Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1], (2E)-3-phenylprop-2-enal [2]

EC Number: 203-213-9 [1], 604-377-8 [2]
CAS Number: 104-55-2 [1], 14371-10-9 [2]

CLH-O-0000006960-70-01/F

Adopted

18 March 2021

18 March 2021

CLH-O-0000006960-70-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1], (2E)-3-phenylprop-2-enal [2]**

EC Number: **203-213-9 [1], 604-377-8 [2]**

CAS Number: **104-55-2 [1], 14371-10-9 [2]**

The proposal was submitted by **Denmark** and received by RAC on **12 February 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Denmark has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **23 March 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 May 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Stine Husa**

Co-Rapporteur, appointed by RAC: **Christine Bjørge**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2021** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	No current Annex VI entry										
Dossier submitters proposal	TBD	cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1], (2E)-3-phenylprop-2-enal [2]	203-213-9 [1], 604-377-8 [2]	104-55-2 [1], 14371-10-9 [2]	Skin Sens. 1A	H317	GHS07 Wng	H317	EUH208	0.02%	
RAC opinion	TBD	cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1], (2E)-3-phenylprop-2-enal [2]	203-213-9 [1], 604-377-8 [2]	104-55-2 [1], 14371-10-9 [2]	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C ≥ 0.01 %	
Resulting Annex VI entry if agreed by COM	TBD	cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1], (2E)-3-phenylprop-2-enal [2]	203-213-9 [1], 604-377-8 [2]	104-55-2 [1], 14371-10-9 [2]	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C ≥ 0.01 %	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) provided a large set of studies including animal and human data and proposed to classify cinnamaldehyde as a skin sensitiser in category 1A (Skin Sens. 1A; H317). The classification was based on the following:

- Animal data: 22 LLNA (Local Lymph Node Assay), 2 LLNA:BrdU-ELISA test, 2 *ex vivo* LLNA:BrdU-ELISA and 3 Guinea Pig Maximisation Tests (GPMT).
- Human data: 46 diagnostic patch test studies, 2 Repeated Open Application Tests (ROAT), 14 Human Repeat Insult Patch Tests (HRIPT), 2 Human Maximisation Tests (HMT) and 3 case studies.

The General Concentration Limit (GCL) for Skin Sens. 1A substances is 0.1% w/v. The DS proposed to set a Specific Concentration Limit (SCL) based on the EC_3 values of 0.2 – 3.1% (w/v) observed in the LLNA studies, indicating a strong to extreme potency , which was also supported by the results of two out of three GPMT tests (100% positive response following a 0.2% intradermal induction dose).

Furthermore, cinnamaldehyde has been identified as a substance of special concern by the SCCS (Scientific Committee of Consumer Safety, 2012) based on the high number of reported human cases under normal exposure conditions. In addition, IFRA (International Fragrance Association) has calculated limits, based on the human and animal data, by which different exposures pose a risk of sensitisation. These limits range from 0.02%-0.4%, where 0.4% is for a product type with limited skin contact (e.g. mouth wash). The DS concluded that an SCL of 0.02% is warranted.

Comments received during consultation

Three Member State Competent Authorities (MSCA) commented during the consultation, all in support of the proposed classification (Skin Sens. 1A). One MSCA supported the proposed SCL of 0.02%, while the other two asked to clarify the basis for the proposed SCL.

One Industry Association disagreed with the proposed SCL but supported the proposed classification as Skin Sens. 1A. They were of the opinion that the GCL should be instead applied and pointed out that:

- the IFRA standards cannot be used to derive a SCL
- the two LLNA studies with EC3 values at the border for extreme potency are unpublished RIFM studies which should not be used for classification, leading to the position that only "strong potency" can be concluded based on the LLNA studies.
- human diagnostic patch test data cannot be used to establish the proposed SCL of 0.02%.

Assessment and comparison with the classification criteria

The sensitising properties of cinnamaldehyde have been intensively studied in animals as well as humans. It is suggested that the mechanism of action may involve the formation of Schiff bases with protein side-chains (Suskind and Majeti 1976).

As cinnamaldehyde showed clear sensitising effects in a range of experimental animal studies and in human patch tests, there is clear evidence that it is a skin sensitiser. RAC considers that the data available for cinnamaldehyde are sufficient for sub-categorisation as Skin Sens. 1A.

Human data

The following human studies with cinnamaldehyde have been assessed:

- 46 patch test studies
- 2 human ROATs (Human repeated open applications tests)
- 14 human HRIPTs (Human Repeat Insult Patch Tests)
- 2 human HMTs (Human Maximation Tests)
- 3 case studies

According to the Guidance on the application of the CLP criteria (CLP guidance), results from human studies can be used for sub-categorisation based on the relatively high, or low, frequency of occurrence of skin sensitisation according to the table below.

***Table**: Relatively high or low frequency of occurrence of skin sensitisation (CLP guidance, table 3.2)*

Human diagnostic patch test data	High frequency	Low/moderate frequency
General population studies	≥ 0.2%	< 0.2%
Dermatitis patients (unselected, consecutive)	≥ 1.0%	< 1%
Selected dermatitis patients (aimed testing)	≥ 2.0%	< 2%
Number of published cases	≥ 100 cases	< 100 cases

With regards to the patch test studies, positive patch test frequencies from the reported diagnostic patch tests are divided in selected and consecutive (unselected) dermatitis patients and range from 0.14% to 34%. The range for the selected dermatitis patients' positive reactions varies from 0.98% to 34% (27 studies), while for the consecutive (unselected) dermatitis patients the positive reactions range from 0.14% to 5.9% (19 studies). The total number of published cases is > 2300 from dermatological clinics in the EU and elsewhere. The test conditions for the diagnostic patch test were typically 1% cinnamaldehyde in petrolatum. Although the observed frequencies show some variations, the results confirm that positive reactions to cinnamaldehyde are commonly observed in dermatitis patients with relatively high frequencies observed in several tests, and the results of these studies can be considered as supportive of a classification for Skin Sens. 1A.

Patch testing with serial dilutions and ROAT are performed on sensitised individuals to assess the degree of sensitivity and safe limits of exposure (CLP guidance, Table 3.1). Two human ROAT were included in the CLH report. In the study by Johansen *et al.* (1996) the lowest threshold concentration (minimum effect level) were 0.02 % for the patch test and 0.1% for the ROAT when 22 cinnamaldehyde allergic patients were tested on the upper back and upper arm respectively with a dilution series of cinnamaldehyde. In the patch test 18/22 had at least 1 positive reaction to cinnamaldehyde and 4/22 had doubtful reactions. In the ROAT use test 8 patients reacted to 0.1% and 5 to 0.8% cinnamaldehyde in ethanol. None reacted to 0.02% cinnamaldehyde in ethanol. Further a total of 13/18 of the patients with a clearly positive patch test reaction to cinnamaldehyde (2% in petrolatum.) also developed a positive reaction in the ROAT test. The 4 patients with doubtful patch test responses to cinnamaldehyde (2% in petrolatum.) were all negative in the ROAT test. The study by Bruze *et al.* (2003) showed that the lowest patch test and ROAT concentrations that gave positive reactions were 0.002% and

0.01% respectively in the 17 cinnamaldehyde allergic patients exposed in the axilla to deodorants containing different concentrations of cinnamaldehyde. In the patch test all 17 patients had at least 1 positive reaction to cinnamaldehyde. In the ROAT test 8/8 patients in the first part of the study and 8/9 patients in the second part of the study gave positive reactions in the axilla when tested with cinnamaldehyde in deodorants. It was concluded in this study that deodorants containing cinnamaldehyde in the concentration range of 0.01–0.32% used 2 times daily on healthy skin can elicit axillary dermatitis within a few weeks. However, it is noted that patch testing with serial dilutions and ROAT are performed solely on sensitised individuals in order to estimate the elicitation threshold of an allergen. This is not a standardised protocol and only provides an indication on the degree on sensitivity (CLP guidance, table 3.1).

The HRIPT and HMT are performed on healthy volunteers in order to assess induction of sensitisation (CLP guidance, Table 3.1). 6 of 14 HRIPT studies showed induction of sensitisation at cinnamaldehyde concentrations between 1 and 3%. Different vehicles were used in these studies: EtOH (4 positive; 4 negative), DEP:EtOH with or without α-tocopherol (1 positive; 4 negative), alcohol SDA 39C (1 positive; 0 negative) and petrolatum (0 positive). Two HMT studies showed positive reactions after exposure to 2-3% cinnamaldehyde. The vehicles used in these studies were butylene glycol and petrolatum, respectively. Human evidence for sub-category 1A can include positive responses at $\leqslant$ 500 µg/cm2 (HRIPT, HMT – induction threshold) (CLP Annex I, 3.4.2.2.2.1)) and human evidence for sub-category 1B can include positive responses at > 500 µg/cm2 (HRIPT, HMT – induction threshold) (CLP Annex I, 3.4.2.2.2.2). Skin exposure is best expressed in dose per unit area, but concentration may be used as a surrogate indicator of exposure when dose per unit are not available. An induction concentration at or below 1% or above 1% would represent a sub-categorisation in category 1A and 1B respectively. It is noted that 3 out of the 6 positive HRIPT studies tested cinnamaldehyde concentrations of 1%. The DS did not have access to robust study summaries, therefore the HRIPT and HMT studies can only be seen as supporting evidence for skin sensitisation in sub-category 1A.

The DS also included 3 case studies. In the study by Guarneri (2010) a 33-year old baker with itching eczematous hand lesions was positively patch tested to Fragrance Mix I (FM I) and cinnamaldehyde. It is noted that standardised fragrance mixtures (FM I and FM II) contained in the European baseline series used for patch testing in dermatological clinics. FM I contains 1% cinnamaldehyde and a total of 8% fragrance allergens (SCCS 2012). The study by Decapite and Anderson (2004) described a 47-year-old man who routinely handled a powder, containing cinnamaldehyde, to mask the vinyl odour from covers used for car seat upholstery suffering from dermatitis of his hands, feet, face and body. He was patch tested positive to cinnamaldehyde and North American Contact Dermatitis Group standard series. In the study by Diba and Statham (2003) a 42-year old woman nurse with rash on her arms showed a positive reaction to fragrance mix containing cinnamaldehyde. A strong urticarial reaction was seen to cinnamaldehyde. After 40 min she developed widespread pruritus and erythema, and 5 min later, started to feel faint. It was concluded that she had immediate, as well as delayed, hypersensitivity to cinnamaldehyde and that this constituent of the fragrance mix was the most likely cause of the anaphylaxis.

Exposure considerations

The CLP Guidance table 3.3 and 3.4 enables the setting of an exposure index to support the assignment of the skin sensitising properties observed in human studies to the sub-categories for classification. An additive exposure index of 1-4 reflects low exposure, whereas 5-6 reflects high exposure.

***Table**: Relative high or low exposure*

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

***Table**: Sub-categorisation decision table*

	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

According to the International Fragrance Association (IFRA Standards amendment 49, 2020) levels of cinnamaldehyde concentrations in consumer products with various degree of skin contact range from 0.014 to 1.8%.

For cinnamaldehyde an additive exposure index of 4 can be calculated based on the following evaluation:

- Concentration/dose: score 0 based on low concentrations reported in products
- Repeated exposure: score 2 based on frequent occurrence in products with estimated daily use
- Number of exposures: score 2 based on anticipated exposures ≥100 times.

The score of 4 indicate a relatively low exposure. Together with a relatively high frequency of occurrence of skin sensitisation a classification as Skin Sens. 1A is supported by the human data.

Animal data

In the CLH report, a large volume of animal data was provided by the DS. These data included the results of 22 LLNA studies, 2 LLNA:BrdU-ELISA tests, 2 *ex vivo* LLNA:BrdU-ELISA and 3 GPMT. The animal studies reported represent guideline studies as well as others based on testing principles that are equivalent to current test guidelines for skin sensitisation. According to the CLP criteria, a classification for skin sensitisation in sub-category 1A or 1B can be based on the following results of a LLNA or GPMT:

	Animal data	
Sub-category 1A	LLNA	EC_3 value ≤2%
	GPMT	≥ 30% responding at ≤0.1% intradermal induction dose, or ≥ 60% responding at >0.1% to ≤1% intradermal induction dose
Sub-category 1B	LLNA	EC_3 value >2%
	GPMT	≥ 30% <60% responding at >0.1% to ≤1% intradermal induction dose, or ≥30% responding at >1% intradermal induction dose

Two out of three GPMTs showed sensitisation with intradermal induction concentrations of 0.2% cinnamaldehyde and a challenge concentration of 0.75%. In the study by Basketter and Scholes (1992) positive reactions were seen in 100% of the animals 24 and 48 hours after challenge, while the study by Basketter (1992) similarly showed positive reactions in 90% and 100% of the

animals at 24 and 48 hours after challenge, respectively. Both studies indicate a strong potency for cinnamaldehyde and support a classification in sub-category 1A. It is however noted that concentrations for intradermal induction lower than 0.2% have not been tested, and it cannot be concluded if cinnamaldehyde could be an extreme sensitiser based on these studies. The third study by Ishihara *et al.* (1986) is not clearly reported and cannot be used for classification.

All the 22 reported LLNA studies showed sensitising effects with a Stimulation Index ≥3. The reported EC_3 values range from 0.2% to 3.1% in these studies. In 20 of these studies the EC_3 values were below 2% which is within the strong potency group, one study reported an EC3 value above 2% (Basketter *et al.*, 2001) while in one study no EC_3 value was calculated (Basketter and Scholes, 1992). It is noted that in two of the LLNA studies the reported EC_3 value is 0.2% which is at the border for extreme potency (unpublished summary report by RIFM 2009, cited in SCCS, 2012).

The reported LLNA studies used a variety of vehicles, and the dermal absorption of cinnamaldehyde could vary accordingly. The two LLNA studies showing the lowest EC_3 values (EC_3=0.2%) used EtOH:DEP as vehicle. According to Betts *et al.* (2007) EtOH:DEP is a suitable alternative to AOO which is a preferred vehicle in the LLNA. In the studies included in the CLH report, EtOH:DEP (with or without α-tocopherol, Trolox C or antioxidant mix) was the most frequently used vehicle, and the 10 studies using this vehicle showed an EC_3 range from 0.2% to 1.4%. In comparison, AOO was used as a vehicle in 4 studies showing an EC_3 range from 0.57% to 3.1%. Other vehicles used in the studies include EtOH:Water (two studies, EC_3 1.2 and 1.6%), DMSO (EC_3 =0.9%), DMF (EC_3=0.5%), MEK (EC_3=1.1%) and PG (EC_3=1.4%). All the tested vehicles show EC_3 values below 2, which confirms the strong potency of cinnamaldehyde regardless of the vehicle used.

Overall, the criteria for the Skin Sens. 1A classification of cinnamaldehyde are fulfilled in several LLNA test and two GPMTs.

Conclusion by RAC

Cinnamaldehyde is a strong sensitiser. This was clearly shown in various sets of data from experimental animals, including several LLNA tests and two GPMTs, and is supported by human data. RAC is therefore of the opinion that a **classification as Skin Sens. 1A; H317 is justified according to the CLP criteria.**

Setting of a specific concentration limit

RAC considers that for cinnamaldehyde there are both animal and human data to support a concentration limit lower than the GCL (0.1%). As regards the animal studies, 2 out of 22 LLNA studies reported EC_3 values of 0.2% which is at the border of extreme potency. The EU expert group on skin sensitisation assessing the classification criteria for skin sensitising potency stated that when EC_3 values are available from several studies, then the lowest value should normally be used (Basketter *et al.*, 2005). It is however noted that the majority of the LLNA studies showed EC_3 values above the border for extreme potency. Less weight is given to the two LLNA studies indicating extreme potency, since only unpublished study-reports pointing to the extreme-potency outcome have been available to the DS. Further, two of the three GPMT-studies available show evidence of strong potency, however it is not possible to conclude on a possible extreme potency based on these studies. It is noted that in these two studies, the induction concentration of 0.2% (injection) showed positive reactions in 100% and 90%/100% of the tested animals.

Two human studies (Patch testing with serial dilutions and ROAT) indicate that a SCL lower than the GCL should be applied based on elicitation thresholds. In the study by Johansen *et al.* (1996) the lowest threshold concentration (minimum effect level) were 0.02% for the patch test and

0.1% for the ROAT. The study by Bruze *et al.* (2005) showed the lowest patch test and ROAT concentrations that gave positive reactions were 0.002% and 0.01% respectively.

Overall, based on the two human studies showing elicitation reaction at concentrations as low as 0.002% and supported by the LLNA studies with EC_3 values as low as 0.2% RAC is of the opinion that, in a weight of evidence assessment, **an SCL of 0.01% (being intermediate between 0.1% and 0.001% in terms of order of magnitude) is justified for cinnamaldehyde**.[1]

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

[1] *Note: because cinnamaldehyde is proposed to be classified as Skin Sens. 1A with an SCL at 0.01%, the supplemental label element EUH208 is obligatory on the packaging of mixtures not classified as skin sensitisers but containing cinnamaldehyde at a concentration ⩾0.001% (CLP Annex II, section 2.8), in order to protect already sensitised individuals.*