Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

**2,4-dimethylcyclohex-3-ene-1-carbaldehyde [1];
(1α,2α,5α)-2,5-dimethylcyclohex-3-ene-1-carbaldehyde [2]; 2,6-dimethylcyclohex-3-ene-1-carbaldehyde [3];
3,5-dimethylcyclohex-3-ene-1-carbaldehyde [4];
3,6-dimethylcyclohex-3-ene-1-carbaldehyde [5];
4,6-dimethylcyclohex-3-ene-1-carbaldehyde [6];
Reaction mass of 3,5-dimethylcyclohex-3-ene-1-carbaldehyde and 2,4-dimethylcyclohex-3-ene-1-carbaldehyde [7]; dimethylcyclohex-3-ene-1-carbaldehyde [8]; Dimethylcyclohex-3-ene-1-carbaldehyde [9];
1,2,4(or 1,3,5)-trimethylcyclohex-3-ene-1-carbaldehyde [10];
1,3,4-trimethylcyclohex-3-ene-1-carbaldehyde [11];
2,2,4-trimethylcyclohex-3-ene-1-carbaldehyde [12];
2,4,6-trimethylcyclohex-3-enecarbaldehyde [13];
isocyclocitral [14]; 3,5,6-trimethylcyclohex-3-ene-1-carbaldehyde [15];
4,6,6-trimethylcyclohex-3-ene-1-carbaldehyde [16];**

EC Number:

268-264-1 [1]; 252-395-6 [2]; - [3]; 268-263-6 [4]; 267-186-5 [5]; 253-139-6 [6]; - [7]; 248-742-6 [8]; 272-113-5 [9]; 276-055-1 [10]; - [11]; - [12]; 215-833-7 [13]; 215-638-7 [14]; 266-810-3 [15]; - [16]

CAS Number:

68039-49-6 [1]; 35145-02-9 [2]; 6975-94-6 [3]; 68039-48-5 [4]; 67801-65-4 [5]; 36635-35-5 [6]; - [7]; 27939-60-2 [8]; 68737-61-1 [9]; 71832-78-5 [10]; 40702-26-9 [11]; 1726-47-2 [12]; 1423-46-7 [13]; 1335-66-6 [14]; 67634-07-5 [15]; 6754-27-4 [16];

CLH-O-0000007204-81-01/F

Adopted
1 December 2022

1 December 2022

CLH-O-0000007204-81-01/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: 2,4-dimethylcyclohex-3-ene-1-carbaldehyde [1];
(1α,2α,5α)-2,5-dimethylcyclohex-3-ene-1-carbaldehyde [2];
2,6-dimethylcyclohex-3-ene-1-carbaldehyde [3];
3,5-dimethylcyclohex-3-ene-1-carbaldehyde [4];
3,6-dimethylcyclohex-3-ene-1-carbaldehyde [5];
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Reaction mass of 3,5-dimethylcyclohex-3-ene-1-carbaldehyde and 2,4-dimethylcyclohex-3-ene-1-carbaldehyde [7];
dimethylcyclohex-3-ene-1-carbaldehyde [8];
Dimethylcyclohex-3-ene-1-carbaldehyde [9];
1,2,4(or 1,3,5)-trimethylcyclohex-3-ene-1-carbaldehyde [10];
1,3,4-trimethylcyclohex-3-ene-1-carbaldehyde [11];
2,2,4-trimethylcyclohex-3-ene-1-carbaldehyde [12];
2,4,6-trimethylcyclohex-3-enecarbaldehyde [13];
isocyclocitral [14];
3,5,6-trimethylcyclohex-3-ene-1-carbaldehyde [15];
4,6,6-trimethylcyclohex-3-ene-1-carbaldehyde [16];

EC Number:-268-264-1 [1]; 252-395-6 [2]; - [3]; 268-263-6 [4]; 267-186-5 [5]; 253-139-6 [6]; - [7]; 248-742-6 [8]; 272-113-5 [9]; 276-055-1 [10]; - [11]; - [12]; 215-833-7 [13]; 215-638-7 [14]; 266-810-3 [15]; - [16]
CAS Number:-68039-49-6 [1]; 35145-02-9 [2]; 6975-94-6 [3]; 68039-48-5 [4]; 67801-65-4 [5]; 36635-35-5 [6]; - [7]; 27939-60-2 [8]; 68737-61-1 [9]; 71832-78-5 [10]; 40702-26-9 [11]; 1726-47-2 [12]; 1423-46-7 [13]; 1335-66-6 [14]; 67634-07-5 [15]; 6754-27-4 [16];

The proposal was submitted by **Germany** and received by RAC on **28 January 2022.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany 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 **28 February 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **29 April2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Veda Varnai**

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 **1 December 2022** 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	2,4-dimethylcyclohex-3-ene-1-carbaldehyde [1]; (1α,2α,5α)-2,5-dimethylcyclohex-3-ene-1-carbaldehyde [2]; 2,6-dimethylcyclohex-3-ene-1-carbaldehyde [3]; 3,5-dimethylcyclohex-3-ene-1-carbaldehyde [4]; 3,6-dimethylcyclohex-3-ene-1-carbaldehyde [5]; 4,6-dimethylcyclohex-3-ene-1-carbaldehyde [6]; Reaction mass of 3,5-dimethylcyclohex-3-ene-1-carbaldehyde and 2,4-dimethylcyclohex-3-ene-1-carbaldehyde [7]; dimethylcyclohex-3-ene-1-carbaldehyde [8]; Dimethylcyclohex-3-ene-1-carbaldehyde [9]; 1,2,4(or 1,3,5)-trimethylcyclohex-3-ene-1-carbaldehyde [10]; 1,3,4-trimethylcyclohex-3-ene-1-carbaldehyde [11]; 2,2,4-trimethylcyclohex-3-ene-1-carbaldehyde [12]; 2,4,6-trimethylcyclohex-3-enecarbaldehyde [13]; isocyclocitral [14]; 3,5,6-trimethylcyclohex-3-ene-1-carbaldehyde [15]; 4,6,6-trimethylcyclohex-3-ene-1-carbaldehyde [16];	268-264-1 [1]; 252-395-6 [2]; - [3]; 268-263-6 [4]; 267-186-5 [5]; 253-139-6 [6]; - [7]; 248-742-6 [8]; 272-113-5 [9]; 276-055-1 [10]; - [11]; - [12]; 215-833-7 [13]; 215-638-7 [14]; 266-810-3 [15]; - [16]	68039-49-6 [1]; 35145-02-9 [2]; 6975-94-6 [3]; 68039-48-5 [4]; 67801-65-4 [5]; 36635-35-5 [6]; - [7]; 27939-60-2 [8]; 68737-61-1 [9]; 71832-78-5 [10]; 40702-26-9 [11]; 1726-47-2 [12]; 1423-46-7 [13]; 1335-66-6 [14]; 67634-07-5 [15]; 6754-27-4 [16];	Skin Sens. 1B	H317	GHS07 Wng	H317			

RAC opinion	TBD	2,4-dimethylcyclohex-3-ene-1-carbaldehyde [1]; (1α,2α,5α)-2,5-dimethylcyclohex-3-ene-1-carbaldehyde [2]; 2,6-dimethylcyclohex-3-ene-1-carbaldehyde [3]; 3,5-dimethylcyclohex-3-ene-1-carbaldehyde [4]; 3,6-dimethylcyclohex-3-ene-1-carbaldehyde [5]; 4,6-dimethylcyclohex-3-ene-1-carbaldehyde [6]; Reaction mass of 3,5-dimethylcyclohex-3-ene-1-carbaldehyde and 2,4-dimethylcyclohex-3-ene-1-carbaldehyde [7]; dimethylcyclohex-3-ene-1-carbaldehyde [8]; Dimethylcyclohex-3-ene-1-carbaldehyde [9]; 1,2,4(or 1,3,5)-trimethylcyclohex-3-ene-1-carbaldehyde [10]; 1,3,4-trimethylcyclohex-3-ene-1-carbaldehyde [11]; 2,2,4-trimethylcyclohex-3-ene-1-carbaldehyde [12]; 2,4,6-trimethylcyclohex-3-enecarbaldehyde [13]; isocyclocitral [14]; 3,5,6-trimethylcyclohex-3-ene-1-carbaldehyde [15]; 4,6,6-trimethylcyclohex-3-ene-1-carbaldehyde [16];	268-264-1 [1]; 252-395-6 [2]; - [3]; 268-263-6 [4]; 267-186-5 [5]; 253-139-6 [6]; - [7]; 248-742-6 [8]; 272-113-5 [9]; 276-055-1 [10]; - [11]; - [12]; 215-833-7 [13]; 215-638-7 [14]; 266-810-3 [15]; - [16]	68039-49-6 [1]; 35145-02-9 [2]; 6975-94-6 [3]; 68039-48-5 [4]; 67801-65-4 [5]; 36635-35-5 [6]; - [7]; 27939-60-2 [8]; 68737-61-1 [9]; 71832-78-5 [10]; 40702-26-9 [11]; 1726-47-2 [12]; 1423-46-7 [13]; 1335-66-6 [14]; 67634-07-5 [15]; 6754-27-4 [16];	Skin Sens. 1	H317	GHS07 Wng	H317			
Resulting Annex VI entry if agreed by COM	TBD	2,4-dimethylcyclohex-3-ene-1-carbaldehyde [1]; (1α,2α,5α)-2,5-dimethylcyclohex-3-ene-1-carbaldehyde [2]; 2,6-dimethylcyclohex-3-ene-1-carbaldehyde [3]; 3,5-dimethylcyclohex-3-ene-1-carbaldehyde [4]; 3,6-dimethylcyclohex-3-ene-1-carbaldehyde [5]; 4,6-dimethylcyclohex-3-ene-1-carbaldehyde [6];	268-264-1 [1]; 252-395-6 [2]; - [3]; 268-263-6 [4]; 267-186-5 [5]; 253-139-6 [6]; - [7]; 248-742-6 [8]; 272-113-5 [9]; 276-055-1 [10]; - [11]; - [12]; 215-833-7 [13];	68039-49-6 [1]; 35145-02-9 [2]; 6975-94-6 [3]; 68039-48-5 [4]; 67801-65-4 [5]; 36635-35-5 [6]; - [7]; 27939-60-2 [8]; 68737-61-1 [9]; 71832-78-5 [10]; 40702-26-9 [11]; 1726-47-2 [12]; 1423-46-7 [13];	Skin Sens. 1	H317	GHS07 Wng	H317			

		Reaction mass of 3,5-dimethylcyclohex-3-ene-1-carbaldehyde and 2,4-dimethylcyclohex-3-ene-1-carbaldehyde [7]; dimethylcyclohex-3-ene-1-carbaldehyde [8]; Dimethylcyclohex-3-ene-1-carbaldehyde [9]; 1,2,4(or 1,3,5)-trimethylcyclohex-3-ene-1-carbaldehyde [10]; 1,3,4-trimethylcyclohex-3-ene-1-carbaldehyde [11]; 2,2,4-trimethylcyclohex-3-ene-1-carbaldehyde [12]; 2,4,6-trimethylcyclohex-3-enecarbaldehyde [13]; isocyclocitral [14]; 3,5,6-trimethylcyclohex-3-ene-1-carbaldehyde [15]; 4,6,6-trimethylcyclohex-3-ene-1-carbaldehyde [16];	215-638-7 [14]; 266-810-3 [15]; - [16]	1335-66-6 [14]; 67634-07-5 [15]; 6754-27-4 [16];							

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

During the Dossier Submitter's manual screening, 48 cyclohex-3-ene-1-carbaldehyde congeners were selected in an initial grouping step. For these substances, a concern for skin sensitisation was identified during the screening and this endpoint was proposed for harmonised classification and labelling. The individual structures, physicochemical properties, and available *in vivo*, *in chemico*/*in vitro*, and human data were taken into consideration to create sub-groups of these congeners. Based on the data analysis, 16 cyclohex-3-ene-1-carbaldehydes containing hydrogen or methyl groups as substituents, with the total number of methyl substituents of either two or three, were selected to be included in a proposal for harmonised classification and labelling for skin sensitisation.

Most of these substances are used as fragrance compounds in similar or the same products/product categories (e.g. cleaning and furnishing care products, laundry and dishwashing products, personal care products, air care products, biocidal products). For some substances of the group, no data on consumer uses are available and there is no information on whether they are on the market. However, also those substances were included in the CLH proposal, e.g. to avoid regrettable substitution.

Data were obtained from:

- the public ECHA dissemination site for the pre-registered substances of the group;
- the REACH lead dossiers of the registered group members;
- literature screening in bibliographic databases (including TOXNET, Web of Science, Embase, PubMed, NTP, ScienceDirect, Wiley Online Library, and Scopus) using as search strings the individual CAS and EC numbers of the group members as well as the name of the structural backbone.

Skin sensitisation is the only hazard class being assessed by the Dossier Submitter in their report, while the data on skin corrosion/irritation are presented for information only. Respiratory sensitisation could not be addressed due to lack of data (namely, one cyclohex-3-ene-1-carbaldehyde congener, EC No. 215-833-7, is self-classified as Resp Sens. 1, but data in support of this classification were found neither in the registration dossier, nor in the published literature).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Skin corrosion/irritation - for information only

In vivo animal data (according to, or similar to OECD TG 404, GLP, non-GLP and pre-GLP, for substances EC No, 268-264-1, 215-833-7, and 215-638-7, and for the 7th congener on the Dossier Submitter's list) and one *in vitro* study (Reconstructed human epidermis EpiDerm Skin Model (EPI-200) for the substance EC No. 248-742-6) showed that the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes produce similar effects and act as skin irritants or are weakly corrosive. Limited human data (for substances EC No. 215-638-7 and 267-186-5; studies cited in Kligman, 1972; RIFM, 1982) did not show skin irritation. However, the Dossier Submitter noted that the tested concentrations were low (2% and 4%).

Skin sensitisation

Animal data

Reliable animal data (LLNA for EC No. 248-742-6 and 215-638-7; GPMT for EC No. 268-264-1) provide strong evidence that the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes cause skin sensitisation *in vivo*.

Based on two reliable LLNA studies (GLP-compliant and in line with OECD TG 429) with congeners EC No. 248-742-6 and 215-638-7, in which EC3>2% was observed (ECHA, 2017c), the Dossier Submitter proposes sub-categorisation in Skin Sens. Cat. 1B. The Dossier Submitter considers that this conclusion is supported by a reliable GPMT test (GLP-compliant and in line with OECD TG 406) with congener EC No. 268-264-1, in which positive response was observed in ≥30% of animals at >1.0% intradermal induction, indicating a skin sensitiser with moderate potency. The Dossier Submitter notes that in this study a strong sensitising potency of the substance EC No. 268-264-1 cannot be excluded, since concentrations for intradermal injection ≤1% were not tested. Nevertheless, the Dossier Submitter is of the opinion that based on the high structural similarity and similar physicochemical properties compared to other di- and tri-methylated congeners, it is expected that EC No. 268-264-1 acts as a moderate skin sensitiser.

Human data

Congener EC No. 272-113-5 elicited skin sensitisation in selected dermatitis patients (diagnostic patch test), but the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes did not induce skin sensitisation in human predictive patch test (HPPT) studies (three human repeated insult patch tests (HRIPT), and four human maximisation tests (HMT)). However, the Dossier Submitter considers that the diagnostic patch test data have a higher weight than the negative results from human predictive patch tests (HMT/HRIPT) since the tested concentrations in HPPTs were relatively low and it cannot be excluded that these substances induce skin sensitisation in humans at higher concentrations.

In chemico and in vitro data

In the registration data of substance EC No. 215-833-7, a reliable *in chemico* direct peptide reactivity assay (DPRA according to OECD TG 442C, GLP-compliant) is described. The DPRA resulted in a positive test result and according to the prediction model, the test substance was assigned in a moderate reactivity class. However, keratinocytes were not activated *in vitro* by the same congener (EC No. 215-833-7), shown in a GLP-compliant test performed according to OECD TG 442D (ARE-Nrf2 Luciferase KeratinoSens Test Method). Using the Integrated Testing Strategies version 1 (ITSv1) and 2 (ITSv2), according to OECD TG 497, the congener EC No. 215-833-7 was identified as a skin sensitiser, but the prediction for the potency of the substance was not possible.

In silico data

All 16 group members are aldehydes that are able to form Schiff bases with amino groups to form potentially allergenic protein-hapten complexes by covalent bonding to proteins. To identify *in silico* alerts for skin sensitisation, the OECD QSAR Toolbox v. 4.3 and v. 4.5 (for EC No. 215-833-7), Derek Nexus v. 6.0.1 and v. 6.1.1 (for EC No. 215-833-7), and the Danish (Q)SAR database were used. For all group members an alert for skin sensitisation was predicted by the OECD QSAR Toolbox v. 4.3 and Derek Nexus v. 6.0.1, but not by the Danish (Q)SAR database.

The Dossier Submitter points out that the profilers used do not represent fully valid (Q)SAR predictions on their own. Rather, they should be seen as indicators of similar hazardous potential within a group/category, which later require verification *in vitro* or *in vivo*.

Grouping and read-across

The Dossier Submitter applied the read-across in line with ECHA's Read Across Assessment Framework (RAAF) (ECHA, 2017d). They chose a category approach (read-across between several substances that have structural similarity; ECHA, 2017d) in order to perform a qualitative read-across (ECHA, 2008), according to Scenario 6 described in ECHA Guidance (ECHA, 2017d) (the read-across hypothesis is based on different compounds with qualitatively similar properties). Details and evaluation of applied approach, including its limitations, are given in the section "Assessment and comparison with the classification criteria".

The **Dossier Submitter concludes** that the skin sensitisation potential of the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes was established in animal studies *in vivo* and a positive *in chemico* direct peptide reactivity assay. (Q)SAR analysis, which also provides a mechanistic explanation (covalent binding of the aldehydes to proteins via Schiff base formation), as well as sensitisation observed in dermatitis patients subjected to diagnostic patch testing, support experimental data.

The Dossier Submitter proposes to classify the di- and trimethylated cyclohex-3-ene-1-carbaldehydes as skin sensitisers with sub-categorisation as **Skin Sens. 1B (H317 - May cause an allergic skin reaction)** and the **GCL of 1% (w/v)**, **based on animal data**. Negative data from HMT/HRIPT support a moderate potency of these congeners[1].

The Dossier Submitter notes that this conclusion is supported by the opinion of the Scientific Committee on Consumer Safety (SCCS), which categorised the congeners EC No. 268-264-1 and 272-113-5 as possible sensitisers, based on structure-activity relationships (SAR) assessment, and isocyclocitral (EC No. 215-638-7) as established contact allergen, based on animal data (other congeners were not considered in this opinion (SCCS, 2011)).

Comments received during consultation

One comment from a MSCA was received in the consultation and it was in support of the Dossier Submitter's proposal. It was asked whether the concentrations used in the sensitisation assays are correctly chosen according to existing guidelines (especially regarding skin irritation). The Dossier Submitter confirmed that available information supports a correct choice of test substances concentration.

Assessment and comparison with the classification criteria

Skin corrosion/irritation - for information only

In vivo animal and *in vitro* data for skin irritation/corrosion for five tested congeners all showed positive results. Limited human data did not show skin irritation, however, the tested concentrations were low (2% and 4%). RAC, therefore, agrees with the Dossier Submitter's conclusion that the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes act as skin irritants or are weakly corrosive.

[1] Negative results of HPPTs at a dose per skin area >500 µg/cm^2 do not allow for classification as skin sensitiser with sub-categorisation as Skin Sens. 1A.

RAC notes that skin corrosion/irritation was tested in animals only using undiluted substance, so based exclusively on these data it is difficult to know if irritation could affect the sensitisation studies. Nevertheless, in the LLNA studies, concentrations of 5% and 10% of the congener EC No. 248-742-6 resulted in SI-values of 2.9 and 4.2 (Anonymous 1, 2012), and EC3 of 7.3% was calculated for congener EC No. 215-638-7 (Anonymous 8, 2006). It is unlikely that significant skin irritation occurred at these low concentrations of congeners. In the GPMT study (for congener EC No. 268-264-1; Anonymous 7, 1998), the highest concentration for intradermal application that causes mild to moderate skin irritation (5%), and the highest non-irritant concentration for topical application were identified in the range-finding study and applied in the main test. Positive diagnostic patch data in humans were performed with 5% concentration of congener EC No. 272-113-5 (Larsen et al., 2001). It is stated in the study that this concentration was "sub-irritant". Additionally, above mentioned human data, although of low reliability (studies were cited from secondary literature and main study information is not available), do not indicate skin irritation of the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes in humans at such a low concentration. In conclusion, although animal data indicate irritative property of the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes, irritation is not expected to play a role in described skin sensitisation studies in animals or in human diagnostic patch test.

Skin sensitisation

Animal data

RAC agrees with the Dossier Submitter that animal data clearly indicate the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes as skin sensitisers.

The results of four LLNA tests and two GPMT tests are available (Table 2) for 5/16 congeners. Out of these, two LLNA studies (for congeners EC No. 248-742-6 and 215-638-7) and one GPMT test (for congener EC No. 268-264-1) are considered adequately reliable (Klimisch score 2: reliable with restriction) for the assessment of skin sensitising potential of the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes.
The reliability of other three animal studies was assessed as Klimisch score 4 (not assignable).

Animal studies with Klimisch score 2:

1) LLNA for congener EC No. **248-742-6** (Anonymous 1, 2012)

It is a GLP study conducted according to OECD TG 429, provided in the registration dossier for the substance EC No. 248-742-6. The test substance was applied to five mice per dose group at concentrations of 1, 2.5, 5, 10, and 25%. Adequate response to a positive control (α-hexyl cinnamaldehyde; historical data used) was reported.

Exposure of animals to EC No. 248-742-6 resulted in SI-values of 2.1, 2, 2.9, 4.2, and 2.7, respectively. Although, according to the authors, an EC3 value could not be calculated since a normal dose range curve was not achieved, concentrations of 5% and 10% of the test substance resulted in SI-values of 2.9 and 4.2, respectively, compared to vehicle control. RAC agrees with the Dossier Submitter that these data support a moderate skin sensitising potency of the substance EC No. 248-742-6.

2) LLNA for congener LLNA EC No. **215-638-7** (Anonymous 8, 2006)

It is a GLP study conducted according to OECD TG 429, provided in the registration dossier for the substance EC No. 215-638-7 (isocyclocitral). The test substance was applied to four mice per dose group at concentrations of 0.5, 1, 2.5, 5, and 10% test substance in EtOH/DEP (1:3). Adequate response to a positive control (HCA in acetone/olive oil) was reported.

Exposure of animals to isocyclocitral resulted in SI-values of 0.8, 1.1, 1.7, 1.8, and 4.4, respectively, with EC3 of 7.3%. RAC agrees with the Dossier Submitter that these data support a moderate skin sensitising potency of the substance EC No. 215-638-7 (isocyclocitral).

3) GPMT for congener EC No. **268-264-1** (Anonymous 7, 1998)

It is a GLP study conducted according to OECD TG 406, provided in the registration dossier for substance No. 7 (no identifier). Based on a range-finding study (to identify the highest concentration for intradermal application that causes mild to moderate skin irritation, and the highest non-irritant concentration for topical application), 5% concentration of EC No. 268-264-1 was applied for intradermal induction and 25% for topical induction.

At a challenge concentration of 50%, 20/20 and 11/20 animals were sensitised at the 24h and 48h readings, respectively. A challenge concentration of 25% resulted in 20/20 and 12/20 positive reactions at 24h and 48h, respectively. RAC agrees with the Dossier Submitter that these results are in line with skin sensitisation of a moderate potency (≥30% responding at >1% intradermal induction dose in a GPMT for sub-category 1B, according to the 2nd ATP of the CLP Regulation) but since concentrations of the substance ≤1% for intradermal induction were not tested, strong sensitising potency cannot be excluded for this congener.

Animal studies with Klimisch score 4:

- In the chemical safety report of substance EC No. 248-742-6, LLNA study with **congener No. 7** (no identifier, registration under REACH) is described, reporting an **EC3 value of 3.3%**. However, no reference was given and further study information are not available.
- In ICCVAM (2011), LLNA with substance **EC No. 215-638-7** is quoted, which showed an **EC3 value of 7.4%**. However, important study information is missing.
- In RIFM (1978), a GPMT conducted with the substance **EC No. 267-186-5** is reported. Intradermal induction at 5% and challenge at 1% **did not result in sensitisation** reactions. However, higher concentrations were not tested. Main experimental details of the study are not available.

Since not enough information on GLP compliance and methodology (e.g. regarding animal species and strain, group size, substance purity) is available, these studies were not further considered in the assessment, both by the Dossier Submitter and the RAC.

Regarding sub-categorisation, reliable LLNA results indicate sub-category **Skin Sens. 1B**, since the EC3 values were well above 2%. Namely, according to Table 3.4.4 in the ECHA CLP Guidance (ECHA, 2017c), sub-category 1A is justified if EC3 value is ≤2% in a LLNA, and sub-category 1B is justified if EC3 value is >2%.

A decision on sub-categorisation cannot be based on GPMT studies, since concentrations of the substance ≤1% for intradermal induction were not tested, and strong sensitising potency cannot be excluded (according to the ECHA CLP Guidance, sub-category 1A is justified if ≥30% animals are responding at ≤0.1% intradermal induction dose or ≥60% animals are responding at >0.1% to ≤1% intradermal induction dose).

<u>Human data</u>

RAC agrees with the Dossier Submitter that human data indicate sensitising potential of the di- and trimethylated cyclohex-3-ene-1-carbaldehydes.

Human data for the di- and trimethylated cyclohex-3-ene-1-carbaldehydes include one diagnostic patch test data and seven human predictive patch tests (three human repeated insult patch tests (HRIPT) and four human maximisation tests (HMT)).

Out of these, five are considered reliable (Klimisch score 2): diagnostic patch test with EC No. 272-113-5; HMT with EC No. 267-186-5, EC No. 272-113-5, and EC No. 215-638-7; and HRIPT with EC No. 248-742-6. The reliability of other three human studies was assessed as Klimisch score 4 (not assignable). It should be noted, however, that all HRIPTs and HMTs followed non-guideline protocols and for the most of them, original reports were not available.

Human studies with Klimisch score 2:

1) Diagnostic patch test with **EC No. 272-113-5**

One human patch test study performed on the di- and trimethylated cyclohex-3-ene-1-carbaldehydes is available in the literature (Larsen et al., 2001). It was performed with congener EC No. 272-113-5 (dimethylcyclohex-3-ene-1-carbaldehyde; dimethyltetrahydrobenz- aldehyde) at **5% in petrolatum**, in 178 selected dermatitis patients previously sensitised to fragrance materials. The test concentration was found to be sub-irritant in 20 control subjects (subjects without clinical evidence of fragrance allergy). Frequency of skin sensitisation in 8 centres that participated in the study is shown in Table 1.

***Table 1**. Human patch test study with EC No. 272-113-5 (Larsen et al., 2001)*

Center	Number of patients	Positive reaction to EC No. 272-113-5	Percent sensitised
England	25	0	0%
Ireland	24	0	0%
Japan	19	0	0%
Sweden	28	1	3.6%
Switzerland	26	1	3.8%
USA Oregon	24	0	0%
USA Pennsylvania	7	0	0%
USA Virginia	25	2	8.0%
TOTAL:	**178**	**4**	**2.3%**
Average (range) percent sensitisation per center:			**1.9 (0% – 8.0%)**

In total 4/178 subjects had a **positive reaction to the test substance** (2.3%), with an average sensitisation rate per center of 1.9 (0-8%).

The limitations include that:

- the number of subjects tested per centre is low;
- data on previous exposure (repeated exposure, number of exposures) to the test substance are lacking; and
- just one diagnostic patch study in humans is available for all congeners.

RAC agrees with the Dossier Submitter that the diagnostic patch data show skin sensitisation property of EC No. 272-113-5, and, by the grouping approach, indicate skin sensitisation property for the di- and trimethylated cyclohex-3-ene-1-carbaldehydes. However, sub-categorisation of skin sensitisation is not possible based on these data due to the above listed limitations.

2) Human predictive patch tests

- HMT with **EC No. 267-186-5** (RIFM, 1982)

The test (according to Kligman and Epstein, 1975) was carried out on 29 healthy male and female volunteers, with congener EC No. 267-186-5 at **2% in pet**. (corresponding to 1500 µg/cm^2). Patch sites were pre-treated (for initial patch only) with 7.5% aqueous sodium lauryl sulphate (SLS) for 24h under occlusion. Test material was applied under occlusion to the same site on forearms of all subjects, for five alternate-day 48 h periods. After a 10-14-day rest period, challenge under occlusion was applied to naive sites for 48 h, with (on the left side) and without (on the right side) pre-treatment with 7.5% aqueous SLS under occlusion. A fifth site challenged with petrolatum served as control.
No sensitisation reaction was observed.

- HMT with **EC No. 272-113-5** (Kligman, 1977)

Congener EC No. 272-113-5 was applied at **4% in pet**. (according to Kligman and Epstein, 1977) to 25 healthy male and female volunteers, using a concentration of 4% in pet. (calculated dose per skin area: 3000 µg/cm^2).
No sensitisation reaction was observed.

- HMT with **EC No. 215-638-7** (Kligman, 1972)

The test (according to Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers, with congener EC No. 215-638-7 (isocyclocitral) at **4% in pet**. (calculated dose per skin area: 2880 µg/cm^2).
No sensitisation reaction was observed.

- HRIPT with **EC No. 248-742-6** (Anonymous 3, 2001)

The test substance at **1% concentration** (vehicle not reported and there is no data to calculate dose per skin area) was performed in 106 volunteers. Nine induction doses under occlusion were applied for 24h at the back of each subject, every Monday, Wednesday and Friday. Challenge dose was applied after 2 weeks at unpatched test site for 24h. At 48h-reading, in one subject mild response (level 1) was observed, and at 72h-reading, the response decreased to barely perceptible (+). No response was found after further 24h.

The authors concluded that there was **no evidence for sensitisation** due to the "transient nature" of the response.

Human studies with Klimisch score 4:

- HMT with **EC No. 215-638-7** (isocyclocitral) is cited in ICCVAM (2011), in which dose per skin area of 2759 µg/cm^2 (vehicle not specified, and other information on this study are not available) **did not induce sensitisation reaction**.
- In the Chemical Safety Report, HRIPT with **congener No. 7** is briefly described. **No sensitisation reaction** was observed with the reaction mass of 3,5-dimethylcyclohex-3-ene-1-carbaldehyde (EC: 268-263-6) and 2,4-dimethylcyclohex-3-ene-1-carbaldehyde (EC: 268-264-1) applied at **5% concentration** (vehicle not specified; no data to calculate dose per skin area). Further information are not available for this study.
- In RIFM reports (2004, 2005), HRIPT is reported with **EC No. 215-638-7** (isocyclocitral) applied at **6%** in 0.5% tocopherol in DEP:EtOH (3:1) (dose per skin area: 7087 µg/cm^2). **No sensitisation reaction** was observed. However, number of subjects tested is not available.

RAC considers that based on the weight-of-evidence (WoE) approach, human data indicate sensitising potential of the di- and trimethylated cyclohex-3-ene-1-carbaldehydes. Diagnostic patch test with congener EC No. 272-113-5 revealed skin sensitisation in 2.3% (4/178) of dermatitis patients previously sensitised to fragrance materials. Positive reactions were found in 3/8 testing centres included in the study (sensitisation rate in these three centres ranged from 3.6% to 8.0%) (Larsen et al., 2001).

Human predictive patch tests (HMT and HRIPT) are non-clinical studies, with only historical relevance, but may be used as the weight of evidence for the sub-categorisation (ECHA, 2017c). RAC is of the opinion that negative human predictive patch tests do not negate a positive result of the human diagnostic patch test mentioned above. It is argued that negative human predictive patch tests results do not mean that sensitisation will not occur in the exposed population, since the assay does not have the power to predict effects in the population, especially if the sensitisation rate is not high (i.e. not above 1%)[2] (Basketter, 2009). According to the ECHA CLP Guidance (ECHA, 2017c), it is considered that the frequency of contact allergy in dermatitis patients is approximately 5 times higher than in the general population. This would imply sensitisation rate of approximately 0.5% in case of di- and tri-methylated cyclohex-3-ene-1-carbaldehydes. Also, in reliable human predictive patch tests (scored as Klimisch 2), negative results were observed at concentrations below the one used in the human diagnostic patch test (1%, 2% and 4% *vs.* 5%, respectively). Therefore, as pointed out by the Dossier Submitter, it cannot be excluded that the congeners tested in human predictive patch tests would not induce skin sensitisation at higher concentrations.

Regarding sub-classification, the diagnostic patch test in selected dermatitis patients (Larsen et al., 2001) showed a relatively high incidence of skin sensitisation (2.3%; according to the ECHA CLP Guidance, the frequency of occurrence of skin sensitisation is considered high if it is ≥2.0%) (ECHA, 2017c). Nevertheless, information on exposure characteristics, with an exception of substance concentration (5%), are not available, so the exposure index (Table 3.3 in ECHA CLP Guidance) cannot be calculated. A sub-categorisation, therefore, cannot be proposed based on this study.

To the RAC opinion, human predictive patch tests, which showed negative results with low exposure [score 3 according to Table 3.3 in the ECHA CLP Guidance: concentration/dose ≥1.0% and ≥500 µg/cm^2 (score 2); repeated exposure <once/daily (score 1); number of exposures <100 exposures (score 0)] and have limitations mentioned above, cannot assist in the decision on sub-categorisation.

RAC concludes that presented human data support **Skin Sens. 1 classification**, and do not justify sub-categorisation.

<u>In chemico and in vitro data</u>

GLP-compliant and reliable (reliability score 1, reliable without restriction) *in chemico* and *in vitro* tests are described for congener **EC No. 215-833-7**, for which *in vivo* or human data are not available:

- *In chemico* Direct Peptide Reactivity Assay (DPRA)[3] , assessing the Key Event 1 (KE1) of the skin sensitisation Adverse Outcome Pathway (AOP) was conducted according to OECD TG 442C. Cysteine peptide depletion was ≥22.62% and ≤42.47%, indicating a **positive test result** and a moderate reactivity class (Anonymous 4, 2015a).

[2] Taking into account that these studies are usually performed with up to 100 healthy volunteers.

[3] DPRA addresses the molecular initiating event of the skin sensitisation AOP by assessing protein reactivity. The test quantifies the reactivity of test chemicals towards synthetic model peptides containing either lysine or cysteine. It quantifies the remaining concentration of cysteine- or lysine-containing peptide following 24 hours incubation with the test chemical. Cysteine and lysine peptide percent depletion values are then calculated and used in a prediction model which allows assigning the test chemical to one of four reactivity classes used to support the discrimination between sensitisers and non-sensitisers (OECD, 2022).

- The ARE-Nrf2 Luciferase KeratinoSens Test Method (which assesses the Key Event 2 (KE2) of the skin sensitisation AOP)[4], was performed according to OECD TG 442D, with a **negative result**: congener EC No. 215-833-7 did not activate keratinocytes *in vitro* (Anonymous 5, 2015b).

RAC notes that a possible reason for these contradictory results from two assays could be a limitation of the KeratinoSens test. Because of the limited metabolic capability of the cell line used and because of the experimental conditions, pro-haptens and pre-haptens may show negative results in this test (OECD, 2022). The SCCS performed SAR analyses on the substances EC No. 268-264-1 and 272-113-5, showing that both act as possible pre-hapten skin sensitisers (SCCS, 2011). Although this type of SAR analysis was not performed for the congener EC No. 215-833-7, there is a possibility that this congener also acts as a pre-hapten.

According to the OECD Guideline Document (GD) 497 (OECD, 2021), Defined Approaches (DA), based on *in chemico* (key event 1 (KE) 1 of the AOP), *in vitro* (KE2/KE3), and *in silico* prediction data may be used to identify the skin sensitisation hazard of test substances and to provide potency sub-categorisation following the Globally Harmonised System for Classification and Labelling.

The Dossier Submitter constructed two versions of the Integrated Testing Strategy (ITSv1 and ITSv2), in order to predict skin sensitisation hazard potential. They used *in silico* predictions (Derek Nexus v.6.1.1 in ITSv1 and OECD (Q)SAR toolbox version 4.5 in ITSv2, with the predictions within the applicability domain), and the DPRA for the substance EC No. 215-833-7. The mean cysteine and lysine depletion of ≥22.62% and <42.47% resulted in score 2, and positive *in silico* predictions resulted in score 1, with a sum score of 3 (Table 3.1 in OECD, 2021). This combined score resulted in a conclusive positive prediction for hazard identification but in an inconclusive prediction for the potency of the test substance (Figure 3.1 in OECD, 2021).

RAC notes that this approach is in line with the OECD TG 497. Although both versions of ITS normally use scores assigned to the quantitative results from the DPRA (KE1) and the h-CLAT (KE3) and from either Derek Nexus v6.1.0 or OECD QSAR TB v4.5, partial information sources (e.g. one *in chemico*/*in vitro* outcome and an *in silico* prediction) may be used to obtain a DA prediction. RAC agrees with the Dossier Submitter that the ITS approach identified congener EC No. 215-833-7 as a skin sensitiser (**Skin Sens. Cat 1**), but that the data do not allow sub-categorisation.

<u>In silico data</u>

For all group members an alert for skin sensitisation was predicted by the OECD QSAR Toolbox (v. 4.3 or v. 4.5) and Derek Nexus (v. 6.0.1 or v. 6.1.1), but not by the Danish (Q)SAR database. The predictions were within the applicability domain.

The following alerts were identified with OECD QSAR Toolbox: Sensitisation - Protein binding potency Lys (DPRA 13%), Protein binding by OECD, Protein binding by OASIS, Protein binding potency Cys (DPRA 13%), Protein binding potency GSH, Protein Binding Potency h-CLAT, Protein binding alerts for skin sensitization according to GHS, Protein binding alerts for skin sensitization by OASIS, Keratinocyte gene expression

Derek Nexus identified an alert "Skin sensitisation mammal".

[4] The assay addresses the second key event of the skin sensitisation AOP - activation of keratinocytes. Small electrophilic substances such as skin sensitisers are able to induce genes that are regulated by the antioxidant response element (ARE). In this assay activation of ARE dependent genes is assessed with the help of luciferase (OECD, 2022).

RAC agrees with the Dossier Submitter that these predictions support the conclusion on skin sensitising property of the evaluated di- and trimethylated cyclohex-3-ene-1-carbaldehydes, within the WoE approach.

Grouping and read-across

RAC agrees with the Dossier Submitter's approach to grouping and read-across. RAC recognises the limitations but considers that they do not significantly affect the grouping approach.

Group members show similar structural pattern and similar physicochemical properties and are expected to cause the same type of effects, namely skin sensitisation. All common and specific assessment elements for Scenario 6 of ECHA's read across assessment framework (ECHA, 2017d) are addressed by the Dossier Submitter and the RAC.

Substance characterisation: The chemical identity (EC/List No. and CAS No., where available), individual structures, and physicochemical properties are described in the CLH Report.

The limitations are:

- The structures for EC No. 272-113-5 and EC No. 215-638-7 (isocyclocitral) are incompletely defined.
- For two substances, most of the physicochemical properties were not available; however, experimental data and human clinical patch test data were available, showing skin sensitisation with a moderate potency (based on animal data).
- Information on tested congener's purity was not always available, even for the studies with Klimisch score 2 (Table 10 in the CLH Report). For *in vivo* studies for which information was available, the purity was 98.1% (LLNA with EC No. 215-638-7; Anonymous 8, 2006) and 99.8% (GPMT with EC No. EC No. 268-264-1; Anonymous 7, 1998). In *in chemico* (direct peptide binding assay) and in *in vitro* (ARE-Nrf2 Luciferase Test Method) studies with EC No. 215-833-7, the purity was 95.7%.
- Regarding impurities profile, there are no information on other compounds available that may be present as impurities and may influence the applicability of the prediction

Structural similarity and differences within the category: Grouping of the 16 substances is based on the same chemical backbone – cyclohex-3-ene-1-carbaldehyde (Figure 1). The members of the group differ in their substituents on various positions of the cyclohexene ring. Membership in the group was restricted to substances with two or three methyl groups as substituents at various positions at the cyclohexene ring, and no further substituents (except hydrogen). RAC considers that the structural similarities among all category members are sufficiently identified and that the structural differences allowed within the category are described.

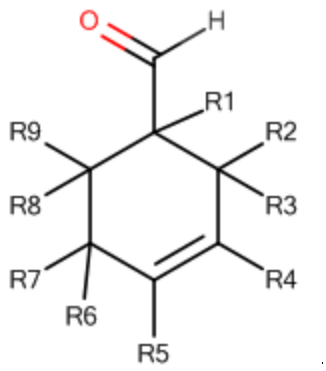

Figure 1*. Definition of the group in terms of chemical structure (copied from the CLH report); R1-R9 = H or methyl, either two or three methyl substituents are present.*

RAC notes that for the two congeners EC No. 272-113-5 and EC No. 215-638-7 the structure could not be explicitly identified. This is a limitation. Nevertheless, it is known that these congeners contain the same chemical backbone (cyclohex-3-ene-1-carbaldehyde) as other group members, and either two (EC No. 272-113-5) or three (EC No. 215-638-7) methyl substituents.

Link of structural similarities and structural differences with the proposed regular pattern of effects: RAC considers that all category members are covered by the category hypothesis: the group members are aldehydes (their structures differ regarding the position of hydrogen or methyl substituents on the cyclohexene ring), which are able to form Schiff bases with amino groups and to form a potentially allergenic protein-hapten complex by covalent bonding to proteins. This is the Molecular Initiating Event (MIE), i.e. the first step in the respective Adverse Outcome Pathway for skin sensitisation.

The results do not indicate that the number of methyl groups and pattern of substitution on the cyclohexene ring affects the sensitisation property or potency of evaluated congeners:

- Reliable positive *in vivo* data (LLNA and GPMT) were available for three group members including congeners with two (EC No. 248-742-6 and 268-264-1) and three methyl substituents (EC No. 215-638-7) at various positions at the cyclohexene ring.
- The reliable *in chemico* assay showed positive result for congener EC No. 215-833-7 with three methyl substituents.
- The reliable human diagnostic patch test showed sensitising potential for congener EC No. 272-113-5, which possess two methyl substituents.
- An alert for skin sensitisation was predicted by the OECD QSAR Toolbox and Derek Nexus for all group members.

These positive results indicate either a moderate skin sensitisation potency (LLNAs) or no sub-categorisation due to study limitations (GPMT, human data, *in chemico*/*in vitro* and ITS data).

Negative results also do not follow a specific pattern regarding the number and/or position of substituents (Table 2).

Consistency of effects in the data matrix: As described above, all type of data (*in vivo*, human, *in chemico*, *in vitro*, *in silico*) do not indicate any specific pattern of skin sensitisation property regarding the number and/or position of substituents. Table 2 presents the data matrix prepared by the Dossier Submitter.

Table 2*. Summary table of available in vivo, in silico, in chemico/in vitro and human data on skin sensitisation for the di- and trimethylated cyclohex-3-ene-1-carbaldehydes (copied from the CLH Report)*

List No.	**1**	**5**	**7**		**8**		**9**	**13**	**14**	
EC No.	268-264-1	267-186-5	-		248-742-6		272-113-5	215-833-7	215-638-7	
CAS No.	68039-49-6	67801-65-4	**-**		27939-60-2		68737-61-1	1423-46-7	1335-66-6	
R1							X			
R2	X		X					X	X	
R3		X		X	X		X			X
R4	X		X			X	X	X	X	
R5				X						X
R6		X			X	X		X	X	X
LLNA			Positive (EC3: 3.3%)		**Positive 5% < EC3 < 10%**				**Positive** EC3: 7.3% **(EC3: 7.4%)**	
GPMT	**100% positive** at 5% intradermal induction and 25%	(Negative at 5% intradermal induction and 1% challenge)								

	or 50% challenge						
HDPT					**2.3% positive**		
HRIPT			Negative (at 5%)	**Negative at 1%**			(Negative at 7087 µg/cm^2)
HMT		**Negative at 2%** (corr. to 1500 µg/cm^2)			**Negative at 4%** (corr. to 3000 µg/cm^2)		**Negative at 4%** (corr. to 2880 µg/cm^2) (Negative at 2759 µg/cm^2)
In vitro/in chemico						**Positive DPRA, Negative Keratino Sens**	
In silico	Alert Skin Sens	Alert Skin Sens	Alert Skin Sens	Alert Skin Sens	Alert Skin Sens	Alert Skin Sens	Alert Skin Sens

Grey background, bold characters – studies with reliability 1 or 2; white background, in brackets – studies with reliability 4; blank – no data

All congeners have a relatively low molecular weight (138.2 to 153.2 g/mol), show log K_{OW} values between 2.7 and 3.3, and vapour pressures of ≤95.7 Pa. Data on skin corrosion/irritation for the di- and tri-methylated cyclohex-3-ene-1-carbaldehydes show that substances produce similar effects and act as skin irritants or are mildly corrosive.

Reliability and adequacy of the source studies: Reliable (Klimisch score 1 or 2) data, either *in vivo*, *in chemico*/*in vitro* or human, are available for 6/16 congeners. *In silico* predictions were within the applicability domain for all group members.

Compounds the test organism is exposed to: A limitation is that for the proposed group there is no information on (bio)transformation products. However, as previously mentioned, in its opinion on fragrance allergens in cosmetic products (SCCS, 2011), the SCCS performed SAR analyses on the substances EC No. 268-264-1 and 272-113-5, showing that both act as possible pre-haptens. SAR analyses on other evaluated congeners were not applied.

Common underlying mechanism, a qualitative aspect: All group members are aldehydes, sharing the same MIE, i.e. they are able to form Schiff bases with amino groups of skin proteins and to form potentially allergenic protein-hapten complexes by covalent bonding to proteins. A QSAR analysis conformed this mechanistic explanation.

Common underlying mechanism, quantitative aspects: The available data do not indicate major differences in skin sensitising potency among congeners. As mentioned previously, positive results from animal and human data indicate either a moderate skin sensitisation potency or no sub-categorisation due to study limitations.

Exposure to other compounds than to those linked to the prediction: As already noted, for the studied congeners there are no information on other compounds that may be present as impurities and may influence the applicability of the prediction.

Occurrence of other effects than those covered by the hypothesis and justification: RAC agrees with the Dossier Submitter that regarding skin sensitisation, there is no additional mechanism expected other than those identified in the hypothesis. This conclusion is supported by the (Q)SAR predictions.

limitation of the applicability of the prediction: RAC considers that the Dossier Submitter sufficiently justified the reasons why other cyclohex-3-ene-1-carbaldehydes were not included in the group. Namely, group members only included substances with hydrogen or methyl groups as substituents, with the total number of methyl substituents of either two or three. Non- or mono-methylated congeners, as well as longer chained alkyl substituents (C3H7, C6H15), were excluded from the group due to lack of data on skin sensitisation and due to the differences in physicochemical properties (Log K_{OW}, vapour pressure) of these congeners compared to the di- and tri-methylated congeners (Table 15 and Figure 2 in the CLH Report).

Conclusions and comparison with the classification criteria

Out of six congeners for which reliable (Klimisch score 1 or 2) data are available *in vivo*, *in chemico*/*in vitro* or human experimental evidence for skin sensitisation property was found for five congeners in:

- two LLNAs, with congeners EC No. 248-742-6 (Anonymous 1, 2012) and EC No. 215-638-7 (Anonymous 8, 2006);
- one GPMT, with congener EC No. 268-264-1 (Anonymous 7, 1998);
- one human diagnostic patch test study (with congener EC No. 272-113-5) (Larsen et al. 2001); and
- one *in chemico* study (DPRA), with congener EC No. 215-833-7 (Anonymous 4, 2015a).

Also, for all group members an alert for skin sensitisation was predicted by the OECD QSAR Toolbox and Derek Nexus.

Negative results in reliable studies were also observed, and they were found in one *in vitro* study (KeratinoSens) and in several human predictive patch tests. The reason for a negative KeratinoSens study is unclear. It could be hypothesised that if this congener acts as a pre-hapten, as it was shown by SAR analysis for other two congeners, KeratinoSens assay could be inadequately sensitive to detect its skin sensitising property. For the same congener (EC No. 215-833-7) positive *in chemico* study is available and ITS approach indicates this congener as a skin sensitiser. RAC is of the opinion that negative human predictive patch tests (HMT and HRIPT with congeners EC No. 267-186-5, EC No. 272-113-5, EC No. 215-638-7, and EC No. 248-742-6) do not negate a positive result of the human diagnostic patch test (with congener EC No. 272-113-5). It is argued that a negative human predictive patch tests result does not mean that sensitisation will not occur in the exposed population, since the assay does not have the power to predict effects in the population (Basketter, 2009). Further, negative results in human predictive patch tests were observed at concentrations below the one used in the human diagnostic patch test, and it cannot be excluded that the congeners tested in human predictive patch tests would not induce skin sensitisation at higher concentrations. In relation to clearly positive results in animal studies, "negative human data should not normally be used to negate positive results from animal studies" according to the ECHA CLP Guidance (ECHA, 2017c).

To summarise, skin sensitisation property for five different congeners was observed in all three reliable animal studies, one diagnostic patch test in humans, in one *in chemico* test evaluating KE1 of the skin sensitisation AOP, and in *in silico* predictions.

Since RAC considers that the grouping approach and read-across for the di- and trimethylated cyclohex-3-ene-1-carbaldehydes are justified and taking into account limitations related to the studies with negative results, RAC concludes, based on the WoE approach, that there is enough evidence to conclude that the 16 evaluated **di- and trimethylated cyclohex-3-ene-1-carbaldehydes are skin sensitisers**.

Regarding sub-categorisation, two LLNAs with two different congeners indicate evidence for Skin Sens. Cat. 1B classification, and there are no data in the available database which would clearly

indicate strong skin sensitising potency (Cat. 1A) of the di- and trimethylated cyclohex-3-ene-1-carbaldehydes (Table 3):

- GPMT showed a high rate of skin sensitisation (up to 100%), but concentrations below 5% for intradermal induction dose were not tested.
- Human diagnostic patch test in selected dermatitis patients showed a relatively high incidence of skin sensitisation (2.3%; according to the ECHA CLP Guidance, the frequency of occurrence of skin sensitisation is considered high if it is ≥2.0%) (ECHA, 2017c). Nevertheless, information on exposure characteristics, with an exception of substance concentration (5%), are not available, so the exposure index (Table 3.3 in ECHA CLP Guidance) cannot be calculated.
- DPRA and *in silico* predictions by DEREK Nexus and OECD (Q)SAR toolbox (ITSv 1 and 2) were inconclusive for sub-categorisation.

Nevertheless, contrary to the Dossier Submitter's proposal for Category 1B, RAC considers that the di- and trimethylated cyclohex-3-ene-1-carbaldehydes should be classified as **Skin Sens. Cat. 1 (H317 - May cause an allergic skin reaction)**, with the GCL of 1% (w/v), since, due to possible variability in potency across the group category, 1A cannot be completely ruled out.

***Table 3**. Comparison of human, animal and in silico/in chemico data for skin sensitisation of the di- and trimethylated cyclohex-3-ene-1-carbaldehydes with CLP criteria (copied from the CLH Report)*

Reference(s)	Criteria acc. to CLP regulation, as laid out in (ECHA, 2017)	Results	Resulting Classification
Animal data			
LLNA (OECD TG 429) EC No. 248-742-6 (Anonymous 1, 2012)	<u>Skin Sens. 1A:</u> 0.2% < EC3 ≤ 2%, Strong sensitiser EC3 ⩽ 0.2%, Extreme sensitiser <u>Skin Sens. 1B:</u> EC3 > 2%, Moderate sensitiser	5% < EC3 <10%	**Skin Sens. 1B** **Moderate potency**
LLNA (OECD TG 429) EC No. 215-638-7 (Anonymous 8, 2006)		EC3: 7.3%	**Skin Sens. 1B** **Moderate potency**
GPMT (OECD TG 406) EC No. 268-264-1 (Anonymous 7, 1998)	<u>Skin Sens. 1A - Extreme potency:</u> ≥ 60% sensitised guinea pigs at ≤ 0.1% intradermal induction <u>Skin Sens. 1A - Strong potency:</u> ≥ 30 - < 60% guinea pigs sensitised at ≤ 0.1% intradermal induction or ≥ 60% guinea pigs sensitised at > 0.1 - ≤ 1.0% intradermal induction <u>Skin Sens. 1B - Moderate potency:</u> ≥ 30 - < 60% guinea pigs sensitised at > 0.1 - ≤ 1.0% intradermal induction or ≥ 30% guinea pigs sensitised at > 1.0% intradermal induction	100% (55 and 60%) responded at 5% intradermal induction	**Skin Sens 1** Strong potency cannot be excluded

Reference(s)	Criteria acc. to CLP regulation, as laid out in (ECHA, 2017)	Results	Resulting Classification
Human data			
Human dermatological patch test Selected dermatitis patients EC No. 272-113-5 (Larsen et al., 2001)	Skin Sens. 1 Relatively low/moderate frequency (< 2.0%) and relatively low exposure or Relatively high frequency (≥ 2.0%) and relatively high exposure Skin Sens. 1A Relatively high frequency (≥ 2.0%) and relatively low exposure Skin Sens. 1B Relatively low/moderate frequency (< 2.0%) and relatively high exposure	4/178 subjects had a positive reaction to the test substance (2.3%); average sensitisation rate per centre: 1.9 (0-8%) Exposure unclear Based on HMT: dose ≥ 500 µg/cm² (score 2); however relative exposure could not be calculated (data on repeated exposure and number of exposures unclear)	**Skin Sens. 1** (not suitable for sub-categorisation due to limitations: low number of subjects tested per centre; unclear previous exposure; only one diagnostic patch study in humans available for all congeners)
Human predictive patch test MHT EC No. 267-186-5 (RIFM, 1982)	Skin Sens. 1 Induction threshold from HRIPT or HMT ≤ 500 or > 500 µg/cm² Skin Sens. 1A Induction threshold ≤ 500 µg/cm² Skin Sens. 1B Induction threshold > 500 µg/cm²	Negative at 1500 µg/cm²	No classification Skin Sens cannot be excluded
Human predictive patch test HMT EC No. 272-113-5 (Kligman, 1977)		Negative at 3000 µg/cm²	
Human predictive patch test HMT EC No. 215-638-7 (Kligman, 1972)		Negative at 2880 µg/cm²	
Integrated Testing Strategy (*in chemico* and *in silico* data)			
DPRA & *in silico* prediction by DEREK Nexus and OECD (Q)SAR toolbox ((OECD, 2021), ITSv 1 and 2) EC No. 215-833-7 (Anonymous 4, 2015a)	Skin Sens 1 Combined score 3-4 Conclusive for hazard, inconclusive for potency Skin Sens 1B Combined score 2 Inconclusive Combined score 0-1	Combined score 3	**Skin Sens. 1** (inconclusive for sub-categorisation)

Additional references

Basketter DA. The human repeated insult patch test in the 21st century: a commentary. Cutan Ocul Toxicol. 2009;28(2):49-53

ECHA (2008): Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.6: QSARs and grouping of chemicals, May 2018, European Chemical Agency

ECHA (2017d): Read-Across Assessment Framework (RAAF), March 2017, European Chemical Agency

OECD (2022): OECD Test Guideline No. 442C - In Chemico Skin Sensitisation Assays addressing the Adverse Outcome Pathway key event on covalent binding to Proteins. 2022-06-30

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).