Comments of BASF SE on the CLH report - Proposal for Harmonised Classification and Labelling of Citral (3,7-dimethylocta-2,6-dienal) provided by the Danish Environmental Protection Agency on 30 August 2017.

General comments

The submitted CLH report for Citral [3,7-dimethylocta-2,6-dienal, CAS Registry Number: 5392-40-5, EC Number: 226-394-6] identifies this substance to be a strong skin sensitizer, that needs to be classified in sub-category 1A. The submitter based its conclusion on high frequencies of skin reactions observed in human diagnostic patch tests and the high number of published cases combined with an estimated low exposure with Citral.

We disagree with the proposal based on the following elements:

- The vast majority of the available reliable animal data meet the SS1B criteria. A weight of evidence approach supports the SS1B classification.
- A large number of human volunteer studies which are all consistent in the conclusion of the SS1B classification.
- Human clinical data, representing only a limited basis for a classification decision due to the uncertainty of the induction exposure conditions, partly meet the SS1B classification criteria.

Comments on animal data

In the CLH report, a weight of evidence approach was applied to compare animal and human data to the CLP criteria and respective guidance. Based on 21 animal studies available, 4 studies were identified by the submitter to fulfill the criteria for a sub-category 1A, whereas 17 studies fulfill the criteria for a sub-category 1B or do not allow subcategorization. Two of these 4 studies (a GPMT and a Bühler test) were considered of being associated with some uncertainty due to lack of dose response relationships and an endpoint of qualitative nature.

We agree, that the animal studies generally confirm the skin sensitization properties of Citral. However, the vast majority of the animal tests clearly define Citral as a weak sensitizer (subcategory 1B) in the sense of definitions laid down in Regulation (EC) No. 1272/2008 (CLP). We agree, that the two guinea pig studies cited are associated with some uncertainty to derive a classification decision, which is due to shortcomings in the study conduct. Both studies were not performed according to the OECD TG 406 and, in particular, the Bühler test cited shows major deviations from current testing protocol such as dosing (9 applications instead of 3 applications for 3 weeks) and animal numbers (5 instead of 20 animals per dose group).

Only 2 out of 14 reported Local Lymph Node Assays showed EC3 values below the 2% (500 µg/cm2) criteria for classification in category 1A (the other 12 supporting an SS1B conclusion). Referring to these two LLNAs, the submitter stated that the studies using EtOH:DEP are of equal reliability to those using AOO as vehicle.

It needs to be pointed out, that 2 of the 3 referred studies with EC3 values below or close to the cut-off criteria of 2% also contained tocopherol or BHT/ tocopherol/ eugenol mixes, which do not represent standard vehicles for LLNA testing. Therefore, the reliability of these studies in determining the classification of Citral cannot be confirmed.

To evaluate the relevance of the LLNA data for the weight of evidence approach, the submitter did not take further details of the study procedure into account besides the choice of the vehicle. It is generally stated, that most studies were reported as being conducted according to or as being equivalent to OECD 429. However, a more detailed assessment of the conducted studies to weigh the evidence is missing. It is not acknowledged by the submitter, that the 3 LLNAs with EC3 values close to or below 2% have been

performed twice by the same testing laboratory with a comparable protocol and the same vehicles (see Table 1) all with different (and higher EC3 values) as described in detail below. Testing of Citral in EtOH:DEP [1:3] led to EC3 values of 1.2% (RIFM, 2004b) and 6.3% (RIFM, 2003a). Application of Citral in EtOH:DEP [3:1] incl. 0.1% tocopherol resulted in EC3 values of 1.5% (RIFM, 2003k) and 6.8% (RIFM, 2003d). Testing in EtOH:DEP [3:1] incl. 0.3% BHT/tocopherol/eugenol resulted in EC3 values of 2.1% (RIFM, 2003l) and 4.6% (RIFM, 2003i). Although the results have been obtained under highly comparable testing conditions, a high variability of the EC3 values obtained can be clearly demonstrated. It is stated by the submitter, that if different EC3 values are available from several studies then the lowest value should normally be used. We would like to emphasize, that the validity of the tests and the reproducibility of the data needs to be taken into account as well. A weighed mean of all available EC3 values has been set at 5.7% or 1420 µg/cm2 to account for this variability of the LLNA database. Given the overall variability in the LLNA the calculated weighted mean value of 5.7% and that the vast majority of studies show EC3 values >2% it is considered reasonable to reach an SS1B conclusion based on the animal data.

Overall, we provide detailed study information for these LLNAs to the submitter in a confidential attachment to this submission to ensure a more in-depth review of the full database.

Table 1. LLNA Summary Data on Citral

Principal Name	CAS Number	LLNA Potency Estimation			References
		EC3 Values (%)	EC3 Value (µg/cm^2)	Vehicle*	
Citral	5392-40-5	1.2	300	EtOH:DEP [1:3]	(RIFM, 2004b)
		1.5	375	EtOH:DEP [3:1] + 0.1%Toc	(RIFM, 2003k)
		2.1	525	EtOH:DEP [3:1] + AO Mix	(RIFM, 2003l)
		3.7	925	EtOH:DEP [3:1] + 0.1%TrlC	(RIFM, 2003m)
		4.6	1150	EtOH:DEP [3:1]	(RIFM, 2003n)
		4.6	1150	EtOH:DEP [3:1] + AO Mix	(RIFM, 2003i)
		5.3	1325	EtOH:DEP [3:1]	(RIFM, 2003c)
		5.8	1400	EtOH:DEP [3:1] + 0.1%TrlC	(RIFM, 2003g)
		6.3	1575	EtOH:DEP [1:3]	(RIFM, 2003a)
		6.8	1700	EtOH:DEP [3:1] + 0.1%Toc	(RIFM, 2003d)
		12.6	3150	AOO	(Basketter, et al., 2012)
		13.0	3250	AOO	(Basketter, et al., 2002a)
		14.1	3525	AOO	(Jung et al., 2012)
		7-15**	NA	AOO	(Basketter and Scholes, 1992)
		***Wt Mean = 5.7	****Wt Mean = 1420		

* Vehicle abbreviations: AOO - Acetone:Olive Oil (4:1); EtOH – Ethanol; DEP – Diethyl phthalate; Toc – Tocopherol; TrlC – Trolox C; AO Mix – 0.3% BHT/tocopherol/eugenol

** Not included in weighted mean calculation due to lack of precise data

*** Wt Mean (%) = (((1,2+6,3)/2)+((1,5+6,8)/2)+((2,1+4,6)/2)+((3,7+5,8)/2)+((4,6+5,3)/2)+((12,6+13+14,1)/3))/6

**** Wt Mean (µg/cm^2) =(((300+1575)/2)+((375+1700)/2)+((525+1150)/2)+((925+1400)/2)+((1150+1325)/2)+((3250+3150+3525)/3))/6

Comments on human data

The dossier submitter cites human data, that would provide substantial evidence of strong sensitizing effects of Citral especially based on 25 diagnostic patch tests. . According to the interpretation of the submitter, these data would confirm a high frequency of occurrence of skin sensitization in unselected (≥ 1.0% in 5 of 14 patch tests) and selected (≥ 2.0% in 10 of 11 patch tests) dermatitis patients and the number of published cases is well above 100. Such tests are considered by the submitter to be the primary source of clinical information on the occurrence of skin sensitization, and the submitter regards the dataset covering the last 3 – 4 decades to be comprehensive.

We disagree with the submitters argumentation. The listed patch tests do not allow to come to a clear decision regarding the induction exposure levels and conditions of the patients in the studies showing a high frequency of reactions to Citral. This represents a major shortcoming of the clinical patch tests for the use in the classification decision as a category 1A or 1B skin sensitizer. The data bear an uncertainty concerning the exposure conditions, that led to the induction of the patient skin sensitization against Citral. In the CLH report it is stated, that these patch tests do not provide specific information on the

previous exposure regime for these patients, and cannot be used to establish an SCL. Due to the clearly defined induction exposure conditions used in the human repeated insult patch tests and the human maximization tests on volunteers, we consider these studies to be a less variable and more useful source for the classification decision as category 1A or 1B skin sensitizer.

It is noted by the submitter, that older volunteer studies (6 HRIPT and 14 HMT studies, respectively), do not indicate a high skin sensitization potency of Citral, although original study information is generally not available. The dossier submitter considers these studies as supportive evidence but states a lower relevance for classification. From the CLH report, it appears, that the lower relevance is justified by the fact, that robust study information and calculations of the estimated induction concentration are not available.

We would like to stress that study details are made available to the submitter in a confidential attachment to this submission and the absence of this info cannot be used to prove a lower relevance for the classification decision. We also question, if the submitter has comparably more detailed information concerning the diagnostic patch tests, that justifies these data to as substantially more relevant evidence for the classification decision.

All of the 20 confirmatory standard tests in human volunteers support a classification of Citral as a weak sensitizer (subcategory 1B) in the sense of definitions laid down in Regulation (EC) No. 1272/2008 (CLP). Four Human Repeated Insult Patch Tests of mixed quality showed no skin sensitization reactions after repeated application of 1400, 1240, 775 & 388 µg Citral/cm^2, whereas positive reactions were observed in some studies carried out at dose levels above the 500 µg/cm^2 threshold for differentiating between categories 1A and 1B.

Two additional publications reporting incidence of contact allergy to Citral in consecutive dermatitis patients have recently been published. Bennike et al (2017)[1] reported data from a single university clinic (University Hospital Herlev-Genofte, Denmark). This reports % positive reactions to Citral of 0.39% from 2010 to 2015. The publication also reports a clear decreasing prevalence trend from 2010 to 2015. Mowitz et al (2017)[2] reported data from southern Sweden between 2009 and 2015. The data shows 1.1% positive reactions from 2009 to 2012 and 1.3% from 2013 to 2015. This additional new data confirms the variation seen in the various studies cited by the submitter in the CLH report on consecutive patients.

Whilst the cumulative data on selected dermatitis patients clearly meets the criteria of a “high frequency” of cases according to the ECHA criteria, a meta-analysis of all data for unselected dermatitis patients does not meet these criteria (Table 2). Consideration of all of the reported cases in the CLH dossier for patch testing at 2% Citral (current recommended patch test concentration), taking into account the information given in the Appendix of this document (Detailed comments on Table 10 of the CLH report) and including the 2 new studies cited above, shows overall 192 reported cases out of a total of 21692 patients tested (0.89%). Furthermore, considering all reported cases independent from the Citral concentration tested (215 reported cases out of 25715 patients) result in a fraction of 0.84%. This would meet the criteria for “low frequency” according to ECHA.

[1]Bennike, N.H et al. (2017); Non-mix fragrances are top sensitizers in consecutive dermatitis patients – a cross-sectional study of the 26 EU-labelled fragrance allergens. Contact Dermatitis, 77, 270-279.

[2]Mowitz, M. et al. (2017); Simultaneous patch testing with fragrance mix I, fragrance mix II and their ingredients in southern Sweden between 2009 and 2015 Contact Dermatitis, 77, 280-287.

Table 2. Meta-analysis of all data for unselected dermatitis patients

	Concentration tested (%)	Positive tested (n)	Total tested (n)	Reference
Patch test data, consecutive (unselected data)	3,5	6	655	Hagvall et al. 2014
	2	28	658	Heydorn et al. 2003
	2	20	1951	Mann et al. 2014
	2	4	1502	Heisterberg et al. 2011
	2	13	2021	Schnuch et al. 2007
	2	12	1701	Frosch et al. 2005
	2	21	1855	Frosch et al. 2002
	2	19	1825	De Groot et al. 2002
	2	23	6004	Bennicke et al. 2017
	2	22	1927	Mowitz et al. 2017
	2	30	2248	Mowitz et al. 2017
	1,5	7	1055	Hagvall et al. 2012
	1	0	192	Frosch et al. 1995
	1	4	228	Michell et al. 1982
Total (tested at 2% Citral)	2	192 (0.89%)	21692	
Total	1-3.5	209 (0.88%)	23822	

Comments on exposure considerations

According to the CLH report, a frequent/daily exposure is anticipated due to the widespread use and the high tonnage of Citral. However, the submitter estimates the overall exposure to Citral to be relatively low based on the current IFRA standard limits (up to 5% in rinse-off cosmetic and other consumer products) and on survey information of actual concentrations in various consumer products (generally lower than 0.1% Citral). However, concentrations of up to 78% Citral in etherical oils and up to 26% in air fresheners have been cited from the DK EPA databaseas well as reference to the SeV report (KEMI 2015) which concludes that Citral concentrations higher than 0.5% are found on the Swedish market. The submitter stated, that it is difficult to avoid exposure. In the submitters exposure considerations, the concentration or dose score has been set to 0 to calculate the additive exposure index justified by the expected and observed concentration <1% Citral in relevant consumer products on the market. The submitter did not justify, why actual and historic exposure with concentrations above 1% Citral still result in a concentration/dose score of 0 instead of 2, which would lead to an additive exposure index of 2+2+2=6 and would define exposure as relatively high.

We agree with the submitter, that Citral is widely used as a fragrance ingredient in cosmetic and household cleaning products. Furthermore, dermal Citral exposure will also occur to an unquantifiable degree via massage oils and essential oils as cited in the CLH submission and via natural food sources such as citrus fruits.

Concerning the positively patch tested patients, it is highly probable that at the time of induction of their allergy, more than once daily exposure (score 2) with more than 100 exposures prior to induction (score 2) occurred regardless of whether they have subsequently chosen to avoid fragranced products. The key to defining the relative exposure level is therefore in knowing if the concentration/dose of Citral at the time of induction was above or below 1.0%/500 µg/cm^2. The diagnostic patch tests do not provide any information concerning induction concentration and other exposure conditions that resulted in skin sensitization against Citral.

The presence of a IFRA standard on Citral which limits exposure to Citral for consumer products is cited as additional evidence of low exposure. This is of low relevance to the exposure considerations for CLH. The induction of sensitization may have occurred either due to exposure to a non-IFRA regulated source

(e.g., non IFRA compliant products, aromatherapy/massage/other unregulated exposure) and/or may have occurred prior to the full implementation of the IFRA standard.

On June 11, 2006 the International Fragrance Association formally issued quantitative limits on the concentration of Citral in different types of consumer product (IFRA, 2006) which would limit the concentration (w/w) of Citral in consumer products below 1% except for the categories "Mouthwash", "Hair styling aids", "Rinse-off cosmetics" and "Household detergents". In terms of the key dose-metric (quantity per unit area), IFRA has used assessment factors[3] that limit exposure to Citral to either one 100th or one 300th of the empirically-derived No Expected Sensitization Induction Level (NESIL) of 1400 µg/cm^2

Prior to these restrictions, the use of Citral was virtually unrestricted by IFRA (requirement to be used in conjunction with some other substances such as Limonene and Pinene). Furthermore, the introduction of these restrictions in June 2006 does not mean that exposure levels to Citral became "relatively low" overnight. When it issued its restrictions on the use of Citral IFRA set timelines for the implementation of these new restrictions. According to these, fragrance manufacturers were to no longer deliver concentrated fragrance formulations to manufactures of cosmetics or household products that do not comply with these new restrictions any longer than 24 months later (i.e. after June 11, 2008).

In a recent submission to the European Commission[4] it was stated "*Based on exchange of data with Cosmetics Europe and manufacturers of finished cosmetic products it can be reasonably assumed that the time needed to reach the shelf in a store is about 12 to 18 months. This time would, for example, cover consumer-product testing for safety, stability, consumer acceptance and performance as well as industrial scale-up and placing on the market. An additional time period to consider is that of products remaining on the shelf when no longer compliant with the most recent version of the Standards. The shelf-life of products is variable but the minimum durability of the majority of cosmetic products may be as long as 36 months. How long a cosmetic product might remain in the hands of the final consumers cannot be assessed, despite recommendations on the product package on the life of the product after opening*". This would mean that it may have been as late as the start of 2013 before stocks of the previously unrestricted products were cleared from retail outlets. Consumers may also have taken a year or so before products they have purchased are used up or discarded.

Hence patients will have been exposed to consumer products containing unrestricted concentrations of Citral, well before and into 2013. Furthermore, their contact allergy to Citral may have been induced many years prior to the clinical patch test studies. With this in mind, it is noteworthy therefore 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.

It is also possible that patients presenting for diagnostic patch testing (although definitely shown to have a contact allergy to Citral) may have suffered dermatitis due to another allergen (as witnessed by the multiple positive patch test reactions experienced by some dermatitis patients indicating the presence of an allergy to Citral but not necessarily its causative role in the case of dermatitis). They may have acquired their allergy to Citral many years previous to their visit to the dermatologist's clinic without actually expressing the clinical signs of contact dermatitis due to Citral[5].

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[3] These Sensitization Assessment Factors that are used to reduce the No Expected Sensitization Induction Level (NESIL) to take account of inter-individual variability, matrix effects from the other constituents of the final product and use considerations (e.g. predisposition of certain regions of the skin) (see Api et al., 2008; Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. Regul Toxicol Pharmacol, 52:3-23).

[4] IDEA Supervisory Group (2016). IDEA project final report on the QRA2. Skin Sensitisation Quantitative Risk Assessment for Fragrance Ingredients, September 30, 2016. Submitted to the European Commission.

[5] Hostynek, J.J. and Maibach, H.I. (2004). Thresholds of elicitation depend on induction conditions. Could low level exposure induce sub-clinical allergic states that are only elicited under the severe conditions of clinical diagnosis? Food Chem. Toxicol., 42: 1859-1865.

It is not possible to come to a clear decision regarding the induction exposure levels of the patients in the studies showing a high frequency of reactions to Citral. This represents a major shortcoming of the clinical patch tests when using it for the classification decision as category 1A or 1B skin sensitizer.

The submitter finally concludes on a classification of Citral as a strong skin sensitizer in sub-category 1A based on high frequencies of skin sensitization observed in human patch and the high number of published cases combined with the estimated low exposure. This refers to section 3.4.2.2.2.1 of Regulation (EC) No. 1272/2008 which states: «Human evidence for sub-category 1A can include: (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».

However, as explained above it cannot be concluded that the current exposure situation would lead to same number of published cases of skin sensitization and if the estimated low exposure preceded the frequencies of skin sensitization observed in the patch tests. Since this causality cannot be demonstrated, the approach taken by the submitter is to be questioned.

Overall, the diagnostic patch tests, that showed sufficiently high frequencies of positive reactions in accordance with recent guidelines have failed to show "*good quality evidence*" that exposure levels were sufficiently low to justify classification in sub-category 1A in accordance with the criteria set by the European Chemicals Agency clarifying Regulation (EC no. 1272/2008) on the classification and labelling of hazardous substances[6].

Conclusion on classification and labelling for skin sensitisation

A high frequency of occurrence of skin sensitization is observed in human patch test studies on selected dermatitis patients, however a meta-analysis of the data on unselected dermatitis patients shows a low frequency of occurrence according to the classification criteria. This, combined with a strong potential for high estimated exposure both from a historical and current perspective provides the justification for a classification in sub-category 1B.

Various predictive tests on animals and several confirmatory tests on healthy volunteers (HRIPT and HMT) indicate that Citral has a moderate potential for sensitization. Animal studies are not uniform in their results, however as discussed a vast majority of the available animal data are consistent with an SS1B classification and this is supported by an overall weight of evidence approach. .

In as much as this regulation calls for the use of "*good quality evidence*" in a "*weight of evidence approach*", it can be concluded that Citral should be placed in sub-category 1B.

[6] Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0. https://echa.europa.eu/documents/10162/13562/clp_en.pdf

Appendix

Detailed comments on Table 10 of the CLH report:

- Heydorn et al. 2003: The study has been listed as patch test with selected patients. However, based on the information given in the publication, the common criteria for the selection of the 658 patients seems to be hand eczema the cause of which was not limited to scented products. The paper defines, that eligible participants were consecutive patients among the referrals in the 3 participating clinical centres, all of whom had eczema on the hands, confined by the wrist, but who were also allowed to have eczema elsewhere on the body. Therefore, we consider this study to be performed on consecutive (unselected) patients.
- Van Oosten et al. 2009: The study has been listed as patch test with consecutive (unselected) patients. However, based on the information given in the publication, the common criteria for the selection of the 320 patients refers to patients with eczema suspected of having a contact allergy to fragrances or cosmetics. The paper defines, that following criteria were used for patient selection: patients with eczema suspected of a contact allergy to fragrances or cosmetics and eczema localized on the face, neck, hands, axillae, genital area, or generalized eczema. Therefore, we consider this study to be performed on selected patients.
- An et al. 2005: The study has been listed as patch test with consecutive (unselected) patients. However, based on the information given in the publication, the common criteria for the selection of the 422 patients refers to patients with eczema suspected of having a contact allergy to fragrances or cosmetics. The paper defines, that patients with suspected contact allergy who visited the hospitals over the period April 2002 to June 2003 were patch-tested in 9 university hospitals in Korea. Therefore, we consider this study to be performed on selected patients.
- De Groot et al. 2002: This citation should be corrected to De Groot et al. 2000.
- Frosch et al. 2005a and 2005b: Only 1 citation has been provided in the chapter References. The citation given there does not contain data for application of Citral.