223.

Detailed study summary and results:

Test type

The Department of Dermatology, University of Groningen, the Netherlands performed a retrospective study of patients with eczema of which a minor part of the patient group were suspected of being contact allergy to fragrances or cosmetics. In the study 320 patients were patch tested with the 26 EU-declared fragrance chemicals, FM I and FM II. The objective of the study was to describe frequencies of contact allergy to these 26 fragrance substances, and to evaluate clinical relevance of these positive reactions.

Description of test method as cited from Van Oosten et al., 2009: "*All 320 patients were tested with the series of 26 EU fragrance ingredients that are labelled. Additionally, the European baseline series (TRUE® test, Mekos laboratories, Denmark), which includes FM I, was tested in 295 patients, and the FM II (Hermal/Trolab, Reinbek, Germany) was tested in 227 patients. The fragrance compounds were obtained from Hermal/Trolab and from other international suppliers (International Flavors & Fragrances, USA; Robertet, France; Givaudan, Switzerland, Milennium Speciality Chemicals Inc., USA; Bedoukian Research Inc., USA; Rhodia, France; Symrise, Germany and Firmenich, Switzerland). All fragrances were dissolved in petrolatum, except for Evernia furfuracea which was dissolved in di-ethyl phthalate (Table 1). Patch tests were performed and read according to the guidelines of the International Contact Dermatitis Research Group (ICDRG) (12). The patches were applied for 2D. Final reading was done on D3. (7, 13). Reading of doubtful reactions was done up to D7 after the application of the patch test material. The relevance of the positive reactions (1+ through 3+) was determined and categorized as certain, probable, possible or not relevant. Contact allergy was defined as clinically relevant according to the following criteria: (i) certain exposure to the sensitizer and (ii) the patients dermatitis can be explained by the exposure (8, 11, 14, 15)".*

The results of the study showed that 0.6% of the consecutive eczema patients (2/320) had positive reactions to citral when tested at 2% in petrolatum.

3.1.2.15 STUDY 17 (Patch test, consecutive) (also cited in REACH registration dossier)

Study reference:

Schnuch A, Uter W, Geier J, Lessmann H, Frosch, PJ: Sensitization to 26 fragrances to be labelled

according to current European regulation. Contact Dermatitis 2007: 57: 1–10.

Detailed study summary and results:

Test type

The IVDK (a network of departments of Dermatology in Germany, Austria and Switzerland) has performed a retrospective study of patch test data from a multicentre project. During 2003-2004, 26 fragrances were patch tested additionally to the standard series in a total of 21325 patients; the number of (consecutive, unselected) patients tested with each of the fragrances ranged from 1658 to 4238.

Description of patch test as cited from Schuch et al., 2007: "*Patch tests are performed in accordance with the recommendations of the International Contact Dermatitis Research Group (12) and the German Contact Dermatitis Research Group (DKG) (13). Patch test material is obtained from Hermal/Trolab, Reinbek, Germany. Patch test preparations are applied for 24 or 48 hr. Readings are done until at least 72 hr using the following grading based on international standards (14), further refined by the German Contact Dermatitis Group (13): neg, ?, +, ++, +++, irritant, follicular. The patch test results of every reading, a standardized history (including age, sex, atopic diseases, current and former occupation(s), presumptive causal exposures), along with final diagnoses and site(s) of dermatitis are assessed and documented.*
All data are transferred to the data centre in Göttingen in an anonymized format every 6 months. During 4 periods of 6 months each, from 1 January 2003 to 31 December 2004, 25 fragrances (Table 1) were successively patch tested additionally to the standard series, i.e. in unselected patients, by departments of the IVDK. In the first period 8, in the second 6, in the third 3, and in the last period 8 compounds were added to the standard series, the number of patients tested with each preparation ranging from 1658 (tree moss) to 4238 (farnesol; tested during 2 periods)."

Statistical analysis of the data was performed using the statistical software package SAS (version 9.1, SAS Institute, Cary, NC, USA).

The results showed that 0.6% (95% CI: 0.3-1.0%) of the consecutive patients (13/2021) were tested positive for citral.

3.1.2.16 STUDY 18 (Patch test, consecutive) (also cited in REACH registration dossier)

Study reference:

An S, Lee A-Y, Lee CH, Kim D-W, Hahm JH, Kim K-J, Moon K-C, Won YH, Ro Y-S, Eun HC: Fragrance contact dermatitis in Korea: a joint study. Contact Dermatitis 2005: 53: 320–323.

Detailed study summary and results:

Test type

A multicentre study was performed by the Korean Society for Contact Dermatitis and Skin Allergy. Nine dermatology departments at university hospitals in Korea took part in this prospective analysis of allergic responses to fragrances where 422 patients (some of which with suspected contact allergy) were patch tested. In addition to the Korean (fragrance) standard and a commercial fragrance series, 18 additional fragrances were patch tested.

Description of patch test as cited from An et al., 2005: *"Test substances: The Korean standard series, which is a variant of the European standard series, and a fragrance series (Table 1) were purchased from Chemotechnique Diagnostics,Malmo, Sweden. We selected additional allergens based on past relevant references and information as to usage frequency. Chemical names, suppliers and test concentrations are summarized in Table 2. The additionally selected 18 fragrances were prepared in batches by the Korean cosmetic company and distributed to researchers at the different hospitals. Patch test method: Finn Chambers on Scanpor fape (Epitest, Tuusula, Finland) tape was used for patch testing, and the results were evaluated according to the recommendation of the International Contact Dermatitis Research Group (15)."*

The results of the study showed that 1.2% of the consecutive patients (5/422) were tested positive for citral at 2% in petrolatum.

3.1.2.17 STUDY 19-20 (Patch test, consecutive)

Study reference:

Frosch PJ, Pirker C, Rastogi SC, Andersen KE, Bruze M, Svedman C, Goossens A, White IR, Uter W, Arnau EG, Lepoittevin J-P, Menné T, Johansen JD: Patch testing with a new fragrance mix detects additional patients sensitive to perfumes and missed by the current fragrance mix. Contact Dermatitis 2005: 52: 207–215. (Frosch et al., 2005a)

Frosch PJ, Rastogi SC, Pirker C, Brinkmeier T, Andersen KE, Bruze M, Svedman C, Goossens A, White IR, Uter W, Arnau EG, Lepoittevin J-P, Johansen JD, Menne T: Patch testing with a new fragrance mix – reactivity to the individual constituents and chemical detection in relevant cosmetic products. Contact Dermatitis 2005: 52: 2016-225 (Frosch et al., 2005b)

Detailed study summary and results:

Test type

Six dermatological departments from Dortmund, Copenhagen, Malmö, Odense, London and Leuven have performed a prospective study of 1701 consecutive patients patch tested with FMI and FMII and their single constituents (SC), including citral, during 2002-2003. The aim of the study was to evaluate the new fragrance mix (FMII) and assess whether FMII can identidy additional patients with a positive fragrance history that are not identified with FMI and to evaluate whether FMII should be added to the European standard series. Citral was obtained from Dragoco/Symrise (Holzminden, Germany). FMII was prepared in 3 test concentrations: 28%, 14% and 2.8% (containing 2.0%, 1.0% and 0.2% citral, respectively).

Description of patch test as cited from Frosch et al., 2005a: "*Consecutive patients attending contact dermatitis clinics at 6 dermatology departments were tested between October 2002 and June 2003 (Dortmund, Copenhagen, Malmö , Odense, London and Leuven). In addition to the standard series, all 3-concentrations of FM II and the SC of 28%FM II and 14% FM II were applied to the skin of the back for 2 days. In all centres, Finn ChambersTM on Scanpor1 tape (Epitest, Tuusula, Finland) were used. Readings were taken atmost centres on day 2 and 4. The second reading, usually at day 3 or 4, was used for the overall evaluation of positive test results. The reactions were categorized according to published guidelines (7).".* Citral was thus tested in individual concentrations of 2.0% and 1.0%.

Further description of patch test of the single constituents as cited from Frosch et al., 2005b: *"The individual constituents of 14% FMII and of 28% FMII were applied simultaneously with the mix. The single constituents of 2.8% FMII were tested only if there was a positive or doubtful (+ or ?) reaction to this concentration of the new mix.*

Statistical analysis of the data was performed using the SAS TM software package (version 8.2, SAS Institute, Cary, NC, USA).

The results showed that 0.35% (6/1701) and 0.7% (12/1701) of the consecutive patients were tested positive for citral at concentrations of 1% and 2% (in petrolatum), respectively.

Higher frequencies of postitive reactions were thus observed with increasing dose of citral.

3.1.2.18 STUDY 21 (Patch test, consecutive)

Study reference:

Frosch PJ, Johansen JD, Menné T, Pirker C, Rastogi SC, Andersen KE, Bruze M, Goossens A, Lepoittevin J-P, White IR: Further important sensitizers in patients sensitive to fragrances*. I. Reactivity to 14 frequently used chemicals. Contact Dermatitis 2002, 47, 78–85.

Detailed study summary and results:

Test type

Six dermatological departments from Dortmund, Copenhagen, Malmö, Odense, London and Leuven have performed a prospective study of 1855 consecutive patients patch tested with FMI and FMII and their single constituents (SC), including citral, during October 1997- October 1998. The aim of the study was to determine the frequency of responses to selected fragrance materials in consecutive patients patch tested in 6 dermatological centres in Europe.

Description of patch test as cited from Frosch et al., 2002: "*Consecutive patients of contact dermatitis clinics at 6 dermatology departments were tested (Dortmund, Copenhagen, Malmo, Odense, London and Leuven) in the time period between October 1997 and October 1998. In addition to the standard series, the 8% FM from the same source and batch (Hermal, Reinbek, Germany) was applied to the back skin for 2days. Finn Chambers on Scanpor tape were used in all centres except Leuven (van der Bend chambers). Readings were taken at most centres on days (D) 2 and 4. The reading at D3 or D4 was used for overall evaluation of positive test results. Test reactions were categorized according to published guidelines (6)".*

The results showed that 1.1% (21/1855) of the consecutive patients were tested positive for citral at 2% (in petrolatum).

3.1.2.19 STUDY 22 (Patch test, consecutive)

Study reference:

De Groot AC, Coenraads JP, Bruynzeel DP, Jagtman BA, van Ginkel CJW, Noz K, van der Valk PGM, Pavel S, Vink J, Weyland JW: Routine patch testing with fragrance chemicals in The Netherlands. Contact Dermatitis 2000: 42: 162-185

Detailed study summary and results:

Test type

A prospective study of 1825 consecutive patients from different dermatological departments in the Netherlands has been performed, data were obtained in the period from September 1998 to April 1999. In this multicentre study 9 fragrance allergens including citral (2%, in petrolatum) were tested in all patients routinely patch tested. The 9 fragrances were selected either because of their widespread use in cosmetics or because they had been identified as relatively frequent allergens.

Description of patch test as cited from de Groot et al., 2000: "*Test procedures were carried out according to internationally accepted criteria. Hydroabietyl alcohol was purchased from Chemotechnique, the other*

fragrances from the Regional Health Inspectorate, Enschede. Test concentrations were chosen on the basis of published data (1) and potential irritancy was excluded in a pilot study involving 200 patients.

The results showed that 1.0% (19/1825) of the consecutive patients were tested positive for citral at 2% (in petrolatum).

3.1.2.20 STUDY 23-24 (Patch test, consecutive)

Study reference:

Frosch PJ, Pilz B, Andersen KE, Burrows D, Camarasa JG, Dooms-Goossens A, Ducombs G, Fuchs T, Hannuksela M, Lachapelle JM, Lahti A, Maibach HI, Menné T, Rycroft RJG, Shaw S, Wahlberg JE, White IR, Wilkinson JD: Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. Con/ac/ Dermalilis, 1995, 33, 333-342.

Detailed study summary and results:

Test type

A prospective multicentre study involving a total of 1323 patients tested in 11 centres was performed. The study involved testing of 48 frequently used constituents of perfumes, including citral, as well as patch testing with a standard series fragrance mix (FM) (not containing citral). 192 patients were patch tested with citral in the Copenhagen center.

Description of patch test as cited from de Frosch et al., 1995: *"In each centre, a minimum of 100 consecutive patients were tested with the allocated FF* (Fenn fragrance) *materials and the 8% FM with its constituents. For each patients positive to any 1 of the FF materials, a questionnaire was filled out regarding clinical relevance and other sensitizations.Patch testing was performed with Finn Chambers on Scanpor tape applied for 2 days to the back. Readings were made following the guidelines of the ICDRG (16) on days 2 and 3, or in some centres on days 2 and 4".*

The results showed that 0% (0/192) of the consecutive patients were tested positive for citral at 0.1% or 1% citral (in petrolatum).

3.1.2.21 STUDY 25 (Patch test, consecutive)

Study reference:

Michell JC, Adams RM, Glendenning WE et al.: Results of standard patch tests with substances abandoned. Contact Dermatitis 1982:8:336-337.

Detailed study summary and results:

Test type

The North American Contact Dermatitis Research Group have tested various fragrance substances on eczema patients as part of the routine testing series. Data obtained in the period 1973-1980 are presented in this publication. No information is provided on the patients nor the methods used and the publication solely gives an overview of the results obtained for the fragrances tested.

The results show that 1.7% of the patients (n = 228) were tested positive for citral tested at a concentration of 1% in pet. Data for citral were obtained in 1973/1974.

3.1.2.22 STUDY 26-30 (HRIPT, 5 studies) (also cited in REACH registration dossier)

Study reference:

Unpublished reports by the Research Institute for Fragrance Materials (RIFM), cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62–73.

Detailed study summary and results:

Test type

A detailed summary of the stydy and results is not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Description of HRIPT tests and results, cited from Lalko and Api, 2008: "*The HRIPT is generally performed utilizing a total of nine 24-h occluded applications over 3-weeks with test material and appropriate controls followed by a 2-week rest period. A single 24-h challenge application is then made to a na¨ıve site with the same materials. Observations at challenge coupled with the patterns of reactivity observed during induction provide the basis for an interpretation of contact allergy (Marzulli and Maibach, 1977; McNamee et al., 2008). Citral has been tested in the HRIPT over a range of concentrations. The following results were obtained. An HRIPT was conducted on 8 female volunteers with 5% citral in alcohol SDA39C. The patches consisted of a 1 in2 webril pad with 0.5 ml of test material; which resulted in a dose of 3876 µg/cm2. Sensitization reactions were observed in 5/8 subjects. Four subjects, who reacted during the initial study, were rechallenged approximately 7 months later with both a patch and a single open application behind one ear. Two subjects (2/4) reacted to the patch at rechallenge, no reactions (0/4) were observed following open application (RIFM, 1964a). No reactions were observed to 1400 µg/cm2 citral in an HRIPT conducted on 101 subjects (30 male and 71 female). The patches consisted of a 25 mm Hill Top Chamber , corresponding to a dosing area of 2.54 cm2, with 0.3 ml of 1.2% citral in 3:1 DEP:EtOH (RIFM, 2004b).*

When 1240 µg/cm2 citral was tested in an HRIPT, no reactions were observed in 50 subjects. The patches consisted of a 1 in2 webril pad with 0.2 ml of 4% citral in petrolatum (RIFM, 1971a). No reactions were observed to 755 µg/cm2 citral in an HRIPT conducted on 40 subjects (11 males and 29 females). The patches consisted of a 1 in2 webril pad with 0.5 ml of 1% citral in alcohol SDA39C (RIFM, 1965). An HRIPT was conducted on 12 male and 29 female volunteers with 0.5% citral in alcohol SDA39C. The patches consisted of a 1 in2 webril pad with 0.5 ml of test material; which resulted in a dose of 388 µg/cm2. No sensitization reactions (0/41) were observed (RIFM, 1964b)."

3.1.2.23 STUDY 31 (HRIPT)

Study reference:

Opdyke DLJ. Citral. Fd. Cosmet Toxicol 1979:17:259-266 *cited in:* Opinion concerning fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. The Scientific Committee on Cosmetic Products and Non-food Products intended for Consumers. SCCFNP 1999.

Detailed study summary and results:

Test type

A detailed summary of the stydy and results is not available in the SCCFNP opinion from 1999 which presents a review of the available data on sensitisation for citral. Only the following data are presented:
"Citral was also studied in the repeated insult patch procedure at 4-8% and sensitized 48% of a panel of 40 human volunteers (33)".

3.1.2.24 STUDY 32-45 (HMT, 14 studies) (also cited in REACH registration dossier)

Study reference:

Unpublished reports by the Research Institute for Fragrance Materials (RIFM), *cited in*: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62–73.

Detailed study summary and results:

Test type

A detailed summary of the stydy and results is not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. The following data are presented:

Description of HMT tests and results, cited from Lalko and Api, 2008: "*The HMT is typically conducted on 25 human subjects by utilizing 5 alternate day 48-h occluded induction applications of test material and appropriate controls. Following a 10 to 14-day rest period 48-hour challenge applications are made to nai*

¨ve sites. Patches may be made with and without pretreatment of sodium lauryl sulfate depending upon the inherent irritancy of the test material. Observations at challenge coupled with the patterns of reactivity observed during induction provide the basis for an interpretation of contact allergy (Kligman and Epstein, 1975). Citral has been tested in the HMT over a range of concentrations. The patches utilized for each of the reported studies consisted of a 14.5 cm2 webril pad with 0.5 ml of test material.

The results of the HMT studies showed that positive reactions generally occurred at concentrations exceeding 500 µg/cm^2. A high percentage of sensitisation reactions were seen in most of the HMT studies except for the one study where no sensitisation reactions occurred (the one study using butylene glycol as a vehicle). The doses tested were in the range 2-8%. Citral was stored and treated under differing conditions in the different studies. A dose-dependant trend can be seen from these studies with sensitisation frequencies generally increasing with the dose as seen from the table below:

Results of 14 HMT studies, unpublished reports from RIFM cited in Lalko and Api, 2008:

Citral, dose	Vehicle	Response in % (no of positive reactions)	RIFM reference in Lalko and Api, 2008
2% (1379 µg/cm^2)	Petrolatum	8.3% (2/24)	1972d
4% (2759 µg/cm^2)	Petrolatum	12% (3/25)	1972b
4% (2759 µg/cm^2)	Petrolatum	12% (3/25)	1972c
4% (2759 µg/cm^2)	Petrolatum	20% (5/25)	1972c
4% (2759 µg/cm^2)	Petrolatum	36%(9/25)	1971c
4% (2759 µg/cm^2)	Petrolatum	16% (4/25)	1971c
4% (2759 µg/cm^2)	Petrolatum	20% (5/25)	1971c
5% (3448 µg/cm^2)	Petrolatum	64% (16/25)	1974a
5% (3448 µg/cm^2)	Petrolatum	56% (14/25)	1974c
5% (3448 µg/cm^2)	Petrolatum	48% (12/25)	1974c
5% (3448 µg/cm^2)	Petrolatum	32% (8/25)	1974c
5% (3448 µg/cm^2)	Petrolatum	45.8% (11/24)	1974d
5% (3448 µg/cm^2)	Butylene glycol	0% (0/25)	1974e
8% (5517 µg/cm^2)	Petrolatum	33.3% (8/24)	1971b

3.1.2.25 STUDY 46 (Case study)

Study reference:

De Mozzi P, Johnston GA: An outbreak of allergic contact dermatitis caused by citral in beauticians working in a health spa. Contact Dermatitis 2014, 70, 376–388.

Detailed study summary and results:

Test type

Due to onset of bilateral hand dermatitis, 9 female beauticians working in the same high-end luxury health spa in the UK were separately and independently referred to the Dermatology Department, University Hospitals of Leicester by their general medical practitioners. The dermatitis was reported to improve with work avoidance. In their job all 9 patients were applying a wide variety of beauty treatment products, including essential oils.

Description of patcth test as cited from De Mozzi and Johnston 2014: *"Patch testing was performed with the British baseline series in all patients, with an additional fragrance series being applied to 7 patients, and a cosmetic series to 4 patients. Allergen series were supplied by Chemotechnique Diagnostics (Vellinge, Sweden), and applied with Finn Chambers® at D0; readings were performed at D2 and D4, according to International Contact Dermatitis Research Group guidelines".*

The results of the patch test showed that 5 of the 9 patients had positive reactions to both FMII as well as to citral 2.0% in pet. A site visit to the healt spa revieled that the predominant brand of products used consisted of a large range of essential oils and spa products all of which contained citral.

3.1.2.26 STUDY 47 (Case study)

Study reference:

Hindle E, Ashworth J, Beck M H: Chelitis from contact allergy to citral in lip salve. Contact Dermatitis 2007: 57: 125-126.

Detailed study summary and results:

Test type

At the Contact Dermatitis Unit, Hope Hospital in Salford, UK, a 30-year-old female with a 5-year history of recurrent chelitis was patch tested with the standard series including FMII, as well as with a range of different other series and products. No further information of the patch test is provided in the reference.

The result of the patch test showed positive reactions to FMII as well as to citral, 2% (in pet.). Based on the testing and information on the products frequently used by this patient the chelitis was attributed to the use of a lip salve containg citral (a Vaseline lip balm with with citral listed as an ingredient on the packaging). Changing to plain Vaseline for lip care and and avoidance of perfume and nail varnish resulted in symptomatic improvement.

3.1.2.27 STUDY 48 (Case study)

Study reference:

Malten KE. Four Bakers showing positive patch-tests to a number of fragrance materials, which can also be used as flavors. Acta Dermato-venereologica 1979:suppl 85:117-121.

Detailed study summary and results:

Test type

The Department of Dermatology in Nijmegen, Holland have described four cases of bakers showing positive patch tests to a number of fragrance materials which can also be used as flavours. All four bakers had developed contact dermatitis on their fingers/hands and one of the also in the face. The development of the contact dermatitis seemed to have a clear time relation with their professional activities although one of the bakers (case no. 4) also had a history of contact dermatitis that could possibly be attributed to non-occupational exposure. One of the four bakers (case no. 3) had a positive reaction to citral in 0.5% pet. He also showed clear positive reactions to certain flavours/spices used for different kinds of sweet bisquits. Following the patch test the person was not seen again at the clinic. In the SCCNFP opinion from 1999 where the study is also cited, the relevance of the study is described as "unknown".

3.1.2.28 STUDY 49 (Patch test, experimental study)

Study reference:

Nagtegaal MJC, Pentinga SE, Kuik J, Kezic S, Rustemeyer T: The role of the skin irritation response in polysensitization to fragrances. Contact Dermatitis, 67 (2012), 28–35.

Detailed study summary and results:

Test type

The Department of Dermatology of the VU University Medical Centre, The Netherlands, has performed a prospective study of 100 selected patients with contact allergy who were patch tested with 25 individual fragrance chemicals and fragrance mixes I and II in the period from 2005-2010. The objective of the study was to to investigate whether enhanced skin irritability is a risk factor for the development of polysensitization to fragrance chemicals.

Description of test method as cited from Nagtegaal et al., 2012:

Patch tests: "*Patch tests were performed in accordance with the recommendations of the ICDRG (12). Preparations of test materials in petrolatum were obtained from Trolab® (Almirall-Hermal, Reinbeck, Germany) or Chemotechnique Diagnostics® (Vellinge, Sweden). Van der Bend® patch test chambers (Van der Bend BV, Brielle, The Netherlands) on Fixomull® tape were used.Test chambers were manually filled by a specially trained investigator. The test substances consisted of 27 commercial patch test materials of fragrance chemicals, including FM I (8%) and FM II (14%), and were coded to ensure that the study could be performed in a double-blind fashion. The materials were supplied in polypropylene syringes, and stored*

in a refrigerator at 5◦C. The patches were applied for 2 days on the upper back, and readings were performed on day 2 (48 hr), day 3 (72 hr), and day 7 (144 hr). Methodological and observer errors were minimized, as preparation and reading of the test were performed by only one specially trained person. Polysensitization was defined as three or more allergic reactions to non-cross-reacting fragrance allergens."

Skin irritation tests: "*This test consisted of the application of SLS at five sites in a row on the non-dominant upper arm for 1 day (24 hr). Van der Bend® patch test chambers on Fixomull® tape were filled with 20 µl of test solution. The SLS test concentrations were 0.0%, 0.45%, 0.67%, 1% and 1.5% in water. New test solutions were prepared every 3 weeks. The participants removed the patches themselves 24 hr after application, after which the test was assessed at day 2, day 3 and day 7 by bioengineering techniques. This included a non-invasive measurement of TEWL by means of a TEWAmeter® (TM300; Courage & Khazaka, Cologne, Germany) and of redness of the skin (erythema index) by means of a DermaSpectrometer® (Cortex Technology, Hadsund, Denmark). The increase in TEWL and erythema index reflects the sensitivity of the skin to SLS irritation. As baseline values of erythema index and TEWL are known to vary day to day, these values were measured every visit. The existing guidelines for assessment of these parameters were followed (13, 14), meaning that the volunteers rested for at least 15 min with uncovered arms before measurement, in a room with a temperature of 20–22◦C, a relative humidity of 35–45%, and no direct incursion of sunlight."*

Statistical analysis: "*All data were analysed for significance by paired samples t-test or Mann–Whitney U-test with SPSS™ statistical software (version 17). The distribution of data was tested by the Shapiro–Wilk normality test. For non-normally distributed data, we applied the Mann–Whitney test. For testing the differences in TEWL between different SLS concentrations and the control site, we used a non-parametric Friedman test followed by Dunn's multicomparison test (p < 0.001)."*

Although not a clinical diagnostic patch test study, patch tests were nevertheless performed according to the guidelines of the International Contact Dermatitis Research Group. The results showed that specifically for citral 9.0% (9/ 100) (95% CI: 4.2-16.4%) of the selected patients had positive reactions when tested at 2% in petrolatum.

Individuals with polysensitization (defined as multiple patch test reactions to > 3 non-related allergens) showed significantly higher irritation responses to SLS 1% and 1.5% (as assessed by transepidermal water loss). It was concluded that an enhanced skin irritation response is associated with polysensitization, and that it could be a phenotype for susceptibility to contact allergy.

4 ENVIRONMENTAL HAZARDS

Classification for environmental hazards is not a part of the CLH proposal for citral.