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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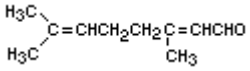
CONTENTS

1 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.

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).

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		
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 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)	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	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral (Key study)	Diliberto et al., 1990
No guideline, GLP compliance not reported. Tissue distribution and time course of excretion in urine, faeces and exhaled 14CO2 measured;	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%)	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral	Phillips et al., 1976

Method	Results	Remarks	Reference
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	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		
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 Sampling: urine and feaces at 2, 4, 6, 8, 12, 16, 24, 32, 48, and 72 hrs, blood at 72 hrs, expired air: continuously	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 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).	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral	Diliberto et al., 1988
No guideline, GLP compliance not reported. Guinea pig (Hartley), female Single dose, dermal	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	**Test material (EC name): citral** Dosed partly as ^{14}C labelled citral	Barbier et al., 1983

Method	Results	Remarks	Reference
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	(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).		
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 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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
					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 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.

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
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 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	Unnamed study report 1978 cited from REACH 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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
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 with citral. Data from IVDK multicentre project (IVDK: Information Network of Departments of Dermatology in Germany, Austria and Switzerland). Data obtained 2005-2013.	**16.2%** were tested positive (n = 1058)	Geier et al., 2015
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,	**6.7%** were tested positive (2/ 30)	Bakker et al., 2011

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		The Netherlands. Data obtained 2008-2011.		
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 the cosmetic dermatitis patient group is assumed to represent selected patients.	0.4% cosmetic dermatitis patients were tested positive (1/ 240) 0.3% non-cosmetic dermatitis patients were tested positive (2/584)	Itoh et al., 1986 and 1988 and Nishimura et al., 1984 cited from Lalko and 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

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
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: Information Network of Departments of Dermatology in Germany, Austria and Switzerland). Data obtained 2003-2004.	0.6% were tested positive (13/2021)	Schnuch et al., 2007 (also cited in REACH reg.)
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

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
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 µg/cm^2) Veh: 3:1 DEP:EtOH	No further information available in cited reference.	0% were tested positive (0/101)	Unpubl. study report from RIFM 2004b 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)	No further information available in cited reference.	62.5% were tested positive (5/8)	Unpubl. study report from RIFM 1964a

[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
	Veh: alcohol SDA39C			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 µg/cm^2) Veh: pet.	No further information available in cited reference.	32% tests were positive (8/25)	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.	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

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
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 µg/cm^2) Veh: pet.	No further information available in cited reference.	20% tests were positive (5/25)	Unpubl. study report from 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,	Citral, 2%	Single-centre, double-blind volunteer study of 100 selected patients	9.0% were tested	Nagtegaal et al.,

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
selected patients	(in pet.)	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.	positive (9/100)	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

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.

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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15 ANNEXES

Annex I: detailed study summaries

Annex II: confidential information on substance identity