CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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];

Index Number: **-**

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Version number: 2 **Date: January 2022**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

During a manual screening of the Dossier Submitter in 2019, 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 were selected to be included in a proposal for harmonised classification and labelling for skin sensitisation. For detailed information on the selection of the sub-group see section Grouping and read-across, see section 10.7.8.

This group of 16 substances comprises substances with the chemical core structure given in **Figure 1** below. The members of the group differ in their substituents on various positions of the cyclohexene ring. Group members only included substances with hydrogen or methyl groups as substituents, while the total number of methyl substituents is either two or three. Public substance names (IUPAC Name) and substance type in terms of composition (mono- or multi-constituent substances or UVCB) are included in Table 1.

In silico predictions revealed an alert for skin sensitisation for all 16 group members. The profilers included in the OECD (Q)SAR toolbox (v. 4.3) also provide a mechanistic explanation. Since the group members are aldehydes, they are able to form Schiff bases with amino groups and, therefore, to form potentially allergenic protein-hapten complexes 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[1].

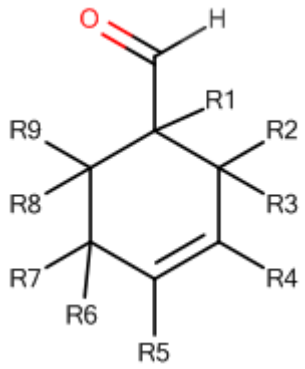

Figure 1: Definition of the group in terms of chemical structure;
R1-R9 = H or methyl, either two or three methyl substituents are present

[1]http://www.oecd.org/env/the-adverse-outcome-pathway-for-skin-sensitisation-initiated-by-covalent-binding-to-proteins-9789264221444-en.htm

Table 1: Cyclohex-3-ene-1-carbaldehyde congeners, position of methyl substituents (P1 – P6), public substance name (IUPAC Name), substance type and composition

List No.	EC No.	CAS No.	Public Substance Name; IUPAC Name	P1	P2	P3	P4	P5	P6	Substance type (mono-, multi-constituent, UVCB)	Constituent of group member(s)	Contains group member(s)
Two methyl substituents at various positions												
1	268-264-1	68039-49-6	2,4-dimethylcyclohex-3-ene-1-carbaldehyde; 2,4-dimethylcyclohex-3-enecarbaldehyde		**X**		**X**			multi		
2	252-395-6	35145-02-9	**(1α,2α,5α)-2,5-dimethylcyclohex-3-ene-1-carbaldehyde; Reaction mass of (1R,2S,5S)-2,5-dimethylcyclohex-3-ene-1-carbaldehyde and (1S,2R,5R)-2,5-dimethylcyclohex-3-ene-1-carbaldehyde**		**X**			**X**		mono	EC No. 248-742-6, 272-113-5	
3	-	6975-94-6	2,6-dimethylcyclohex-3-enecarbaldehyde		**X**				**X**	multi		
4	268-263-6	68039-48-5	3,5-dimethylcyclohex-3-ene-1-carbaldehyde; 3,5-dimethylcyclohex-3-enecarbaldehyde			**X**		**X**		multi		
5	267-186-5	67801-65-4	3,6-dimethylcyclohex-3-ene-1-carbaldehyde; 3,6-dimethylcyclohex-3-enecarbaldehyde			**X**			**X**	multi		
6	253-139-6	36635-35-5	4,6-dimethylcyclohex-3-ene-1-carbaldehyde; 4,6-dimethylcyclohex-3-enecarbaldehyde				**X**		**X**	multi	EC No. 248-742-6, 272-113-5	
7	-	-	Reaction mass of 3,5-dimethylcyclohex-3-ene-1-carbaldehyde and 2,4-di-methylcyclohex-3-ene-1-carbaldehyde;		**X**		**X**			multi		EC No. 268-263-6, 268-264-1
			reaction mass of 3,5-dimethylcyclohex-3-enecarbaldehyde and 2,4-dimethylcyclohex-3-enecarbaldehyde			**X**		**X**				
8	248-742-6	27939-60-2	Dimethylcyclohex-3-ene-1-carbaldehyde; Reaction mass of rel-(1R,6R)-4,6-dimethylcyclohex-3-			**X**			**X**	multi		
			ene-1-carbaldehyde and rel-(1R,6R)-3,6-dimethylcyclohex-3-ene-1-carbaldehyde				**X**		**X**			
9	272-113-5	68737-61-1	Dimethylcyclohex-3-ene-1-carbaldehyde	Incompletely defined						multi		
three methyl substituents at various positions												
10	276-055-1	71832-78-5	1,2,4 (or 1,3,5)-	**X**	**X**		**X**			multi		

List No.	EC No.	CAS No.	Public Substance Name; IUPAC Name	P1	P2	P3	P4	P5	P6	Substance type (mono-, multi-constituent, UVCB)	Constituent of group member(s)	Contains group member(s)
			trimethylcyclohex-3-ene-1-carbaldehyde									
11	-	40702-26-9	1,3,4-trimethylcyclohex-3-enecarbaldehyde	**X**		**X**	**X**			multi		
12	-	1726-47-2	pseudocyclocitral; 2,2,4-trimethylcyclohex-3-enecarbaldehyde		**X X**		**X**			mono		
13	215-833-7	1423-46-7	2,4,6-trimethylcyclohex-3-enecarbaldehyde		**X**		**X**		**X**	multi	EC No. 215-638-7	
14	215-638-7	1335-66-6	isocyclocitral	Incompletely defined						multi		
15	266-810-3	67634-07-5	3,5,6-trimethylcyclohex-3-ene-1-carbaldehyde; 3,5,6-trimethylcyclohex-3-enecarbaldehyde			**X**		**X**	**X**	mono		
16	-	6754-27-4	4,6,6-trimethylcyclohex-3-enecarbaldehyde				**X**		**X X**	multi		

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 2: Proposed harmonised classification and labelling for the 16 di- and tri-methylated cyclohex-3-ene-1-carbaldehydes

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					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 existing entry in Annex VI of CLP									
Dossier submitter's proposal	tba	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]	[1] 268-264-1 [2] 252-395-6 [3] - [4] 268-263-6 [5] 267-186-5 [6] 253-139-6 [7] - [8] 248-742-6 [9] 272-113-5 [10] 276-055-1 [11] - [12] - [13] 215-833-7 [14] 215-638-7 [15] 266-810-3 [16] -	[1] 68039-49-6 [2] 35145-02-9 [3] 6975-94-6 [4] 68039-48-5 [5] 67801-65-4 [6] 36635-35-5 [7] - [8] 27939-60-2 [9] 68737-61-1 [10] 71832-78-5 [11] 40702-26-9 [12] 1726-47-2 [13] 1423-46-7 [14] 1335-66-6 [15] 67634-07-5 [16] 6754-27-4	Skin Sens. 1B	H317	GHS07 Wng	H317		

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

Table 3: Reason for not proposing harmonised classification and status under consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Among the 16 di- and trimethylated cyclohex-3-ene-1-carbaldehydes addressed in this dossier, the substances with EC No. 215-833-7, 248-742-6, and substance No. 7 (no identifiers) are registered under REACH. Furthermore, the group includes a high number of substances pre-registered under REACH (Table). Currently, none of the congeners has a harmonised classification and labelling in Annex VI to the CLP regulation.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The Dossier Submitter (DS) proposes a harmonised classification of all 16 cyclohex-3-ene-1-carbaldehydes listed in Table 2 **Error! Reference source not found.** as skin sensitisers with moderate potency (Skin Sens. 1B). For some congeners, self-classifications for skin sensitisation (Skin Sens. 1 or 1B) have been notified. However, contradicting entries in self-classification from different notifiers in the C&L Inventory and/or the registration dossiers for the same substances were noted. Furthermore, the DS disagrees with some notifiers and/or registrants, who did not self-classify the cyclohex-3-ene-1-carbaldehydes as skin sensitisers. Due to the high structural similarity among the 16 cyclohex-3-ene-1-carbaldehydes, it is expected that all group members act as skin sensitisers with moderate potency (see section 10.7.8).

Harmonised classification as Skin Sens. 1B would ensure adequate perception of the skin sensitisation hazard associated with these substances, and ensure an adequate level of protection, *inter alia* by setting the concentration limit for the classification of mixtures containing the above cyclohex-3-ene-1-carbaldehydes to 1 %.

Table 4 Self-classification of cyclohex-3-ene-1-carbaldehydes. For 12 members of the 16 substituted cyclohex-3-ene-1-carbaldehydes in the group, data were available from the ECHA dissemination site, including information on the registration status (registered, pre-registered, or none of both), and self-classification from the C&L notifications (the data are shown for the notified hazard class relative to the number of all notifiers; last accessed 2nd December 2021).

No	EC No.	CAS No.	R1	R2	R3	R4	R5	R6	Registration under REACH	Acute Tox. 4	Skin Irrit. 2 (H315)	Skin Sens. 1/ 1B (H317)	Eye Irrit. 2 (H319)	Resp. Sens. 1 (H334)	STOT SE 3 (H335)	Not classified
1	268-264-1	68039-49-6		X		X			Pre	1/2358	2351/ 2358	2351/ 2358	959/2358	-	1/2358 (inhalation respiratory tract)	4/2358
2	252-395-6	35145-02-9		X			X		Pre	-	-	-	-	-	-	-
3	-	6975-94-6		X				X	-	-	-	-	-	-	-	-
4	268-263-6	68039-48-5			X		X		Pre	18/151	151/151	151/151	133/151	-	-	-
5	267-186-5	67801-65-4			X			X	Pre	1576/ 1579	1576/ 1579	1352/ 1579	212/ 1579	-	-	-
6	253-139-6	36635-35-5				X		X	Pre	-	-	-	-	-	-	-
7	-	-		X		X			X	-	11/11	11/11	-	-	-	-
					X		X									
8	248-742-6	27939-60-2			X			X	X	22/405	404/405	404/405	384/405	-	-	1/405
						X		X								
9	272-113-5	68737-61-1	Incompletely defined						Pre	-	348/348	348/348	320/348	-	-	-
10	276-055-1	71832-78-5	X	X		X			Pre	-	233/233	-	233/233	-	-	-
11	-	40702-26-9	X		X	X			-	-	-	-	-	-	-	-
12	-	1726-47-2		X X		X			-	-	-	-	-	-	-	-
13	215-833-7	1423-46-7		X		X		X	X	-	28/170	132/170	132/170	38/170	-	-
14	215-638-7	1335-66-6		X		X		X	Pre	-	1327/ 1709	1706/ 1709	1703/ 1709	-	-	3/1709
						X	X	X								
15	266-810-3	67634-07-5			X		X	X	Pre	-	-	113/113	113/113	-	-	-
16	-	6754-27-4				X		X X	-	-	-	-	-	-	-	-

5 IDENTIFIED USES

Available data for most of the substances show uses as fragrance compounds in similar or the same products/product categories.

The substance EC No. 215-638-7 is used as a fragrance in the following products: Cleaning and furnishing care products, laundry and dishwashing products, personal care products, and air care products (and plastic and rubber products not covered elsewhere) (EPA, 2020). Furthermore, it is used as food additive (ICCVAM, 2011). There are indications that substance EC No. 231-452-9 is used as fragrance as well (US EPA, 2021).

Information on consumer uses for EC No. 215-833-7, 248-742-6, and substance No. 7 (no identifier is available from the ECHA dissemination site) was taken from the ECHA dissemination site (as of 14 December 2021). Registrants have registered uses in the following products: Air care products, biocides (e.g. disinfectants, pest control products), perfumes and fragrances, polishes and waxes, washing and cleaning products, and cosmetics and personal care products. However, none of these substances are registered as active substances in biocides (Regulation (EU) No. 528/2012). Release to the environment of these substances is likely to occur from indoor and outdoor (not for substance No. 7) use as processing aid.

Furthermore, widespread uses by professional workers are reported on the ECHA dissemination site for the substances EC No. 215-833-7, 248-742-6, and No. 7, no identifier. They are used by professionals in washing and cleaning products, polishes and waxes, and cosmetics and personal care products (EC No. 215-833-7 only).

The substance EC No. 268-264-1 was identified as one of the most important fragrance compounds in terms of volumes used (SCCS, 2011).

Finally, the substance EC No. 272-113-5 is supposed to be used as fragrance ingredient (Larsen et al., 2001).

According to the Scientific Committee on Consumer Safety, the substances EC No. 268-264-1 and 272-113-5 are possible sensitisers, based on a structure-activity relationship assessment, while isocyclocitral (EC No. 215-638-7) was categorised as an established contact allergen, based on animal data (SCCS, 2011). Other group members were not investigated in this opinion.

In Table , the identified consumer uses for the group members are summarised. Sources of information were registrations, but also external databases (Mintel Global New Product Database for Europe, SPIN, IFRA, public available SDS, CosIng, literature databases).

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, substances were included in the CLH proposal, e.g. to avoid regrettable substitution.

Table 5: Use categories of uses for group members (only consumer uses are listed)

List No.	EC No.	CAS No.	Use category description						
			PC 3: Air care products	PC 28: Perfumes, fragrances	PC 31: Polishes and wax blends	PC 35: Washing and cleaning products	PC 39: Cosmetics, personal care products	PC 8: Biocidal products (e.g. disinfectants, pest control)	Pet products
1	268-264-1	68039-49-6	x	x		x	x	x	x
2	252-395-6	35145-02-9							
3	-	6975-94-6							
4	268-263-6	68039-48-5		x			x		
5	267-186-5	67801-65-4	x	x			x	x	
6	253-139-6	36635-35-5							
7	-	-		x	x	x	x		
8	248-742-6	27939-60-2							
9	272-113-5	68737-61-1	x	x		x	x		
10	276-055-1	71832-78-5							
11	-	40702-26-9							
12	-	1726-47-2							
13	215-833-7	1423-46-7	x	x	x	x	x	x	
14	215-638-7	1335-66-6	x	x		x	x		
15	266-810-3	67634-07-5					x		
16	-	6754-27-4							

6 DATA SOURCES

Data were received from the public ECHA dissemination site for the pre-registered substances of the group, and the REACH lead dossiers of the registered group members. Furthermore, results from a literature screening in bibliographic databases were considered, including TOXNET, Web of Science, Embase, PubMed, NTP, ScienceDirect, Wiley Online Library, and Scopus[2] using as search strings the individual CAS and EC numbers of the group members as well as the name of the structural backbone (in the various spellings allowed according to the IUPAC nomenclature, e.g. 3-cyclohexene-1-carbaldehyde, cyclohex-3-ene-1-carbaldehyde, 3-cyclohexene-1-carboxaldehyde etc.). 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) (https://qsartoolbox.org), Derek Nexus v. 6.0.1 and v. 6.1.1 (for EC No. 215-833-7) (https://www.lhasalimited.org/products/derek-nexus.htm), and the Danish (Q)SAR database (http://qsar.food.dtu.dk/) were used.

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[2] https://toxnet.nlm.nih.gov/; https://apps.webofknowledge.com; https://www.embase.com; https://www.ncbi.nlm.nih.gov/pubmed/; https://ntp.niehs.nih.gov; https://www.sciencedirect.com/; https://onlinelibrary.wiley.com/; https://www.scopus.com

7 PHYSICOCHEMICAL PROPERTIES

Table 6 Physicochemical properties of di- and tri-methylated cyclohex-3-ene-1-carbaldehydes

List No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**		**8**	
EC No.	268-264-1	252-395-6	-	268-263-6	267-186-5	253-139-6	-		248-742-6	
CAS No.	68039-49-6	35145-02-9	6975-94-6	68039-48-5	67801-65-4	36635-35-5	-		27939-60-2	
R1										
R2	**X**	**X**	**X**				**X**			
R3				**X**	**X**			**X**	**X**	
R4	**X**					**X**	**X**			**X**
R5		**X**		**X**				**X**		
R6			**X**		**X**	**X**			**X**	**X**
MW (g/mol)	138.21	138.21	138.21	138.21	138.21	138.21	multi-constituent		multi-constituent	
Relative density (g/cm^3 at 20 °C)	0.935 (predicted)	0.938 (predicted)	0.944 (predicted)	0.948 (predicted)	0.946 (predicted)	0.958 (predicted)	0.929		0.9305	
Boiling point (°C)	196 (predicted)	191 (predicted)	79-80 (20 Torr) 191 (predicted at 760 Torr)	196 (predicted)	194 (predicted)	196 (predicted)	195 (760 Torr)		195.4 (760 Torr)	
Vapour pressure (Pa)	51.86 (predicted)	75.72 (predicted)	78.53 and 95.72 (both predicted)	62.26 (predicted)	62.13 (predicted)	57.47 (predicted)	36 (20 °C)		66.1 (24 °C)	
Water solubility (mg/L)	1086.33‡ (predicted: 7.86x 10-3 mol/L; range: 2.25x10-3 to 1.12x10-2 mol/L)	1324‡ (predicted: 9.58x 10-3 mol/L; range: 2.91x10-3 to 1.31x10-2 mol/L)	3.25 x 10+5‡ (predicted: 2.35 mol/L; range: 1.29x10-2 to 7.00 mol/L)	1285.35‡ (predicted: 9.30x 10-3 mol/L; rang: 2.25x10-3 to 1.46x10-2 mol/L)	1006.17‡ (predicted: 7.28x 10-3 mol/L; range: 2.25x10-3 to 1.12x10-2 mol/L)	4.85 x 10+5‡ (predicted: 3.51 mol/L; range. 1.11x10-2 to 7.00 mol/L)	910 (20 °C)		381.8 (24 °C)	
Part. coefficient n-octanol/water	2.77 (predicted)	2.62 (predicted)	2.58 (predicted)	2.76 (predicted)	2.74 (predicted)	2.7 (predicted)	2.7		3.1 (25 °C)	
References	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/ https://www.echemportal.org/echemportal/substance-search https://canadachemicals.oecd.org/Search.aspx https://pubchem.ncbi.nlm.nih.gov/	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/	ECHA-Registration Dossier		ECHA-Registration Dossier https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf	

‡ Water solubility in mg/L was calculated from predicted average value (mol/L at 25°C)

List No.	9	10	11	12	13	14	15	16
EC No.	272-113-5	276-055-1	-	-	215-833-7	215-638-7	266-810-3	-
CAS No.	68737-61-1	71832-78-5	40702-26-9	1726-47-2	1423-46-7	1335-66-6	67634-07-5	6754-27-4
R1	Incompletely defined	X	X					
R2		X		X X	X	X		
R3			X			X	X	
R4		X	X	X	X	X		X
R5						X	X	
R6					X	X X	X	X X
MW (g/mol)	138.21	152.23	152.23	152.23	152.23	multi-constituent	152.23	152.23
Relative density (g/cm^3 at 20 °C)	ND	0.936 (predicted)	0.9325 (420 °C) 0.947 (predicted)	0.933 (13 °C) 0.918 (Predicted)	0.9203	ND	0.929 (predicted)	0.935±0.06 (predicted)
Boiling point (°C)	ND	213 (predicted)	64-67 (5 Torr) 215 (predicted)	211 (predicted)	203 (257 Torr)	ND	213 (predicted)	92.0-93.5 (20 Torr)
Vapour pressure (Pa)	ND	64.93 (predicted)	62.93 (predicted)	47.46 (predicted)	83 (25 °C) 52 (20 °C)	ND	40.13 (predicted)	50.92 (predicted)
Water solubility (mg/L)	ND	5.34 x10+5‡ (predicted: 3.51 mol/L range: 1.01x10-2 to 7.00 mol/L)	5.34 x10+5‡ (predicted: 3.51 mol/L; range: 1.23x10-2 to 7.00 mol/L)	3.56 x 10+5‡ (predicted: 2.34 mol/L; range: 3.86x10-3 to 7.00 mol/L)	218 (20 °C) (predicted: 1.75 mol/L; range: 7.84x10-4 to 7.00 mol/L)	ND	2.66 x10+5‡ (predicted: 1.75 mol/L; range: 7.84x10-4 to 7.00 mol/L)	ND
Partition coefficient n-octanol/water	2.85 (predicted)	3.10 (predicted)	3.13 (predicted)	3.06 (predicted)	3.1	3.27 (predicted)	3.09 (predicted)	3.31 (predicted)
References	https://chem.nlm.nih.gov/chemidplus/ https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/ https://canadachemicals.oecd.org/Search.aspx https://www.echemportal.org/echemportal/substance-search	https://www.epa.gov/	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/	ECHA-Registration Dossier https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf	https://chem.nlm.nih.gov/chemidplus/ https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/ https://www.echemportal.org/echemportal/substance-search https://pubchem.ncbi.nlm.nih.gov/ https://canadachemicals.oecd.org/Search.aspx	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://www.epa.gov/	https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf https://pubchem.ncbi.nlm.nih.gov/ https://canadachemicals.oecd.org/Search.aspx https://www.echemportal.org/echemportal/substance-search

ND – No data

The physicochemical properties of the di- and trimethylated cyclohex-3-ene-1-carbaldehydes in this group are listed in Table . All group members show similar physicochemical properties:

- The molecular weights range from 138.21 to 152.23 g/mol. Other substances are reactions masses of di- or trimethylated congeners (MW: 138.21 to 152.23 g/mol). Some mixtures are multi-constituent substances of group members of known molecular weight.
- All group members show similar boiling points and vapour pressures. For some, only predicted boiling points and/or vapour pressure values were available, which are comparable to measured values of other group members (measurement at 760 Torr for boiling point and at 24 °C for vapour pressure). Lower values of measured boiling points or vapour pressures for some substances are most probably due to measurements at lower pressures or temperature, respectively (5 and 20 Torr for boiling points or 20 °C for vapour pressure; see also section 10.7.8, Figure 2).
- Log K_{OW} (logarithm of the n-octanol/water partition coefficient) values were comparable among all group members, ranging from 2.7 to 3.3, including measured and predicted values.
- Predicted values for water solubility (mg/L) varied considerably, which can be explained by different prediction sources, while measured values of water solubility were more comparable, ranging from 218 to 910 mg/L.

Altogether, group members show similar physicochemical properties and are suggested to cause the same type of effects, namely skin sensitisation. The substances have a low molecular weight, with a range from 138.2 to 153.2 g/mol. According to ((ECHA, 2017b), Table R.7.12-3), the optimal molecular weight for dermal absorption is < 100 g/mol, whereas at a molecular weight above 500 g/mol, dermal uptake is less likely due to the size of the molecule. Furthermore, all group members show log K_{OW} values between 2.7 and 3.3, suggesting that the substances are comparable and sufficiently lipophilic to cross the *stratum corneum*. Substances with log K_{OW} between 1 and 4 favour dermal absorption (values between 2 and 3 are optimal) (ECHA, 2017b). Furthermore, partition from the *stratum corneum* into the epidermis is given by the sufficient water solubility of the cyclohex-3-ene-1-carbaldehyde congeners (≥ 218 mg/L, measured). In general, for a water solubility between 100 - 10 000 mg/L, absorption is expected to be moderate to high. There are substances with a (predicted) water solubility above 10 000 mg/L (with log K_{OW} values > 1), for which a high dermal uptake can be expected (ECHA, 2017b). Vapour pressures for all of the substances are ≤ 95.7 Pa, therefore, acc. to ECHA, substances are likely to be well-absorbed (ECHA, 2017b).

For two substances, most of the physicochemical properties were not available (EC No. 272-113-5, EC No. 215-638-7). However, available experimental data, including positive data from human clinical patch tests and a local lymph node assay, were available for both. These data show that these substances induce skin sensitisation with a moderate potency (based on animal data), as shown for the other group members as well.

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Experimental toxicokinetic data regarding the absorption, distribution, metabolism and excretion of the cyclohex-3-ene-1-carbaldehydes are not available.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not assessed in this dossier.

10.2 Acute toxicity - dermal route

Not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Not assessed in this dossier.

10.4 Skin corrosion/irritation – for information only

Data on skin corrosion/irritation for di- and trimethylated cyclohex-3-ene-1-carbaldehydes show that substances produce similar effects and act as skin irritants or are weakly corrosive.

Table 7: Summary table of animal studies on skin corrosion/irritation for di- and trimethylated cyclohex-3-ene-1-carbaldehydes

List No.	Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels duration of exposure	-Observations and time point of onset -Mean scores/animal -Reversibility	Results	Reference
1	According to OECD TG 404 GLP-compliant Test substance: EC No. 268-264-1, CAS No. 68039-49-6 Purity: 99.0% Reliability: 2, reliable with restriction No study report available	Rabbit, N=4 (strain not specified)	0.5 mL test material (undiluted) was applied semi-occlusive at flank for 4h; observation period: 1h, 1d, 2d, 3d, and 7d	Erythema score (average 24/48/72 h): 2 (max. 2) for 3 animals, 2.2 (max. 2.5) for 1 animal); Erythema score (7d): 1 (1 animal, 1.5 (2 animals), 2 (1 animal) Oedema score (average 24/48/72 h): 0.83 (max.1) for 1 animal), 1.2 (max. 2) for 2 animals, 1.5 (max. 2) for 1 animal; Oedema score (7d): 0 (1 animal), 0.5 (3 animals) Primary irritation index (sum erythema 24/48/72 h + sum oedema 24/48/72 h)/ (3*No. of animals) = 3.21 Desquamation from the skin in 2/4 animals at 7 d	**Positive** **Skin Irrit. Cat. 2**	(ECETOC, 1995) Reg. Dossier for No. 7 (no identifier)

List No.	Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels duration of exposure	-Observations and time point of onset -Mean scores/animal -Reversibility	Results	Reference
1	Similar to OECD TG 404 GLP: no information Test substance: EC No. 268-264-1, CAS No. 68039-49-6 Purity: no information Reliability: 2, reliable with restriction	Rabbit, New Zealand White; N=6 (3 male, 3 female)	Test material, 0.5 mL, applied to the clipped, intact skin under a gauze patch and loosely covered with impervious sheeting; 4h contact period; excess sample is removed after contact; skin reaction is scored by the method of Draize, at 5 h, 1 d, 2 d, 3 d, 7 d, and 10 and 14 d after dosing (depending on local skin reaction)	Minor to moderate erythema and minor to moderate oedema (score 1-2; 6/6 animals); necrosis (4/6 animals; 3 animals after 1-2d, 1 animal after 7d); desquamation in 5/6 animals at 10d; erythema persisted on 2 animals after 14d	**Positive** **Skin Corr. Cat. 1C**	(BRRC, 1986)
1	Non-guideline study Similar to OECD TG 402 pre GLP Test substance: 2,4-dimethyl-cyclohex-3-ene-1-carbaldehyde; EC No. 268-264-1 CAS No. 68039-49-6 Purity: No information Reliability: 4, not assignable No details on test guideline provided	Rabbit, N= 10/ treated group No controls Sex and strain not specified	Dose: 5.0 g/kg bw; Duration of observation period following administration: 14 days Irritation was assessed according to the following irritation parameters: skin redness, skin oedema, skin sloughing of exposure area and skin (hard/thickness); necropsy observation were performed No further information (e.g. exposure time)	2/10 animals died on day 1 of the 14 day observation period Toxic signs: Anorexia, decreased mobility due to severe oedema & eschar of exposure site, ptosis ("droopiness" of a body part) - Skin irritation: moderate redness (1/8), severe redness (7/8), moderate oedema (4/8) and severe oedema (4/8). At necropsy: yellow exudate in nose/mouth (2/10), red areas in intestines (3/10), yellow areas in intestines (1/10), bloated intestines (1/10), intestines containing dark green substance (1/10), liver dark (5/10), liver mottled (1/10), liver white nodules (2/10), lungs with areas dark (2/10), kidney mottled (2/10), kidney pale (1/10), skin sloughing of exposure area (1/10), skin oedema (8/10), skin redness (9/10), skin hard/ thick (6/10)	**Positive** Skin irritating effects observed No conclusion on classification	(Anonymous 6, 1978) Reg. Dossier No. 7 (no identifier)
7	Similar to OECD TG 404 Non-GLP Test substance: No. 7, no identifier Purity: No information Reliability: 2,	Rabbits, New Zealand White, female, N=4	Test material (0.5 mL, no vehicle) was applied on the skin under a surgical patch (covered with elastic adhesive bandage); Exposure period: 4 h (semi-occlusive); after removal of the tapes, the treated sites were	Observation, 24 h: moderate erythema (all animals, score 2), slight to moderate oedema (1 animal score 2, 1 animal score 1.5, 2 animals score 1); 48 h: moderate erythema (all animals score 2), slight to moderate oedema (2 animals score 1.5, 2 animals score 1); 72 h: oedema effects decreased (3 animals score 1, 1 animal score 0.5), the erythema effects stayed at the	**Positive** **Skin Irrit. Cat 2**	(Anonymous 9, 1987) Reg. Dossier EC No. 248-742-6

List No.	Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels duration of exposure	-Observations and time point of onset -Mean scores/animal -Reversibility	Results	Reference
	reliable with restriction No study report available No observation during 14 days		cleansed by gentle swabbing with cotton wool soaked in warm water; Observation of animals at 24, 48 and 72 h after patch removal, until 7d	same level (1 animal score 2.5, 3 animals score 2); 7 d: still signs of both erythema (1 animal score 2, 2 animals score 1.5 and 1 animal score 1) and oedema (3 animals score 0.5, 1 animal score 0). Study was not continued until the complete disappearance of effects (and erythema were still moderate). According to the Registrant, obtained scores were not sufficient for classification. However, the effects did not disappear until the last observation (7 d)		
13	Similar to OECD TG 404 Pre-GLP Test substance: EC No. 215-833-7, CAS No. 1423-46-7 Purity: No information Reliability: 2, reliable with restriction No observation during 14 days	Rabbits, New Zealand White, sex not specified, N=8	Primary irritation to the skin is measured by a patch-test technique on the clipped dorsum under semi-occlusive conditions; 0.5 mL undiluted test material; application for 4h; assessments immediately after removal, 24, 48 and 72 hours	Overall irritating score (time points 4, 24, 48, and 72 h; a score of max. 7 was used): 3/7 (mild irritation in 3 animals), 6/7 (severe irritation in 3 animals), 4/7 (moderate irritation in 2 animals), not fully reversible within 72 h (8/8 animals) 1/8 animal with pale brown tissue 24 h after treatment, at 72 h most sites showed slight/moderate to moderate responses with 3/8 animals showing brown tissue	**Positive** **Skin Irrit. Cat 2**	(Anonymous 10, 1984)
14	Non-guideline study Pre-GLP Test material: Isocyclocitral; EC No. 215-638-7 CAS No. 1335-66-6 Purity: No information Reliability: 4, not assignable Cited from (Opdyke, 1976)	Rabbit, no further information	Test substance applied "full strength" (assumed undiluted) to intact or abraded skin for 24h under occlusion No further information	Test substance was mildly irritating. No further information	**Positive** Skin irritating effects observed No conclusion on classification	(Levenstein, 1973)

There is evidence from animal *in vivo* studies that di- and trimethylated cyclohex-3-ene-1-carbaldehydes produce damage to the skin. An *in vivo* study according to OECD TG 404 and in line with GLP principles was conducted with EC No. 268-264-1 (purity: 99.0%, undiluted), showing that

(semi-occlusive) application of the undiluted substance for a 4 h contact period resulted in mild to moderate erythema and oedema (N=4 animals), with erythema still present at the end of the observation period (7d) (ECETOC, 1995). However, the study report was not available and information on the rabbit strain were lacking.

In another study similar to OECD TG 404 (no information on GLP) application of the substance EC No. 268-264-1 (purity not given) produced minor to moderate erythema and oedema in all animals, while erythema persisted (on 2 animals) after 14 days. Furthermore, necrosis developed in more than half of the animals (N=4/6) and animals showed desquamation of the skin (5/6 animals) and scab formation (3/6 animals) (BRRC, 1986). The study author designated the test substance as "corrosive". However, for this study, a more severe response in animals was observed when compared to the OECD TG 404 study of (ECETOC, 1995) performed with substance EC No. 268-264-1.

In a third *in vivo* study conducted with the substance EC No. 268-264-1 and similar to OECD TG 402, dermal application for an unknown exposure time resulted in moderate oedema (4/8) and severe oedema (4/8) (Anonymous 6, 1978). Study was precluded from further assessment since main study information were not available to the DS.

Congener No. 7 (no identifier) was applied to rabbit skin (4 h, semi-occlusive) in a study performed similar to OECD TG 404 (Anonymous 9, 1987). Observations revealed moderate erythema (all animals) and slight to moderate oedema. The study was not continued until the complete disappearance of effects and the scores for erythema were still moderate at the end of the observation period of 7d.

Primary irritation to the skin was investigated after application (4 h, semi-occlusive) of the undiluted test substance EC No. 215-833-7 to rabbit skin (Anonymous 10, 1984). Observation showed a slight/moderate to moderate response with three animals showing brown tissue (72 h), which were interpreted as suspected necrosis.

The test substance isocyclocitral (EC No. 215-638-7) was applied to rabbit skin for 24h under occlusion, resulting in mild irritations (Levenstein, 1973). This study was cited from second literature and main study information were lacking.

Table 8: Summary table of human data on skin corrosion/irritation

List No.	Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
5	Closed-patch test in humans Reliability: 4, not assignable Cited from (Letizia, 2000)	EC No. 267-186-5 CAS No. 67801-65-4 Purity: No information 2% in pet.	Test substance was applied at 2% in petrolatum on the backs of 29 healthy, male and female volunteers for 48h; No further information	No skin irritation observed	(RIFM, 1982)
14	Closed-patch test in humans Reliability: 4, not assignable Cited from (Opdyke, 1976)	Isocyclocitral EC No. 215-638-7 CAS No. 1335-66-6 Purity: No information 4% in pet.	Test substance was applied at 4% in petrolatum for 48 h; No further information	No skin irritation observed	(Kligman, 1972)

Human data on skin irritation/corrosion was retrieved from the open literature for substances EC No. 215-638-7 and 267-186-5 (Kligman, 1972; RIFM, 1982). Application of 4% and 2%, respectively in a closed patch test to human skin did not result in skin irritation. Studies were cited from secondary literature and main study information was not available to the DS.

Further information on skin irritation/corrosion produced by cyclohex-3-ene-1-carbaldehyde congeners in humans was not available.

Table 9: Summary table of other studies relevant for skin corrosion/irritation

List No.	Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Results	Reference
8	According to OECD TG 431 GLP-compliant Test substance: EC No. 248-742-6, CAS No. 27939-60-2 Purity: No information Reliability: 2, reliable with restriction	*In vitro* Reconstructed human epidermis (RHE) EpiDerm Skin Model (EPI-200)	The liquid test item was applied undiluted (50 µL) directly on top of the tissue; Exposure: 3 min and 1h; after exposure the skin tissue is thoroughly rinsed to remove the test item followed by immediate determination of the cytotoxic effects.	Tissue viability (1 h): 14%, (3 min): 124% Skin corrosive category 1B	**Positive** **Skin Corr. Cat. 1B**	(Anonymous 2, 2016)

An in *vitro* skin irritation/corrosion study using reconstructed human epidermis was performed with substance EC No. 248-742-6. Application of the substance resulted in severely reduced tissue viability indicating corrosive properties.

10.5 Serious eye damage/eye irritation

Not assessed in this dossier.

10.6 Respiratory sensitisation

One cyclohex-3-ene-1-carbaldehyde congener, EC No. 215-833-7 is self-classified as respiratory sensitiser (Resp Sens.1). However, data in support of this classification were found neither in the registration dossier, nor in the published literature. This endpoint could not be addressed due to lack of data.

10.7 Skin sensitisation

Skin sensitisation by low-molecular-weight chemicals is an immunological process that consists of two phases. During induction, the chemical forms a hapten-protein-complex in the skin of naive individuals. This is followed by a sequential set of events, leading to the production of allergen-specific memory T-cells. In the second phase (elicitation), exposure of the sensitised individual to the allergen leads to proliferation and activation of these T-cells, secretion of cytokines and mobilisation of other inflammatory cells resulting in the clinical outcome of allergic contact dermatitis (ECHA, 2017a).

10.7.1 Animal data on skin sensitisation

Table 10: Summary table of animal studies on skin sensitisation for di- and trimethylated cyclohex-3-ene-1-carbaldehydes

List No.	Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure, observation	Results[4]	Reference
			LLNA			
7	OECD TG 429 LLNA GLP: No information Reliability: 4, not assignable Cited from CSR for EC No. 248-742-6, no further information	No data available	**Reaction mass of 3,5-dimethylcyclohex-3-ene-1-carbaldehyde and 2,4-dimethylcyclohex-3-ene-1-carbaldehyde** **No. 7, no identifier** (Constituent 1: EC No. 268-263-6, CAS No. 68039-48-5 Constituent 2: EC No. 268-264-1, CAS No. 68039-49-6)	**EC3: 3.3%** No further information	**Positive**	(Anonymous, 2018)
8	According to OECD TG 429 LLNA GLP-compliant Reliability: 2, reliable with restriction No study report available	Mouse, CBA:J; female N=5/dose	**Dimethylcyclohex-3-ene-1-carbaldehyde** **EC No. 248-742-6** **CAS No. 27939-60-2** Purity: No data	**1, 2.5, 5, 10, and 25%** in ethanol/diethylphthalate (EtOH/DEP) (1:3), resulted in **SI-values of 2.1, 2, 2.9, 4.2, and 2.7**, respectively. According to the authors, an EC3 value could not be calculated since a normal dose range curve was not achieved. **5% < EC3 < 10%** Positive control (historical data used): 5, 15, and 35% α-hexyl cinnamaldehyde (HCA, CAS No. 101-86-0) in EtOH/DEP (1:3) gave SI values of 1.8, 4.0, and 7.5, respectively.	**Positive** **Skin Sens. 1B**	(Anonymous 1, 2012)
14	According to OECD TG 429	Mouse, CBA/Ca and	**Isocyclocitral**	0.5, 1, 2.5, 5, and 10% test substance in EtOH/DEP (1:3), resulting in stimulation index (SI) values of 0.8, 1.1, 1.7,	**Positive**	(Anonymous 8, 2006)

[4] Classification acc. to the DS's interpretation of the study results

List No.	Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure, observation	Results[4]	Reference
	LLNA GLP-compliant Reliability: 2, reliable with restriction No study report available	CBA/Ca/Ola/Hsd female N=4/dose	**EC No. 215-638-7** **CAS No. 1335-66-6** Purity: 98.1%	1.8, and 4.4, respectively. **EC3: 7.3%** (corresponding to 1825 µg/cm^2) Control: 5%, 10%, and 25% HCA in acetone/olive oil (4:1); 10 and 25% preparations caused skin sensitisation	**Skin Sens. 1B**	Reg. Dossier: EC: 215-833-7
14	LLNA GLP: No information Reliability: 4, not assignable Cited from (ICCVAM, 2011)	No data available	**Isocyclocitral** **EC No. 215-638-7** **CAS No. 1335-66-6**	Test substance in EtOH/DEP (1:3), no further information **EC3: 7.4%** (corresponding to 1838 µg/cm^2)	**Positive**	(RIFM, 2007)
			GPMT			
1	According to OECD TG 406 GPMT GLP compliant Reliability: 2, reliable with restriction No study report available	Guinea pig, Albino Dunkin Hartley, female N=20/test group N=10/control group	**2,4-dimethylcyclohex-3-ene-1-carbaldehyde** **EC No. 268-264-1** **CAS No. 68039-49-6** Purity: 99.8%	Concentration based on a range-finding test. Intradermal induction: (a) 5% test substance in arachis oil BP (highest concentration that caused mild to moderate skin irritation) (b) 5% test substance in Freund's Complete Adjuvant (FCA)/water (1:1), (c) FCA/water (1:1) Topical induction: 25% in EtOH/DEP (1:1) Topical challenge: 50% (highest non-irritant concentration) and 25% in EtOH/DEP (1:1) Mild to moderate skin irritation in most animals, after	**Positive** **Skin Sens 1**	(Anonymous 7) Reg. Dossier: No. 7 (no identifier)

List No.	Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure, observation	Results[4]	Reference
				intradermal and topical induction Readings after challenge: **24 h (50%): 20/20 positive** (well-defined or moderate to severe erythema and very slight or slight oedema) **48 h (50%): 11/20 positive** (8/20, very slight or well-defined erythema; 11/20, very slight to slight oedema) **24 h (25%): 20/20 positive** (very slight to moderate to severe erythema and very slight to slight oedema) **48 h (25%): 12/20 positive** (9/20 very slight or well-defined erythema; 12/20, very slight or slight oedema) Negative control 24/48 h (25/50% challenge): No signs of erythema or oedema		
2	GPMT GLP: No information Reliability: 4, not assignable Only secondary literature available, cited from (Letizia, 2000)	Guinea pig, Pirbright white, female N=20/test group	**3,6-Dimethyl-3-cyclohexene-I-carboxaldehyde** **EC No. 267-186-5** **CAS No. 67801-65-4**	Intradermal induction: (a) Two injections of 0.1 ml 50% Freund's complete adjuvant (FCA), (b) Two injections of 0.1 ml of a 5% solution of the test substance in olive oil, (c) Two injections of 0.1 ml of a 5% aqueous suspension of the test material in FCA (1:1); One week later, topical induction (occluded for 48 h): 5% test substance in petrolatum After 14 days, challenge application (on the shaved flank, occluded, for 24 h): 1% of test substance in ethanol Examination after 24 and 48 h after patch removal No sensitisation reaction was observed.	**Negative**	(RIFM, 1978)

A local lymph node assay (LLNA) conducted according to OECD TG 429 and in compliance with GLP was provided in the registration dossier for the substance EC No. 248-742-6 (Anonymous 1, 2012). The test substance EC No. 248-742-6 was applied to five mice per dose group using concentrations of 1, 2.5, 5, 10, and 25%. Exposure of animals resulted in SI-values of 2.1, 2, 2.9, 4.2, and 2.7, respectively. According to the authors, an EC3 value could not be calculated since a normal dose range curve was not achieved. Nevertheless, concentrations of 5% and 10% of the substance EC No. 248-742-6 resulted in an almost 3-fold or higher than 3-fold increase of the proliferation of local lymph node lymphocytes (SI-values of 2.9 and 4.2, respectively), compared to vehicle control. Therefore, data support a moderate skin sensitising potency of the substance EC No. 248-742-6. Another LLNA was described in the chemical safety report of substance EC No. 248-742-6, which, however, was not performed with EC No. 248-742-6, but with congener No. 7 (no identifier, registration under REACH), revealing an EC3 value of 3.3%. However, no reference was given and further study information was not available to the DS. Therefore, this information was not considered further by the DS. Notably, EC No. 248-742-6 is a constituent (> 1%) of multi-constituent substance No. 7 (no identifier), which might indicate that No. 7 (no identifier) has skin sensitisation potential as well.

Furthermore, in an LLNA performed according to OECD TG 429 and in line with the GLP principles, the sensitising potency of substance EC No. 215-638-7 (not registered under REACH) was investigated. Four mice per dose group were induced with the substance EC No. 215-638-7 at concentrations of 0.5, 1.0, 2.5, 5 and 10%. An EC3 value of 7.3% was calculated showing a moderate skin sensitising potency of the substance EC No. 215-638-7 (Anonymous 8, 2006). Another LLNA conducted with substance EC No. 215-638-7 was available from secondary literature (ICCVAM, 2011). An EC3 value of 7.4% was reported for the test substance. However, important study information is missing.

In the registration dossier for substance No. 7 (no identifier) the registrant provided a guinea pig maximisation test (GPMT), performed with substance EC No. 268-264-1 (pre-registration under REACH). In this GPMT, conducted according to OECD TG 406 and in compliance with GLP, the skin sensitising potency of substance EC No. 268-264-1 was investigated using concentrations of 5% for intradermal induction and 50% for topical challenge (Anonymous 7, 1998). Test concentrations based on a range-finding test to identify the highest concentration that caused mild to moderate skin irritation (intradermal application) and the highest non-irritant concentration (topical application) of the test substance. Application of EC No. 268-264-1 in the main study resulted in 20/20 and 11/20 sensitised animals at the 24 and 48 hour-readings, respectively. A challenge concentration of 25% resulted in 20/20 and 12/20 positive reactions at 24 and 48 hours, respectively. Data show that substance EC No. 268-264-1 elicits skin sensitisation with a moderate potency. However, concentrations of the substance ≤1% for intradermal induction were not tested and strong a sensitising potency cannot be excluded.

Finally, a GPMT conducted with the substance EC No. 267-186-5 (pre-registered under REACH) was available from secondary literature (RIFM, 1978). Exposure of animals with 5% of the test substance for intradermal induction and 1% for challenge did not result in sensitisation reactions, while higher concentrations were not investigated. This study is of low reliability, because main experimental details were not available to the DS. Therefore this study was not considered further.

10.7.2 Human data on skin sensitisation

Human data for di- and trimethylated cyclohex-3-ene-1-carbaldehydes include diagnostic patch test data, human repeated insult patch tests (HRIPT), and human maximisation tests (HMT), summarised in Table .

Table 11: Summary table of human data on skin sensitisation for di- and trimethylated cyclohex-3-ene-1-carbaldehydes

<table>
<tr><th>List No.</th><th>Type of data/report</th><th>Test substance</th><th>Relevant information about the study (as applicable), observation</th><th>Results[5]</th><th>Reference</th></tr>
<tr><td colspan="6">Diagnostic patch test study</td></tr>
<tr><td>9</td><td>Diagnostic patch test study

Selected dermatitis patients

Reliability: 2, reliable with restriction

Publication</td><td>Dimethyltetrahydrobenzaldehyde (Isomeric mixture: 2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde and 3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde)

EC No. 272-113-5
CAS No. 68737-61-1

5% in petrolatum (pet.)</td><td>Over a 3-month period, 178 volunteers (age or sex not reported) with sensitisation to fragrance materials, were patch-tested in 8 centres in J, NI, USA, GB, CH, and SE.
Sensitisation to fragrance ingredients was established by previous patch-testing to fragrance allergens in all subjects.
Patch-testing was performed to 20 new fragrance ingredients.
Patch-testing with serial dilutions of the individual materials were performed in 20 control subjects without clinical evidence of fragrance allergy, to prove that the test concentrations were sub-irritant.
Patch test results from 8 centres:

<table><tr><td>Centre</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>all</td></tr><tr><td>No. patients</td><td>25</td><td>24</td><td>19</td><td>28</td><td>26</td><td>24</td><td>7</td><td>25</td><td>178</td></tr><tr><td>No. positive</td><td>0</td><td>0</td><td>0</td><td>1</td><td>1</td><td>0</td><td>0</td><td>2</td><td>4</td></tr></table></td><td>Positive reactions: 2.3% (4/178)

Low/moderate to high frequency

Skin Sens 1</td><td>(Larsen et al., 2001)</td></tr>
</table>

[5] Classification acc. to the DS's interpretation of the study results

Human predictive patch test					
5	Human maximisation test (HMT) Reliability: 2, reliable with restriction	**3,6-dimethyl-3-cyclohexene-1-carboxaldehyde; 3,6-dimethylcyclohex-3-ene-l-carbaldehyde; cyclovertal** **EC No. 267-186-5** **CAS No. 67801-65-4** **2% in pet. (corr. to 1500 µg/cm^2)**	A HMT (according to Kligman and Epstein, 1975) was carried out on 29 healthy, male and female volunteers (no information on age). Concentration (2%) based on a reported maximum use concentration of 0.2% in consumer products (Volume: 300 µl, patch test area 4 cm^2). Pre-treatment of patch sites, for initial patch only with 7.5% aqueous sodium lauryl sulphate (SLS) under occlusion, for 24 h; Application of test material under occlusion to the same site on forearms of all subjects, for five alternate-day 48 h periods; After a 10-l4 day rest period, challenge under occlusion to naive sites for 48 h; applications were preceded by 30 min applications of 7.5% aqueous SLS under occlusion on the left side, test material was applied without SLS treatment on the right side. A fifth site challenged with pet. served as control. Dose/skin area (DSA): 1500 µg/cm^2 (calculated) No sensitisation reaction was observed in humans.	**Negative** **Skin Sens 1B cannot be excluded**	(RIFM, 1982)
7	Human repeated insult patch test (HRIPT) Reliability: 4, not assignable Primary report not identified, number of test subjects not available	**Reaction mass of 3,5-dimethylcyclohex-3-ene-1-carbaldehyde and 2,4-dimethylcyclohex-3-ene-1-carbaldehyde** **No. 7, no identifier** **(Constituent 1:** **EC: 268-263-6, CAS No. 68039-48-5** **Constituent 2:** **EC: 268-264-1, CAS No. 68039-49-6)** **5%** (vehicle not specified)	No sensitisation reaction was observed in humans. No further information DSA (µg/cm^2) not reported, no data to calculate DSA	**Negative** **Skin Sens 1B cannot be excluded**	(Anonymous, 2018)

8	HRIPT Reliability: 2, reliable with restriction Primary report not assignable	**Dimethylcyclohex-3-ene-1-carbaldehyde** **EC No. 248-742-6** **CAS No. 27939-60-2** **1% in 0.2 mL** (vehicle not specified)	Out of 110 volunteers (18 males, 92 females; age: 21 to 69 years), 106 subjects finished the study[6]; Induction: at the back of each subject, under occlusion, every Monday, Wednesday and Friday, for nine applications. Bandages were removed after 24 h. Challenge after 2 weeks: At unpatched test site for 24 h 48 h-reading: 1/106 subjects with mild response (level 1); 72 h-reading: Response decreased to barely perceptible (+), no response after further 24 h The authors concluded that there was no evidence for sensitisation due to the "*transient nature*" of the response. Another subject with a mild and transient response was re-challenged (approx. 6 weeks after end of study): "*The results indicated that the substance is not associated with an allergic dermatitis response.*" DSA (µg/cm^2) not reported, no data to calculate DSA (µg/cm^2)	**Negative** **Skin Sens 1B cannot be excluded**	(Anonymous 3, 2001)
9	HMT Reliability: 2, reliable with restriction	**Dimethyltetrahydrobenzaldehyde (Isomeric mixture: 2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde and 3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde)** **EC No. 272-113-5** **CAS No. 68737-61-1** **4% in pet. (corresponding to 3000 µg/cm^2)**	An HMT (acc. to Kligman and Epstein, 1977) was carried out on 25 healthy, male and female volunteers (no information on age), using a concentration of 4% (Volume: 300 µL; area: 4 cm^2). DSA: 3000 µg/cm^2 (calculated) No sensitisation reaction was observed in humans.	**Negative** **Skin Sens 1B cannot be excluded**	(Kligman, 1977)
14	HMT Reliability: 2, reliable with restriction Cited from (Opdyke, 1976)	**Isocyclocitral** **EC No. 215-638-7** **CAS No. 1335-66-6** **4% in pet. (corr. to 2880 µg/cm^2)**	An HMT (acc. to Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers; no information on age (Volume: 300 or 1000 µL; area: 4 or 14.5 cm^2). DSA: 2880 µg/cm^2 (calculated) The test material produced no sensitisation reaction.	**Negative** **Skin Sens 1B cannot be excluded**	(Kligman, 1972)

[6] Four subjects (4/110) discontinued the study for personal reasons.

14	HMT Reliability: 4, not assignable Cited from (ICCVAM, 2011) Primary report not identified, number of test subjects not available, vehicle not available	**Isocyclocitral** **EC No. 215-638-7** **CAS No. 1335-66-6** 2759 µg/cm^2 (vehicle not specified)	No sensitisation reaction was observed in humans. NOAEL: 2759 µg/cm^2 Dose reported reflects the highest concentration tested. No further information	**Negative** **Skin Sens 1B cannot be excluded**	(RIFM, 2007)
14	HRIPT Reliability: 4, not assignable	**Isocyclocitral** **EC No. 215-638-7** **CAS No. 1335-66-6** **6% in 0.5% tocopherol in DEP:EtOH (3:1)**	Number of subjects tested is not available for this test report. No sensitisation reaction was observed in humans. NOAEL: 7087 µg/cm^2 Dose reported reflects the highest concentration tested.	**Negative** **Skin Sens 1B cannot be excluded**	(RIFM, 2004) (RIFM, 2005)

Human diagnostic patch test studies cover the elicitation phase and indicate previous sensitisation to a test substance in humans. There is one human patch test study from the literature, including selected dermatitis patients tested to substance EC No. 272-113-5 (5% in pet.) in eight clinical centres (Larsen et al., 2001). Out of 178 volunteers previously sensitised to fragrance materials, four subjects showed positive reactions for this substance, resulting in a frequency of sensitised subjects of 2.3%. Based on the low number of subjects tested per centre and due to the fact that just one HDPT study for all congeners is available, the relative frequency might be seen between a relatively low/moderate (< 2%, selected dermatitis patients) and a relatively high frequency (≥ 2%) of the occurrence of skin sensitisation (ECHA, 2017c). Data are insufficient to establish previous exposure to the test substance (lacking data on repeated exposure and number of exposures).

Furthermore, a high number of human predictive patch tests (HPPT) performed with several congeners were available. HPPT including human maximisation tests (HMT) and human repeated insult patch tests (HRIPT) were conducted as induction studies. In general, HPPT followed non-guideline protocols and mostly original reports were not available. According to CLP Regulation (EC) No 1272/2008, data from HPPT may be used as weight of evidence for sub-categorisation (ECHA, 2017c). Human predictive studies performed with the substances were evaluated and considered for assessment, if essential information was available.

Human maximisation tests were retrieved from the literature for the substances EC No. 267-186-5, EC No. 272-113-5, and EC No. 215-638-7 (Kligman, 1972; Kligman, 1977; RIFM, 1982). In neither of these studies, sensitisation reactions were observed at the tested concentrations (2%, 4% and 4%, respectively). Due to the availability of essential information (identification of the primary reference, substance identity, and number of test subjects), data were considered for further assessment. Based on the available data, applied doses per skin areas (DSA) were calculated and corresponded to 1500, 3000, and 2880 µg/cm^2 for EC No. 267-186-5, EC No. 272-113-5, and EC No. 215-638-7, respectively. Another HMT conducted with isocyclocitral (EC No. 215-638-7) (RIFM, 2007) was of low reliability due to non-availability of essential study information. Isocyclocitral applied at a DSA of 2759 µg/cm^2 did not result in sensitisation in humans, however, the original report for this HMT was not identified, and the number of test subjects was not available to the DS, resulting in exclusion of this study for further assessment.

A human repeated insult patch test (HRIPT) conducted with substance EC No. 248-742-6 was provided in the registration dossier (Anonymous 3, 2001). In 106 volunteers tested with EC No. 248-742-6 at a concentration of 1%, no sensitisation reaction was observed in humans. This human study was considered for assessment, because essential information was available, including the identification of the primary reference, substance identity, and number of test subjects. Furthermore, HRIPTs were performed with substances EC No. 215-638-7 and No. 7, no identifier (Anonymous, 2018; RIFM, 2007). The substances did not produce any skin sensitisation reactions at the tested concentrations (7087 µg/cm^2 and 5%, respectively). However, these tests were precluded from further assessment, due to the lack of information (e.g. original study report not identified, number of test subjects not available).

In summary, substance EC No. 272-113-5 elicited skin sensitisation in selected dermatitis patients, but di- and tri-methylated cyclohex-3-ene-1-carbaldehydes did not induce skin sensitisation in human predictive patch test studies. Notably, tested concentrations in HPPTs were relatively low and it cannot be excluded, that these substances induce skin sensitisation in humans at higher concentrations. Negative results of HPPTs at a DSA > 500 µg/cm^2 do not allow for classification as skin sensitiser with sub-categorisation as Skin Sens. 1A, but classification as skin sensitiser 1B can be justified.

10.7.3 Other studies relevant for skin sensitisation

Table 12: Summary table of other studies relevant for skin sensitisation for di- and trimethylated cyclohex-3-ene-1-carbaldehydes

List No.	Type of study/data	Test substance,	Relevant information about the study (as applicable), observation	Results	Reference
13	According to OECD TG 442C (*in chemico* Direct Peptide Reactivity Assay (DPRA)) GLP-compliant Reliability: 1, reliable without restriction	**Isocyclocitral Tech, corresponding to 3-Cyclohexene-1-carboxaldehyde, 2,4,6-trimethyl-** **EC No. 215-833-7** **CAS No. 1423-46-7** Purity: 95.7% Supplier: Anonymous 4; Lot/batch No. VE00154585	Experimental study, direct peptide binding assay *in chemico:* Test substance (25 mM, in acetonitrile) incubated with Lys-peptide (0.5 mM, in ammonium acetate buffer (pH 10.5) with 25% of acetonitrile); Test substance (5 mM in acetonitrile) incubated with Cys-peptide (0.5 mM in phosphate buffer (pH 7.5) with 25% of acetonitrile); After 24 h of incubation the remaining peptide is quantified with HPLC-UV: Cys-peptide depletion: 25.5 ± 2.2% Lys-peptide depletion: 52.0 ± 0.2% Average depletion Cys- and Lys-peptide: 38.7% Positive and negative control: valid	**Positive**	(Anonymous 4, 2015a)
13	According to OECD TG 442D (*in vitro* ARE-Nrf2 Luciferase Test Method) GLP-compliant Reliability: 1, reliable without restriction	**Isocyclocitral Tech, corresponding to 3-Cyclohexene-1-carboxaldehyde, 2,4,6-trimethyl-** **EC No. 215-833-7** **CAS No. 1423-46-7** Purity: 95.7% Supplier: Anonymous 5; Lot/batch No. VE00154585	Experimental study, activation of keratinocytes *in vitro*; Substance tested at 12 concentrations, range from 0.98 to 2000 µM. Test substance did not induce the luciferase gene above the threshold of 1.5 (Average I_{max} values indicating maximal fold-induction up to a concentration of 1000 µM: 1.29, N=3); Concentration for 50% reduction of cellular viability (IC50): 596 µM Positive (Cinnamic aldehyde), vehicle and negative controls: valid	**Negative**	(Anonymous 5, 2015b)

An *in chemico* and an *in vitro* test were submitted for substance EC No. 215-833-7 (Table). The *in chemico* Direct Peptide Reactivity Assay (DPRA) addresses the molecular initiating event of the skin sensitisation Adverse Outcome Pathway (AOP) by assessing protein reactivity. It quantifies the reactivity of test chemicals towards synthetic model peptides containing either lysine or cysteine. On the basis of percent peptide depletion values the test substance is categorised into one of four classes of reactivity enabling the discrimination between skin sensitisers and non-sensitisers (OECD, 2020). Test results of a DPRA conducted according to OECD TG 442C with substance EC No. 215-833-7 revealed a cysteine peptide depletion ≥ 22.62% and ≤ 42.47%, indicating a positive test result and a moderate reactivity class (Anonymous 4, 2015a).

The ARE-Nrf2 Luciferase KeratinoSens Test Method addresses the second key event of the skin sensitisation AOP, namely 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. In a test performed according to OECD TG 442D (ARE-Nrf2 Luciferase Test Method) substance EC No. 215-833-7 did not activate keratinocytes *in vitro* (Anonymous 5, 2015b).

For the substance EC No. 215-833-7, *in vivo* or human data were not available.

According to 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 (sub-category 1A = strong/moderate sensitisers; sub-category 1B = weak sensitisers, and No Classification (NC = non-sensitiser).

Using the "2 out of 3" DA, intended for skin sensitisation hazard (but not potency) identification, the discordant results from the DPRA and KeratinoSens assay and the lack of further *in vitro* data (activation of dendritic cells, h-CLAT) do not allow to conclude on whether the substance EC No. 215-833-7 should be classified as a sensitiser or not ((OECD, 2021), Figure 2.1).

The Integrated Testing Strategies version 1 (ITSv1) and 2 (ITSv2) were constructed for prediction of the skin sensitisation hazard potential and potency sub-categorisation. Using the ITSv1 and ITSv2, positive *in silico* predictions (Derek Nexus v.6.1.1 and OECD (Q)SAR toolbox version 4.5, prediction in applicability domain), resulting in score 1, and the positive DPRA for the substance EC No. 215-833-7, with a mean cysteine and lysine depletion (%) of ≥ 22.62 and < 42.47, resulting in score 2 ((OECD, 2021), Table 3.1) are combined to an overall score of 3. This combined score results in a conclusive positive prediction for hazard identification but in an inconclusive prediction for the potency of the test substance (Skin Sens 1; (OECD, 2021), Table A2.2).

Altogether, the substance EC No. 215-833-7 was identified as a skin sensitiser. However, data do not allow for sub-categorisation.

10.7.4 *In silico* alerts for skin sensitisation

The following *in silico* tools were used to identify alerts for skin sensitisation of the congeners. 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:

- OECD QSAR Toolbox v. 4.3 (and v. 4.5 for substance EC No. 215-833-7) (https://qsartoolbox.org)

  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 v. 6.0.1 (and v. 6.1.1 for substance EC No. 215-833-7) (https://www.lhasalimited.org/products/derek-nexus.htm)

  Skin sensitisation mammal

- Danish (Q)SAR database

  No alert for skin sensitisation

It is important to note that the profilers used do not represent fully valid (Q)SAR predictions on their own. They should be seen as indicators of similar hazardous potential within a group/category, which later require verification *in vitro* or *in vivo*.

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

Table 13: Summary table of available *in vivo*, *in silico*, *in chemico/in vitro* and human data on skin sensitisation for di- and trimethylated cyclohex-3-ene-1-carbaldehydes[7]

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			(EC3: 3.3%)		**5% < EC3 < 10%**				EC3: 7.3% **(EC3: 7.4%)**	
GPMT	**100% positive at 5% intradermal induction**	(Negative at 5% intradermal induction)								
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	

[7] Grey background, bold characters – studies with reliability ≤ 2
white background, in brackets – studies with reliability > 2
blank – no data

There is evidence from reliable animal studies and a human diagnostic patch test study that di- and tri-methylated cyclohex-3-ene-1-carbaldehydes act as skin sensitisers. Reliable human induction studies performed with the congeners, including HRIPTs and HMTs, did not result in skin sensitisation. However, the tested concentrations were relatively low and a skin sensitisation potential cannot be excluded from HPPTs.

For substance EC No. 248-742-6, a positive LLNA is available, performed according to OECD TG 429 and in compliance with GLP (reliable with restriction). The exposure of mice to the test substance at concentrations of 1, 2.5, 5, 10, and 25%, resulted in SI-values of 2.1, 2, 2.9, 4.2, and 2.7, respectively. Animal data suggest a moderate skin sensitising potency of the test substance (Anonymous 1, 2012). A HRIPT, conducted with substance EC No. 248-742-6 did not result in sensitisation in humans (Anonymous 3, 2001). However, the concentration used for the HRIPT (1%) was relatively low and below the concentration that resulted in a positive test result in the LLNA (5 and 10% of the test substance resulting in SI-values of 2.9 and 4.2, respectively).

Furthermore, in a GLP-compliant LLNA performed according to OECD TG 429 (reliable with restrictions), application of the test substance EC No. 215-638-7 resulted in an EC3-value of 7.3% (Anonymous 8, 2006), revealing a moderate skin sensitising potency. Furthermore, for an LLNA of low reliability (cited from second literature) an EC3 value of 7.4% was reported (ICCVAM, 2011) for this substance. In a reliable HMT, 4% (DSA: 2880 µg/cm^2) of the substance EC No. 215-638-7 did not produce sensitisation reactions in 25 volunteers (Kligman, 1972). These results support that the test substance does not act as a skin sensitiser with a strong or extreme potency (< 500 µg/cm^2; (ECHA, 2017c; European Parliament, 2008) paragraph 3.4.2.2.2.1 and 3.4.2.2.2.2), however a moderate skin sensitising potency cannot be excluded. Further HRIPT or HMT conducted with this congener did not result in indications of sensitisation in humans at the applied dose (HRIPT: 7087 µg/cm^2, HMT: 2759 µg/cm^2 (RIFM, 2007)). However, studies were excluded from further assessment due to lacking study information (original study report, number of test subject, and vehicle not available).

Further reliable animal data show a skin sensitisation potential of cyclohex-3-ene-1-carbaldehyde congeners. For substances EC No. 268-264-1, a GPMT was performed in line with OECD TG 406 and GLP principles (Anonymous 7, 1998). Application of 5% of the test substance for intradermal induction and 50% challenge concentration resulted in 100% and 55% animals with positive reactions, at the 24 and 48 h readings, respectively. Data do allow to classify the substance EC No. 268-264-1 in a moderate potency group. As lower concentrations (< 1%) of EC No. 268-264-1 were not tested, a strong potency cannot be excluded. However, due to the close structural similarity to other di- and tri-methylated cyclohex-3-ene-1-carbaldehydes, a moderate skin sensitising potency is supported.

In another GPMT, 5% of the test substance EC No. 267-186-5 for intradermal induction and 1% for challenge did not result in skin sensitisation reactions in guinea pigs (RIFM, 1978). This result is inconsistent compared to the above *in vivo* data of the congeners. However, essential study information was not available (e.g. lacking information on the testing guideline and (non)-irritant concentrations) and this study was not further assessed. In a HMT, 2% (DSA: 1500 µg/cm^2) of the substance EC No. 267-186-5 did not produce skin sensitisation reactions in 29 healthy volunteers (RIFM, 1982), however this was a relatively low test concentration. Therefore, induction of skin sensitisation at higher concentration cannot be excluded.

Evidence from human data that cyclohex-3-ene-1-carbaldehyde congeners elicit skin sensitisation was available from a dermatological patch test study. Patch-testing with 5% of the substance EC No. 272-113-5 in patients with a previously determined sensitisation to fragrance materials resulted in an occurrence of frequency of skin sensitisation barely exceeding the value of ≥ 2%, i.e. being in the border between a relatively low/moderate to a high frequency. In an HMT performed with substance EC No. 272-113-5, no skin sensitisation was observed using 4% test substance (corr. to DSA: 3000 µg/cm^2).

Finally, the registration data of substance EC No. 215-833-7 include an *in chemico* direct peptide reactivity assay (DPRA) according to a GLP-compliant OECD TG 442C (Anonymous 4) study. 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 substance EC No. 215-833-7, shown in a test performed according to OECD TG 442D and in line with GLP principles (Anonymous 5, 2015b). According to OECD GD 497 using the ITSv1 and ITSv2, the substance EC No. 215-833-7 was identified as a skin sensitiser. However, the prediction for the potency of the substance was not possible.

Altogether, reliable animal data indicate that cyclohex-3-ene-1-carbaldehyde congeners act as skin sensitisers *in vivo* and allow sub-categorisation in a moderate potency group (Skin Sens 1B). Human data from patch-testing in selected dermatitis patients show that these substances are able to elicit skin sensitisation, as an indicator of previous sensitisation. Further available human data, including HRIPT or HMT do not show positive skin reactions at the tested concentrations. However, test concentrations of substances were relatively low (below concentrations resulting in a positive test result in animals). Available *in chemico* data and *in silico* prediction identify a skin sensitisation hazard.

10.7.6 Comparison with the CLP criteria

Reliable (at least reliability 2) and relevant experiments for animal, human, and *in silico/in chemico*/*in vitro* data are compared with the CLP criteria, as laid down in the Guidance on the Application of the CLP criteria (Table).

Table 14: Comparison of human, animal and *in silico/in chemico* data for skin sensitisation of di- and trimethylated cyclohex-3-ene-1-carbaldehydes with CLP criteria

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)	Skin Sens. 1A: 0.2% < EC3 ≤ 2%, Strong sensitiser EC3 ≤ 0.2%, Extreme sensitiser Skin Sens. 1B: 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)	Skin Sens. 1A - Extreme potency: ≥ 60% sensitised guinea pigs at ≤ 0.1% intradermal induction Skin Sens. 1A - Strong potency: ≥ 30 - < 60% guinea pigs sensitised at ≤ 0.1% intradermal induction or ≥ 60% guinea pigs sensitised at > 0.1 - ≤ 1.0% intradermal induction Skin Sens. 1B - Moderate potency: ≥ 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	Frequency borderline between "relatively low/moderate" to "relatively high" 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)
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)
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	

Reliable animal data give strong evidence that di- and trimethylated cyclohex-3-ene-1-carbaldehydes cause skin sensitisation *in vivo*. LLNAs conducted according to OECD TG 429 and in line with GLP principles with substances EC No. 248-742-6 and 215-638-7 prove that these substances act as moderate skin sensitisers (EC3 > 2%, (ECHA, 2017c), Table 3.6). Furthermore, a GPMT performed according to OECD TG 406 and in compliance with CLP indicates that substance EC No. 268-264-1 acts as a skin sensitiser with moderate potency (> 1.0% intradermal induction and ≥ 30% animals sensitised, Table 3.7, (ECHA, 2017c)). The treatment with concentration of 5% of EC No. 268-264-1 for intradermal induction elicited skin sensitisation in 100% animals, but these results should be taken with care. A strong sensitising potency of the substance EC No. 268-264-1 cannot be excluded, because concentrations for intradermal injection ≤ 1% were not tested in this GPMT. However, based on the high structural similarity and similar physicochemical properties compared to other di- and trimethylated congeners, it is expected that EC No. 268-264-1 acts as a

moderate skin sensitiser.

There is evidence from patch test studies that the substance EC No. 272-113-5 elicits skin sensitisation in patients with contact dermatitis to fragrance materials. Patch test results are at the border between a relatively low/moderate frequency and a relatively high frequency of occurrence of skin sensitisation (< 2.0%, relatively low/moderate frequency; ≥ 2.0%, relatively high frequency ((ECHA, 2017c), Table 3.2).

Reliable HRIPTs and HMTs were conducted with several di- and trimethylated cyclohex-3-ene-1-carbaldehydes (EC No. 267-186-5, 272-113-5, and 215-638-7. Studies performed with relatively low concentrations (1, 2, 4, and 4% corr. to unknown, 1500, 3000, and 2880 µg/cm^2, respectively) did not result in skin sensitisation reactions in humans. The negative test results of the HPPTs show that the congeners do not act as skin sensitisers with a strong (or extreme) potency (sub-categorisation 1A). However, negative HPPTs results ≥ 500 µg/cm^2 do not mean that the substances do not have the potential to act as skin sensitisers with a moderate potency as seen in *in vivo* assays of these congeners.

An *in chemico* DPRA conducted with substance EC No. 215-833-7 and addressing the MIE of protein reactivity within the skin sensitisation Adverse Outcome Pathway (AOP) revealed a positive test result with a moderate reactivity class. However, in an *in vitro* keratinocytes activation assay, addressing the second key event of the skin sensitisation AOP, exposure of the substance EC No. 215-833-7 gave negative test results. Predictions for skin sensitisation hazard identification and potency sub-categorisation using the ITSv1 and ITSv2 of OECD TG 497 identified EC No. 215-833-7 as a skin sensitiser. However, the prediction regarding the potency of the test substance was inconclusive.

Experimental data are supported by the mechanistic explanation of the profilers included in the OECD (Q)SAR toolbox. 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. This is the Molecular Initiating Event, i.e. the first step in the respective AOP for skin sensitisation.

In summary, the skin sensitisation potential of di- and trimethylated cyclohex-3-ene-1-carbaldehydes was established in animal studies *in vivo* and a positive *in chemico* direct peptide reactivity assay, addressing the molecular initiating event leading to the skin sensitisation. Experimental data are supported by (Q)SAR analysis, which also provide for a mechanistic explanation (covalent binding of the aldehydes to proteins via Schiff base formation). Sensitisation also has been observed in dermatitis patients subjected to diagnostic patch testing. In the view of the DS, these data have higher weight than the negative results from human predictive patch tests (HMT/HRIPT) which were obtained using comparatively low test concentrations. Therefore, it is proposed by the DS to classify di- and trimethylated cyclohex-3-ene-1-carbaldehydes as skin sensitisers with a moderate potency (sub-categorisation 1B), based on animal data. Data from human predictive patch tests (HMT/HRIPT) support a moderate potency of these congeners.

The conclusion for classification of di- and trimethylated cyclohex-3-ene-1-carbaldehydes as skin sensitisers is further supported by the opinion of Scientific Committee on Consumer Safety, categorising the substances EC No. 268-264-1 and 272-113-5 as possible sensitisers, based on structure-activity relationship 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)).

10.7.7 Conclusion on classification and labelling for skin sensitisation

In conclusion, the DS proposes to classify 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).

10.7.8 Grouping and read-across

A concern for skin sensitisation of cyclohex-3-ene-1-carbaldehyde congeners was identified during a manual screening activity of the DS, and this endpoint was proposed for harmonised classification and labelling. In total, 48 congeners were selected in the initial grouping step of the manual screening. All initial group members shared the same chemical backbone (cyclohex-3-ene-1-carbaldehyde), with different substituents on various positions of the cyclohexene ring, including hydrogen, alkyl, or alkenyl groups as substituents. Alkyl substituents included methyl, propyl, and 4-methyl-4-pentyl chains. Alkenyl substituents were 1-allyl, 1-ethenyl, 1-methylallyl, 1-methylvinyl, and 2-methylpropen-1-yl, 4-methyl-3-pentenyl, 4-methyl-4-pentenyl, and 2,2,3-trimethylcyclopent-3-en-1-yl chains.

With the aim to identify a group of structural highly similar substances for harmonized classification, the group members were investigated for individual structures, physicochemical properties, and available *in vivo*, *in chemico/in vitro*, and human data. Notably, for all 48 group members an alert for skin sensitisation was predicted using the OECD QSAR Toolbox v. 4.3 and Derek Nexus v. 6.0.1. However, the Danish (Q)SAR database did not result in an alert for skin sensitisation for any group member. Furthermore, a potential for skin irritation/corrosion for all congeners was predicted using the OECD QSAR Toolbox v. 4.3 and Danish (Q)SAR database (not all substances). Derek Nexus did not show alerts for skin irritation/corrosion for any of the substances. The available data for all 48 group members investigated in the manual screening are summarised in Table 15.

Of the initial 48 group members investigated during the manual screening, 16 were selected for a CLH group proposal on skin sensitisation.

This is based on reliable animal *in vivo* data, *in chemico*/*in vitro* and human data that were mainly available for alkylated cyclohex-3-ene-1-carbaldehydes (with two or three methyl substituents). For cyclohex-3-ene-1-carbaldehydes with alkenyl substituents, reliable data on skin sensitisation were available for a very low number of substances (including one LLNA with a much higher EC3, compared to EC3 of alkylated congeners). Furthermore, alkenyl-substituted substances mainly differed in their physicochemical properties (data shown in Table 15), compared to (di- and trimethylated) congeners and differences in the effect on skin sensitisation compared to alkyl-substituted congeners cannot be ruled out. Therefore, alkenyl substituents were precluded from the further grouping and read-across.

For alkylated group members, no data on skin sensitisation were available for congeners with just one methyl substituent at various positions, or longer chained alkyl substituents (C3H7, C6H15). As shown in Figure 2, congeners with no or just one methyl substituent differ in their physicochemical properties (increased vapour pressures, lower log K_{OW}), compared to congeners with two or three methyl substituents. Based on this fact and since no data on skin sensitisation were available for non- or mono-methylated congeners, these substances were excluded from grouping. Furthermore, substances with longer-chain alkyl substituents (C3H7, C6H15) also showed different physicochemical properties (increased log K_{OW}) relative to group members with two or three methyl substituents. Based on the lack of data on skin sensitisation for congeners with just one methyl substituent at various positions and for longer chain alkylated (C3H7, C6H15) substances and the differences in physicochemical properties, compared to di and trimethylated congeners, these substances were excluded from the group as well.

In conclusion, the grouping and read-across hypothesis was established for cyclohex-3-ene-1-carbaldehydes with two or three methyl substituents at various positions. For further detail, please see the assessment below.

Table 15: Substance identity, structure, and type of the 48 cyclohex-3-ene-1-carbaldehydes from manual screening[8]

EC No.	CAS No.	P1	P2	P3	P4	P5	P6	MW (g/mol)	Relative density g/cm3(20 °C)	Boiling point (°C)	Vapour pressure (Pa)	Water solubility (mg/L)	Part. Coefficient n-octanol/ water	LLNA	GPMT	HDPT	HRIPT	HMT	In chemic/ in vitro	QSAR predict. f. skin sensitisation	Skin irrit/ corr. (exp. data)	QSAR predict. f. skin irrit./corr.
Alkyl																						
202-858-3	100-50-5							110.15	0.9647	164	252 (25 °C)	11.5 x 10+3 (pH 3.9, 20 °C)	1.89 predicted	ND						+	positive	+
-	931-96-4	CH3						124.18	0.9447 predicted	174 predicted	163.98 predicted	2.36 mol/L predicted corr. to 2.93 x 10+5	2.15 predicted	ND						+	ND	+
231-452-9	7560-64-7				CH3			124.18	0.999 predicted	190 predicted	101.59 predicted	3.51 mol/L predicted corr. to. 4.36 x 10+5	2.17 predicted	ND						+	ND	+
201-953-7	89-94-1						CH3	124.18	0.900	181 predicted	131.98 predicted	3.51 mol/L predicted corr. to. 4.36 x 10+5	2.08 predicted	ND						+	ND	+
-	36635-33-3						CH3	124.18	0.983 (20 °C) predicted	70-72 (19 Torr)	ND			ND						+	ND	+
-	36635-34-4						CH3	124.18	0.983 predicted	171.3± 29.0 (760 Torr) predicted	ND			ND						+	ND	+

[8] Grey background, bold characters – studies with reliability ≤2
white background, in brackets – studies with reliability >2
ND – no data

EC No.	CAS No.	P1	P2	P3	P4	P5	P6	MW (g/mol)	Relative density g/cm3(20 °C)	Boiling point (°C)	Vapour pressure (Pa)	Water solubility (mg/L)	Part. Coefficient n-octanol/ water	LLNA	GPMT	HDPT	HRIPT	HMT	In chemic/ in vitro	QSAR predict. f. skin sensitisation	Skin irrit/ corr. (exp. data)	QSAR predict. f. skin irrit./corr.
280-869-2	83803-51-4			C6H15				194.31	0.933 predicted	259 predicted	4.47 predicted	3.50 mol/L predicted corr. to. 6.80 x 10+5	4.38 predicted	ND						+	ND	+
280-868-7	83803-50-3				C6H15			194.31	0.933 predicted	259 predicted	4.47 predicted	3.50 mol/L predicted corr. to. 6.80 x 10+5	4.38 predicted	ND						+	ND	+
945-920-1	-			C6H15				multi-constit.	0.894	274 (1008 hPa)	0.59 (20 °C) 1.1 (25 °C)	1.28 mol/L (20 °C)	5.3 (35 °C, pH 7)	ND						+	**positive**	+
					C6H15																	
266-314-7	66327-54-6	CH3			C6H15			208.34	0.893 predicted	265 predicted	1.28 predicted	1.76 x 10-4 mol/L predicted corr. to: 36.7	4.92	ND						+	**positive**	+
268-264-1	68039-49-6		CH3		CH3			138.21	0.935 predicted	196 predicted	51.86 predicted	7.86 x 10-3 mol/L predicted corr. to: 1086.33	2.77 predicted	ND	**100% positive, 5% intra-dermal induction**	ND				+	**positive**	+
252-395-6	35145-02-9		CH3			CH3		138.21	0.938 predicted	191 predicted	75.72 predicted	9.58 x 10-3 mol/L predicted corr. to: 1324	2.62 predicted	ND						+	ND	+
-	6975-94-6		CH3				CH3	138.21	0.944 (20 °C, predicted	79-80 (20 Torr)	78.53 and 95,72 (both predicted)	2.35 mol/L predicted corr. to. 3.25 x 10+5	2.58 predicted	ND						+	ND	+

EC No.	CAS No.	P1	P2	P3	P4	P5	P6	MW (g/mol)	Relative density g/cm3(20 °C)	Boiling point (°C)	Vapour pressure (Pa)	Water solubility (mg/L)	Part. Coefficient n-octanol/ water	LLNA	GPMT	HDPT	HRIPT	HMT	In chemic/ in vitro	QSAR predict. f. skin sensitisation	Skin irrit/ corr. (exp. data)	QSAR predict. f. skin irrit./corr.
268-263-6	68039-48-5			CH3		CH3		138.21	0.948 predicted	196 predicted	62.26 predicted	9.30 x 10-3 mol/L predicted corr. to: 1285.35	2.76 predicted	ND						+	ND	+
267-186-5	67801-65-4			CH3			CH3	138.21	0.946 predicted	194 predicted	predicted	7.28 x 10-3 mol/L predicted corr. to: 1006.17	2.74 predicted	ND	(Negative at 5% intra-dermal induction)	ND		**Negative at 2%**	ND	+	ND	+
253-139-6	36635-35-5				CH3		CH3	138.21	0.958 predicted	196 predicted	57,47 predicted	3.51 mol/L predicted corr. to: 48.51 x 10+5	2.7 predicted	ND						+	ND	+
-	-		CH3		CH3			multi-constit.	0.929	195	36 (20 °C)	910 (20 °C)	2.7	(EC3: 3.3%)	ND		(Negative at 5%)	ND		+	**positive**	+
				CH3		CH3																
248-742-6	27939-60-2			CH3			CH3	ND	0.9305	195.4	66.1 (24 °C)	381.8 (24 °C)	3.1 (25 °C)	**5% < EC3< 10%**	ND		**Negative at 1%**	ND		+	**positive**	+
					CH3		CH3															
272-113-5	71832-78-5	dimethylcyclohex-3-ene-1-carbaldehyde (incompletely defined)						ND					2.85 predicted	ND		**2.3%, rel. low/ moderate to high frequency**	ND	**Negative at 2700 µg/cm²**	ND	+	ND	+
276-055-1	276-055-1	CH3	CH3		CH3			152.24	0.936 predicted	213 predicted	64.93 predicted	3.51 mol/L predicted corr. to: 5.72 x 10+5	3.10 predicted	ND						+	ND	+

EC No.	CAS No.	P1	P2	P3	P4	P5	P6	MW (g/mol)	Relative density g/cm3(20 °C)	Boiling point (°C)	Vapour pressure (Pa)	Water solubility (mg/L)	Part. Coefficient n-octanol/ water	LLNA	GPMT	HDPT	HRIPT	HMT	In chemic/ in vitro	QSAR predict. f. skin sensitisation	Skin irrit/ corr. (exp. data)	QSAR predict. f. skin irrit./corr.
-	40702-26-9	CH3		CH3	CH3			152.23	0.9325 (420 °C)	64-67 (5 Torr) 215 predicted	62.93 predicted	3.51 mol/L predicted corr. to: 5.34 x 10+5	3.13 predicted	ND						+	ND	+
-	1726-47-2		2 x CH3		CH3			152.23	0.933 (13 °C)	211 predicted	47.46 predicted	2.34 mol/L predicted corr. to: 3.26 x 10+5	3.06 predicted	ND						+	ND	+
215-833-7	1423-46-7		CH3		CH3		CH3	152.23	0.9203	203	83 (25 °C) 52 (20 °C)	218 (20 °C)	3.1	ND					**Positive DPRA, Negative Keratino Sens**	+	**positive**	+
215-638-7	1335-66-6		CH3		CH3		CH3	ND						**EC3: 7.3%** (EC3: 7.4%)	ND		(Negative at 7087 µg/cm²)	(Negative at 2759 µg/cm²)	ND	+	**positive**	+
				CH3		CH3	CH3															
266-810-3	67634-07-5			CH3		CH3	CH3	152.23	0.929 predicted	213 predicted	40.13 predicted	1.75 mol/L predicted corr. to: 2.66 x 10+5	3.09 predicted	ND						+	ND	+
-	6754-27-4				CH3		2 x CH3	152.23	0.935±0.06 predicted	92.0-93.5 (20 Torr)	50.92 (25 °C) predicted	ND	3.31 predicted	ND						+	ND	+
254-267-5	39067-36-		CH3		CH3		C3H7	180.29	0.902 predicted	243 predicted	7.73 predicted	2.33 mol/L predicted corr. to: 4.20 x 10+5	4.05 predicted	ND						+	ND	+

EC No.	CAS No.	P1	P2	P3	P4	P5	P6	MW (g/mol)	Relative density g/cm3(20 °C)	Boiling point (°C)	Vapour pressure (Pa)	Water solubility (mg/L)	Part. Coefficient n-octanol/ water	LLNA	GPMT	HDPT	HRIPT	HMT	In chemic/ in vitro	QSAR predict. f. skin sensitisation	Skin irrit/ corr. (exp. data)	QSAR predict. f. skin irrit./corr.
-	68140-54-5			CH3		CH3	C3H7	180.29	0.909 predicted	240 predicted	6.45 predicted	3.50 mol/L predicted corr. to: 6.31 x 10+5	3.99 predicted	ND						+	ND	+
Alkenyl																						
807-589-0	1049017-63-1	C2H3						136.19	1.08 predicted	195 predicted	203.98 predicted	3.50 mol/L predicted corr. to: 4.78 x 10+5	2.34 predicted	ND						+	ND	+
242-015-7	18126-38-0		C4H7					164.24	0.9348	230 predicted	11.03 predicted	3.50 mol/L predicted corr. to: 5.75 x 10+5	3.09 predicted	ND						+	ND	+
257-943-8	52475-89-5			C6H11				192.30	0.932 predicted	267 predicted	7.33 predicted	1.37x10-3 mol/L predicted corr. to: 263.5	4.27 predicted	ND						+	ND	+
253-617-4	37677-14-8				C6H11			192.30	0.933	267 predicted	7.31 predicted	1.37x10-3 mol/L predicted corr. to: 263.5	4.27 predicted	ND				**Negative at 1944 µg/cm²**	ND	+	ND	+
915-650-9	-			C6H11				multi-constit.	0.8949	213	0.59 (24 °C)	24 (24 °C)	4.7 (25 °C)	**EC3: 24.0%**	ND					+	**positive**	+
					C6H11																	
268-810-9	68140-59-0	CH3	C3H5					164.24	0.965 predicted	227 predicted	60.13 predicted	3.50 mol/L predicted corr. to: 5.75 x 10+5	3.18 predicted	ND						+	ND	+

EC No.	CAS No.	P1	P2	P3	P4	P5	P6	MW (g/mol)	Relative density g/cm3(20 °C)	Boiling point (°C)	Vapour pressure (Pa)	Water solubility (mg/L)	Part. Coefficient n-octanol/ water	LLNA	GPMT	HDPT	HRIPT	HMT	In chemic/ in vitro	QSAR predict. f. skin sensitisation	Skin irrit/ corr. (exp. data)	QSAR predict. f. skin irrit./corr.
257-941-7	52474-60-9	CH3		C6H11				206.32	0.928 predicted	271 predicted	3.06 predicted	2.33 mol/L predicted corr. to: 4.81 x 10+5	4.48 predicted	ND						+	ND	+
257-942-2	52475-86-2	CH3			C6H11			206.32	0.927 predicted	275 predicted	3.01 predicted	3.56x10-4 mol/L predicted corr. to:70	4.66 predicted	ND						+	ND	+
-	67746-28-5	CH3			C6H11			206.32	0.933 predicted	275 predicted	3.17 predicted	3.50 mol/L predicted corr. to: 7.22 x 10+5	4.54 predicted	ND						+	ND	+
915-712-5	-	CH3		C6H11				multi-constit.	0.918	275	0.58 (20 °C) 1 (25 °C)	3.19 (20 °C, pH 8.1)	4.8 (35 °C)	ND	**Negative, 5% intra-dermal induction**	ND				+	**positive**	+
		CH3			C6H11																	
261-901-4	59742-21-1			C6H11			CH3	206.32	0.932 (predicted)	278 (predicted)	5.37 (predicted)	3.50 mol/L (predicted) corr. to: 7.22 x 10+5	4.45 (predicted)	ND						+	ND	+
261-900-9	59742-20-0				C6H11		CH3	206.32	0.9198	278 (predicted)	11.14 (predicted)	3.50 mol/L (predicted) corr. to: 7.22 x 10+5	4.45 (predicted)	ND						+	ND	+
266-313-1	66310-72-3	C3H4			C6H11			232.36	0.933 (predicted)	312 (predicted)	0.49 (predicted)	3.50 mol/L (predicted) corr. to: 8.13 x 10+5	5.16 (predicted)	ND						+	ND	+

EC No.	CAS No.	P1	P2	P3	P4	P5	P6	MW (g/mol)	Relative density g/cm3(20 °C)	Boiling point (°C)	Vapour pressure (Pa)	Water solubility (mg/L)	Part. Coefficient n-octanol/ water	LLNA	GPMT	HDPT	HRIPT	HMT	In chemic/ in vitro	QSAR predict. f. skin sensitisation	Skin irrit/ corr. (exp. data)	QSAR predict. f. skin irrit./corr.
-	80296-03-5	R,S-4-methyl-1-(1-methyl-2-methylenecyclopentyl)-cyclohex-3-enecarbaldehyde						ND						ND						+	ND	+
-	945-049-7	Reaction mass of 3-methyl-1-(2,2,3-trimethylcyclopent-3-en-1-yl)cyclohex-3-ene-1-carbaldehyde and rel-(1R)-4-methyl-1-[(1R)-2,2,3-trimethylcyclopent-3-en-1-yl]cyclohex-3-ene-1-carbaldehyde and rel-(1R)-4-methyl-1-[(1S)-2,2,3-trimethylcyclopent-3-en-1-yl]cyclohex-3-ene-1-carbaldehyde						multi-constit.	ND					ND						+	ND	+
255-858-0	42507-55-1		C4H7		CH3	CH3		192.30	0.978±0.06 predicted	258.9±40.0 (760 Torr) predicted	12.33 predicted	3.50 mol/L predicted corr. to: 6.73 x 10+5	4.17 predicted	ND						+	ND	+
255-859-6	42507-56-2		C4H7		CH3	CH3		192.30	0.979 predicted	255 predicted	12.33 predicted	3.50 mol/L predicted corr. to: 6.73 x 10+5	4.17 predicted	ND						+	ND	+
255-860-1	42507-57-3		C4H7		CH3	CH3		192.30	0.979 predicted	255 predicted	12.33 predicted	3.50 mol/L predicted corr. to: 6.73 x 10+5	4.17 predicted	ND						+	ND	+
255-861-7	42507-58-4		C4H7		CH3	CH3		192.30	0.979 predicted	255 predicted	12.33 predicted	3.50 mol/L predicted corr. to: 6.73 x 10+5	4.17 predicted	ND						+	ND	+
268-809-3	68140-58-9	C3H4	CH3		CH3		CH3	192.30	0.908 predicted	247 predicted	5.03 predicted	3.50 mol/L predicted corr. to: 6.73 x 10+5	4.21 predicted	ND						+	ND	+

LLNA – Local lymph node assay;
GPMT – Guinea pig maximisation test;
DPRA – Direct peptide reactivity assay;
KeratinoSens – Are-Nrf2 Luciferase KeratinoSens Test Method;
HDPT – Human diagnostic patch test, HRIPT – Human repeated insult patch test, HMT – Human maximisation test

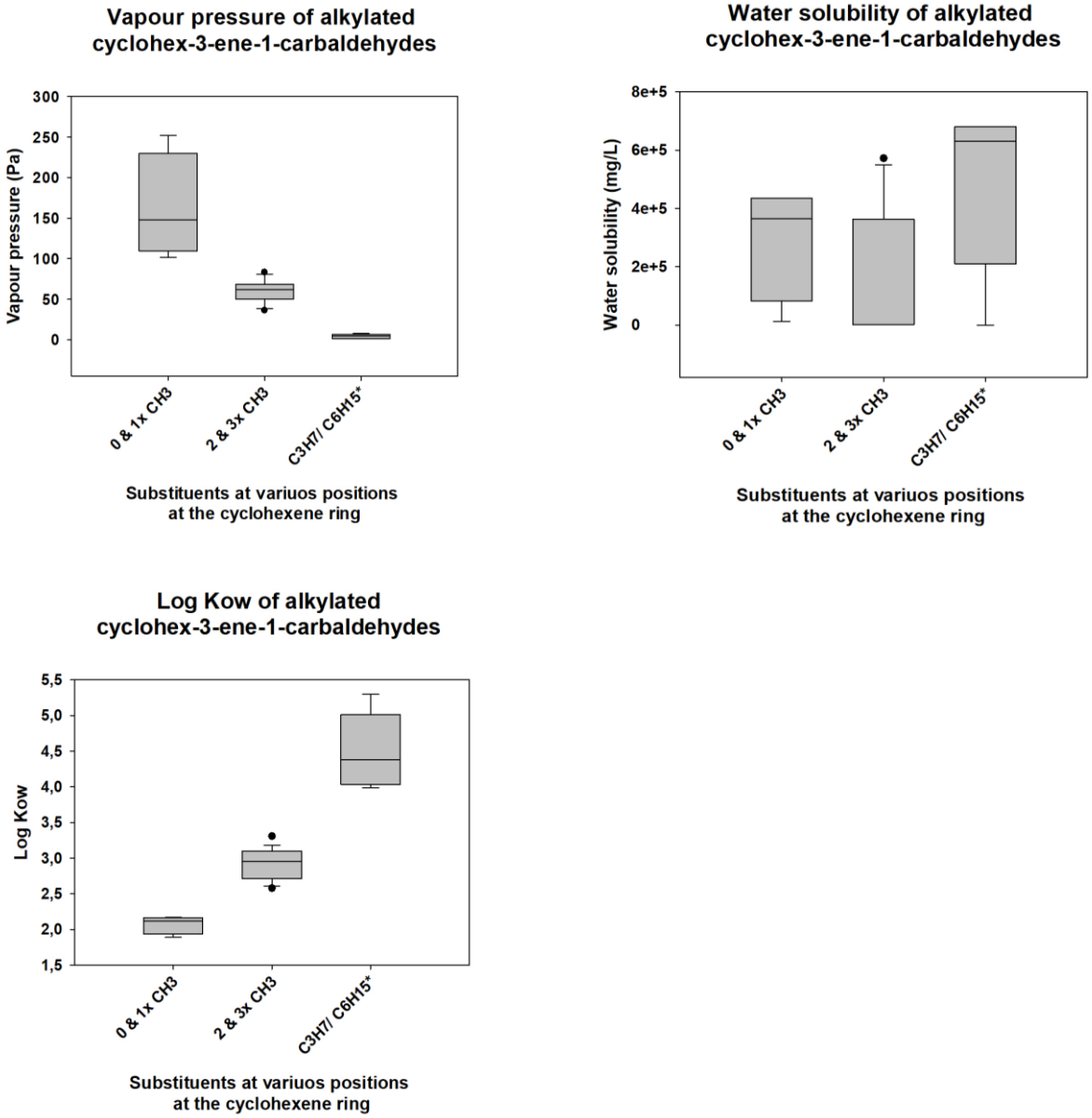

Figure 2: Physicochemical data of alkylated cyclohex-3-ene-1-carbaldehydes.

Vapour pressures of non- or mono-methylated congeners show much higher values compared to di- or trimethylated and longer-chain alkylated (C3H7, C6H15) group members. Outliers among di- and tri-methylated substances include vapour pressures for EC No. 215-833-7 (83 °C) measured at 25 °C and 215-833-7 (36 °C), measured at 20 °C. Vapour pressures for other members of the sub-group, laying in between, were mainly predicted (except for EC No. 248-742-6, vapour pressure: 66.1 °C, measured at 24 °C). There were no major differences in water solubility between the sub-groups. However, water solubility (mg/L) was predicted for most of the congeners showing a wide range, which can be explained by different prediction sources. Log K_{OW} is higher for di-and trimethylated congeners and when compared to non-or mono-methylated substances, and even higher for longer-chain alkyl substituted group members. While one outlier at the lower end of the sub-group of di-and trimethylated group members represents the measured coefficient for substance No. 7 (no identifier, log K_{OW} = 2.7), the outlier at the upper end is the predicted coefficient for CAS No. 6754-27-4 (log K_{OW} = 3). For most of the other di- and trimethylated substances predicted log K_{OW} values were available (except for EC No. 248-742-6, log K_{OW} = 3.1 measured and EC No. 215-833-7, log K_{OW}: 3.1 measured). * Congeners with 2 x CH_3 & C_3H_7 and no or 1 x CH_3 & C_6H_{15} at various positions

10.7.9 Assessment of the reliability of the read-across (in line with the ECHA Read-Across Assessment Framework, RAAF)

The read-across hypothesis (category approach) is based on different compounds, which have the same type of effect. Studies showing a skin sensitising potential of (different) cyclohex-3-ene-1-carbaldehydes (source substances) are used to predict skin sensitisation that would be observed in studies with similar congeners (target substances) if they were to be conducted. Effects observed for several cyclohex-3-ene-1-carbaldehydes (source substances) reveal no relevant differences in strength of effects, namely in the potency of skin sensitisation and allow sub-categorisation in a moderate potency group.

Assessment elements for scenario 6 of ECHA's read across assessment framework were investigated.

10.7.9.1 Substance characterisation

The chemical identity (EC/List No. and CAS No.) and individual structures, physicochemical properties, available *in vivo*, *in chemico/in vitro*, human date and (positive) *in silico* alerts for skin sensitisation of the investigated cyclohex-3-ene-1-carbaldehydes are listed in Table 15.

10.7.9.2 Structural similarity and category hypothesis

Grouping of the 16 substances is based on the same chemical backbone, namely cyclohex-3-ene-1-carbaldehyde. The chemical core structure of the group is given in Figure 1 above. 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).

10.7.9.3 Link of structural similarities and structural differences with the proposed regular pattern

All group members are aldehydes, but differ in their position of hydrogen or methyl substituents on the cyclohexene ring.

Reliable *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. Furthermore, reliable *in chemico*/*in vitro* assays were performed for one group member with three methyl substituents (EC No. 215-833-7). Reliable human data, including diagnostic patch tests and predictive patch tests were available for four congeners. Studies were performed with substances containing two or three methyl substituents at various positions (EC No. 267-186-5, 248-742-6, 272-113-5, and 215-638-7). The DS concludes that predictions can be made for cyclohex-3-ene-1-carbaldehydes with two and three methyl substituents at various positions at the cyclohexene ring.

10.7.9.4 Consistency of effects in the data matrix

Reliable local lymph node assays (LLNAs) from two congeners (one substance with two methyl (EC No. 248-742-6) and one substance with three methyl substituents (EC No. 215-638-7)) show that the substances induce skin sensitisation resulting in EC3 values of 7.3% (Anonymous 8, 2006) and 5% < EC3 < 10% (Anonymous 1, 2012), respectively. In a reliable guinea pig maximisation test (GPMT) conducted with a substance with two methyl substituents (EC No. 268-264-1,), 5% of the test substance for intradermal induction and 50% for topical challenge resulted in 100% (24 h reading) and 55% (48 h reading) animals with positive sensitisation reactions (Anonymous 7, 1998).

Animal data indicate that cyclohex-3-ene-1-carbaldehydes with two and three methyl substituents at various positions at the cyclohexene ring cause the same type of effects, namely skin sensitisation with a moderate potency.

In a human patch test study, substance EC No. 272-113-5 (two methyl substituents) elicited skin reactions in selected dermatitis patients (2.3% positive reactoins, using 5% test substance in pet.). Reliable human predictive patch tests (HPPTs) show that relatively low test concentration (1 – 4%) of congeners with two and three methyl substituents at various positions (EC No. 267-186-5, 248-742-6, 272-113-5, and 215-638-7) do not induce skin sensitisation. However, concentrations of test substances used in HPPTs were relatively low, supporting that cyclohex-3-ene-1-carbaldehydes do not act as skin sensitisers with a strong or extreme potency, but appear to have a moderate skin sensitising potential, as shown in the above *in vivo* studies.

In an *in chemico* Direct Peptide Reactivity Assay (DPRA; acc. to OECD TG 442C), addressing the MIE of skin sensitisation, the substance EC No. 215-833-7 was predicted as skin sensitiser. However, the substance did not activate keratinocytes *in vitro* (ARE-Nrf2 Luciferase Test Method, acc. to OECD TG 442D). *In vitro*/ *in chemico* data are inconclusive and, based on missing data from other *in vitro* studies (activation of dendritic cells), *in vivo* or human studies, do not allow to classify substance EC No. 215-833-7 as skin sensitiser. However, due to the high structural similarity and similar physicochemical properties compared to other group members, a skin sensitisation potential of EC No. 215-833-7 is expected.

Altogether, group members show similar structural pattern and similar physicochemical properties and are expected to cause the same type of effects, namely skin sensitisation.

The congeners have a relatively low molecular weight, with a range from 138.2 to 153.2 g/mol. According to (ECHA, 2017b) the optimal molecular weight for dermal absorption is < 100, while a molecular weight above 500 g/mol, the molecule may be too large for dermal uptake (Table R.7.12-3). Furthermore, all group members show log K_{OW} values between 2.7 and 3.3, suggesting that the substances are sufficiently lipophilic to cross the *stratum corneum*. Substances with a partition coefficient between 1 and 4 favour dermal absorption (values between 2 and 3 are optimal), particularly if the water solubility is high (ECHA, 2017b). Partition from the *stratum corneum* into the epidermis is given by the sufficient water solubility of the cyclohex-3-ene-1-carbaldehydes (≥ 218 mg/L). In general, for substances that show a water solubility between 100 - 10,000 mg/L, absorption is moderate to high. There are substances with a (predicted) water solubility above 10,000 mg/L (with log p-values > 1), so high dermal uptake might be expected (ECHA, 2017b).

Vapour pressures for all of the congeners are ≤ 95.7 Pa. Because substances with vapour pressures in that range are likely to be well absorbed (ECHA, 2017b), significant dermal absorption for di- and trimethylated cyclohex-3-ene-1-carbaldehydes is expected.

For two substances, only limited information on the physicochemical properties was available (EC No. 272-113-5, EC No. 215-638-7). However, available experimental data, including positive data from human clinical patch test data and local lymph node assay show that the substances induce skin sensitisation with a moderate potency (based on animal data), as shown for the other group members as well.

Furthermore, data on skin corrosion/irritation for di- and tri-methylated cyclohex-3-ene-1-carbaldehydes show that substances produce similar effects and act as skin irritants or are corrosive (see section 10.4). Substances that are skin irritants or corrosive may lead to enhanced skin penetration due to damage of the skin surface (ECHA, 2017b).

10.7.9.5 Reliability and adequacy of the source studies

Studies on skin sensitisation were available for several di- and tri-methylated congeners. Only those *in vivo* studies were used for assessment and read across that were at least reliable with restriction (reliability 2). Data include LLNAs performed according to OECD TG 429 and a GPMT in line with OECD TG 406. Furthermore, human diagnostic patch test (HDPT) and predictive patch tests (HPPT) were taken into account. HPPTs, in general, are not followed guideline protocols. The DS included HPPTs for assessment and read-across, based on available information (identification of the original study report, substance identity, number of test subjects, test concentration or DSA are given). For one congener, *in chemico*/*in vitro*

data were obtained from a study performed according to OECD TG 442C/D and considered reliable without restriction (Reliability 1).

Studies are adequate for the purpose of classification and labelling.

10.7.9.6 Compounds the test organism is exposed to

For the group in question, no information on (bio) transformation products is available, which could be formed after exposure of the organism. However, in its opinion on fragrance allergens in cosmetic products (SCCS, 2011), the SCCS performed structure activity relationships (SAR) analyses on the substances EC No. 268-264-1 and 272-113-5, revealing that both act as possible prehapten skin sensitiser, based on structural alerts. A prehapten is a chemical that itself is non- or low-sensitising, but that is transformed into a hapten outside the skin by simple chemical transformation (e.g. air oxidation) and without the requirement of specific enzymatic systems (in contrast to a prohapten, which forms a new or more potent allergen by enzymatic activation). SAR analyses on other congeners addressed in this proposal were not applied.

10.7.9.7 Common underlying mechanism, qualitative aspect

All group members are aldehydes, which 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. This is the Molecular Initiating Event (MIE), i.e. the first step in the respective Adverse Outcome Pathway for skin sensitisation[9]. This mechanistic explanation was provided by the profilers included in the OECD (Q)SAR toolbox.

10.7.9.8 Common underlying mechanism, quantitative aspects

It is likely that the cyclohex-3-ene-1-carbaldehydes are dermally absorbed, due to their relatively low molecular weight, moderate partition coefficient n-octanol/water, and moderate to high water solubility, and vapour pressure lower than 100 Pa (ECHA, 2017b). However, small differences in their physicochemical properties might affect the amount of substance dermally absorbed. Nevertheless, absorption at similar rates and extent is expected, as shown by results of *in vivo* studies with the source substances, resulting in a comparable potency for skin sensitisation (Skin Sens. 1B).

10.7.9.9 Exposure to other compounds than to those linked to the prediction

For the group of congeners, there are no information on other compounds available that may be present as impurities and may influence the prediction (see also 10.7.9.6).

10.7.9.10 Occurrence of other effects than covered by the hypothesis and justification

Regarding skin sensitisation, there is no additional mechanism expected other than those identified in the hypothesis.

Studies addressing effects of the congeners after repeated oral exposure (studies for acute toxicity, repeated dose toxicity, developmental and reproductive toxicity, specific target organ toxicity) were not investigated because they were not considered of relevance for skin sensitisation, i.e. the endpoint of interest in this proposal.

10.7.9.11 Bias that influences the prediction

There was no bias in selecting the group members, i.e. no theoretical group members with an actual indication of use were excluded.

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[9] http://www.oecd.org/env/the-adverse-outcome-pathway-for-skin-sensitisation-initiated-by-covalent-binding-to-proteins-9789264221444-en.htm

10.8 Germ cell mutagenicity

Not assessed in this dossier.

10.9 Carcinogenicity

Not assessed in this dossier.

10.10 Reproductive toxicity

Not assessed in this dossier.

10.11 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Not assessed in this dossier.

10.13 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

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