Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

Hydroxyisohexyl 3-cyclohexene carboxaldehyde

Reaction mass of 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde and 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [1]; 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [2]; 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [3]

EC number: - [1]; 250-863-4 [2]; 257-187-9 [3]
CAS number: - [1]; 31906-04-4 [2]; 51414-25-6 [3]

CLH-O-0000003906-67-03/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 March 2014

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

EC Number: 250-863-4; 257-187-9

CAS Number: 31906-04-4; 51414-25-6

Index Number: Not available

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Version number: Version 2 **Date:** 5 June 2013

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE

CONTENTS

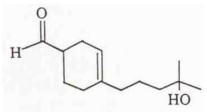
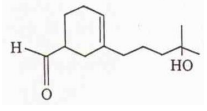
Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
International chemical identifier:	Hydroxyisohexyl 3-cyclohexene carboxaldehyde (INCI); reaction mass of 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde and 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [1]; 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [2]; 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [3]
EC number:	-[1]; 250-863-4 [2]; 257-187-9 [3]
CAS number:	- [1]; 31906-04-4 [2]; 51414-25-6 [3]
CAS name:	Not available
IUPAC name:	Reaction mass of 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde and 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [1]; 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [2]; 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [3]
Molecular formula:	$C_{13}H_{22}O_2$
Molecular weight range:	210.3
Structural formula:	Major isomer  Minor isomer 

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE

Annex VI Index number:	Not listed in Annex VI
Degree of purity:	≥ 98% (w/w)
Impurities:	Data not available

Trade names and abbreviations: *Lyral®,Kovanol®,Landolal®,Mugonal®,Cyclohexal®, HydroxyEmpetal®, Leerall® and Lydoucal®; HMPCC.*

The INCI name of [1] is hydroxyisohexyl 3-cyclohexene carboxaldehyde. The short form of the INCI name, HICC, will be used throughout this report. Thus, HICC is a multi-constituent substance composed of two isomers with two different CAS numbers: CAS No. 31906-04-4, major isomer [2] and CAS No. 51414-25-6, minor isomer [3]. HICC is composed of 70% of the major isomer and 30% of the minor isomer. There is no specific CAS number available for the multi-constituent form [1]. However, although not strictly correct CAS No. 31906-04-4 is used for the multi-constituent form as well as for the major isomer. Data on sensitization is only available for the multi-constituent form and for the major isomer. According to the notifications to the Inventory both isomers, with the different CAS numbers, have been self-classified as either Skin Sens. 1 or Skin Sens 1B. The difference in structure between the two isomers is in the 3- and 4-position of the aldehyde group. Generally, binding to proteins is a key step in the sensitisation process and HICC is expected to do this by a Schiff base formation (Patlewicz, *et al*, 2002), which is independent of the position of the aldehyde group in HICC. Further, there is no data to indicate any difference in potency between the two isomers. Therefore, the same classification, Skin Sens. 1A is proposed for both isomers, [2] and [3], as well as for the reaction mass [1].

Table 2: Impurities/Contaminants

Contaminants	Typical concentration	Concentration range
Information not available	-	-

1.2 Harmonized classification and labelling proposal

Table 3: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Not available; not included in Annex VI, Table 3.1	Not available; not included in Annex VI, Table 3.2.
Current proposal for consideration by RAC	Skin Sens. 1A; H317 GHS07, Warning; SCL=0.01%.	Xi; R43
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Sens. 1A; H317 GHS07, Warning; SCL=0.01%.	Xi; R43

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 4: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	-	-	-	n.e.
2.2.	Flammable gases	-	-		n.e.
2.3.	Flammable aerosols	-	-	-	n.e.
2.4.	Oxidizing gases	-	-	-	n.e.
2.5.	Gases under pressure	-	-	-	n.e.
2.6.	Flammable liquids	-	-	-	n.e.
2.7.	Flammable solids	-	-	-	n.e.
2.8.	Self-reactive substances and mixtures	-	-	-	n.e.
2.9.	Pyrophoric liquids	-	-	-	n.e.
2.10.	Pyrophoric solids	-	-	-	n.e.
2.11.	Self-heating substances and mixtures	-	-	-	n.e.
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	n.e.
2.13.	Oxidizing liquids	-	-	-	n.e.
2.14.	Oxidizing solids	-	-	-	n.e.
2.15.	Organic peroxides	-	-	-	n.e.
2.16.	Substance and mixtures corrosive to metals	-	-	-	n.e.
3.1.	Acute toxicity - oral	-	-	-	n.e.
	Acute toxicity - dermal	-	-		n.e.
	Acute toxicity - inhalation	-	-	-	n.e.
3.2.	Skin corrosion / irritation	-	-	-	n.e.
3.3.	Serious eye damage / eye irritation	-	-	-	n.e.
3.4.	Respiratory sensitization	-	-	-	n.e.
3.4.	Skin sensitization	Skin Sens. 1A, H317	0.01%	-	-
3.5.	Germ cell mutagenicity	-	-	-	n.e.
3.6.	Carcinogenicity	-	-	-	n.e.
3.7.	Reproductive toxicity	-	-	-	n.e.

3.8.	Specific target organ toxicity –single exposure	-	-	-	n.e.
3.9.	Specific target organ toxicity – repeated exposure	-	-	-	n.e.
3.10.	Aspiration hazard	-	-	-	n.e.
4.1.	Hazardous to the aquatic environment	-	-	-	n.e.
5.1.	Hazardous to the ozone layer	-	-	-	n.e.

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification
n.e. = not evaluated.

Labelling: Signal word: Warning
Hazard statements: H317
Precautionary statements: P261, P272, P280

Proposed notes assigned to an entry: -

Table 5: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	-	-	-	n.e.
Oxidizing properties	-	-		n.e.
Flammability	-	-	-	n.e.
Other physico-chemical properties *[Add rows when relevant]*	-	-	-	n.e.
Thermal stability	-	-	-	n.e.
Acute toxicity	-	-	-	n.e.
Acute toxicity – irreversible damage after single exposure	-	-	-	n.e.
Repeated dose toxicity	-	-	-	n.e.
Irritation / Corrosion		-	-	n.e.
Sensitization	Skin sensitizer, Xi; R43	0.01%	-	-
Carcinogenicity	-	-	-	n.e.
Mutagenicity – Genetic toxicity	-	-	-	n.e.
Toxicity to reproduction – fertility	-	-	-	n.e.
Toxicity to reproduction – development	-	-	-	n.e.
Toxicity to reproduction – breastfed babies. Effects on or via lactation	-	-	-	n.e.
Environment	-	-	-	n.e.

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification
n.e. = not evaluated.

Labelling: Indication of danger: Xi
R-phrases: R43
S-phrases: S24, S37

2. BACKGROUND TO THE CLH PROPOSAL

2.1. History of the previous classification and labelling

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has not been discussed for harmonized classification and labelling under the CLP.

The Scientific Committee on Consumer Safety (SCCS 2012) has concluded HICC to be an established contact allergen in humans. It belongs to fragrances of special concern due to the high number of published cases of allergy in scientific literature, more than 1500 cases since 1999. The SCCS considered the number of cases reported over the last decade to be exceptionally high and that the earlier recommendation by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP, 2003) to limit HICC in consumer products to a maximum of 200 ppm (0.02%) is not sufficiently protective. Thus their recommendation was that HICC should not be used at all in consumer products.

International Fragrance Association, IFRA, recommended a maximum limit of 1.5% HICC in consumer products in 2003. In 2009 the recommendation was adjusted and lowered to 0.02-0.2%, depending on type of product.

2.2. Short summary of the scientific justification for the CLH proposal

HICC is a synthetic fragrance which has been in the market since 1960 (Rieger, 2001). It is one of the most ubiquitous fragrance substances used in manufacture of various consumer products, like household products and cosmetics.

HICC was identified as a cause of skin allergy in early and late 90's from clinical case reports in some European clinics (de Groot *et al*, 1987; de Groot, *et al*, 1989; Handley and Burros, 1994; Hendriks, 1999; Giemenez-Arnau, *et al*, 2002; LeCoz and Goldberg, 2002). These studies drew attention for further clinical and epidemiological studies in Europe. Accordingly multicentre studies in Europe, involving thousands of dermatitis patients have demonstrated HICC to be a major and leading cause of allergic contact dermatitis (ACD) in Europe with a frequency ranging between 2 and 3% (Frosch, *et al*, 1999; Schnuch, *et al*, 2012a; Thyssen, *et al*, 2012). Subsequently it has been included in routine diagnostic patch test screening in national and European baseline series in many member states (Geier, *et al*, 2002; Bruze, *et al*, 2008).

Although the concentrations of HICC in products are low, varying between 0.01 and 0.63% and despite recommendations to limit the levels by the International Fragrance Association, IFRA, and the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers, SCCNFP (2003), recent evaluations have demonstrated that the incidence of skin sensitization due to HICC hasn't shown any considerable change in the number of new cases in member states up till now (Heisterberg, *et al*, 2012; Schnuch, *et al*, 2012a; Thyssen, *et al*, 2012).

Animal data are limited. However in an LLNA study the EC3 value was 17.1% indicating its sensitizing properties. QSAR results show a high protein affinity of HICC (Patlewicz, *et al*, 2002; Bonefeld, *et al*, 2011). Besides it has been shown that HICC is phototoxic (Eun, *et al*, 2004).

Based on its wide distribution in consumer products, high prevalence of allergic contact dermatitis as demonstrated by diagnostic patch testing in different European clinics and exposure to low concentrations of HICC in consumer products a harmonized classification for skin sensitization in sub-category 1A with a specific concentration limit (SCL) of 0.01% is proposed.

2.3. Current harmonised classification and labelling

2.3.1. Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Not included in Annex VI, Table 3.1.

2.3.2. Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Not included in Annex VI, Table 3.2.

2.4. Current self-classification and labelling

In the C&L Inventory at ECHA's website (last visited 15 May 2013) HICC has been self-classified in two different CAS numbers: CAS No. 31906-04-4, major isomer and CAS No. 51414-25-6, minor isomer as described in Part B, 2.1 of this document.

2.4.1. Current self-classification and labelling based on the CLP Regulation criteria

CAS No. 31906-04-4, 4-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde was self-classified by 1084 notifiers in 10 aggregated notifications as follows..

Self-Classification	Number of notifiers	Hazard statement	Pictogram	Signal word	Hazard statement (Labelling)
Skin Sens. 1	1070	H317	GHS07	Wng	H317
Skin Sens. 1B	14	H317	GHS07	Wng	H317
Eye Irrit. 2	749	H319	GHS07	Wng	H319
Aquatic Chronic 3	1060	H412	GHS07	Wng	H412

CAS No. 51414-25-6, 3-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde has been self-classified by 78 notifiers in two aggregated notifications as follows.

Self-Classification	Number of notifiers	Hazard statement	Pictogram	Signal word	Hazard statement (Labelling)
Skin Sens. 1	65	H317	GHS07	Wng	H317
Skin Sens. 1B	13	H317	GHS07	Wng	H317
Aquatic Chronic 3	78	H412	GHS07	Wng	H412

2.4.2. Current self-classification and labelling based on DSD criteria

No self-classification based on DSD criteria is available.

3. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Broad use at low concentration

HICC has a wide use in various consumer products (such as cleaning products and detergents, cosmetics, scented products and room fresheners), in Europe. In a Dutch study it was found at a rate of 33% of the 300 products analyzed (de Groot, *et al*, 1994). Later on Frosch, *et al* (1999) have detected it in 53% of the 73 randomly selected consumer products from European markets with a level of 1-1847 ppm content. Buckley (2007) identified HICC in 30 % of the 300 consumer products in the UK. HICC was found in 223 of 2207 (10%) cosmetic and household products in Germany (G. Mildau G, cited in Schnuch, *et al*, 2009); 151 (18%) of 843 products in another study contained HICC (K. Schumacher, cited in Schnuch, *et al*, 2009).

Rastogi, *et al*, in 1998 found HICC in 50% of the products they studied in Denmark (Rastogi, *et al*, 1998) and following this in 1999 reported its abundance in children's cosmetics and cosmetic toys at a range of 540 to 6300 ppm (0.054-0.63%) and mean of 2,570 ppm (0.26%) (Rastogi, *et al*,1999). Later on in 2001 they identified it in 10 (17%) of the 59 occupational and household products at a range of 36 to103 ppm (0.0036-0.01%) (Rastogi, *et al*, 2002). No decline in usage was observed in 2001 (Rastogi, *et al*, 2001). In a subsequent study from Danish EPA, Pors and Fuhlendorff (2003) reported its dominance in air fresheners; they found it at a range of 310 to62000 ppm (0.031-6.2%) in 9 of the 24 products. In patient product analyses made together with patch tests Frosch, *et al* (2005a,b) have chemically detected HICC in 19 (79.2%) of the 24 products with a concentration range of 0.017 to 3.832%. Wijnhoven, *et al* (2008) referred to a Dutch study which found up till 2790 ppm (0.28%) in consumer products ranking it 3rd in prevalence of fragrances after limonene and linalool and further concluded that it is one of the most frequently used fragrances in the Netherlands.

On top of this, Heisterberg, *et al* (2011a) reported that HICC was commonly found in Denmark in 53 (24.9%) of 213 deodorants, 33 (17.6%) of 188 scented lotions, 32 (22%) of 144 fine fragrances, 12 (12.5%) of 96 shampoos and 16 (19%) of 84 liquid soaps. Nardelli, *et al* (2011) found HICC in 48 of 301causal products collected in Belgium with an increasing trend from 9% in 2005 to 13% in 2009. It was also a common ingredient in consumer products sold in Swedish markets (Yazar, *et al*, 2010).

Earlier studies showed that it was among the 'top 25' materials in personal care products, household products and detergents analyzed from 400 consumer goods in the US in 1989 being the 13th in the list with a frequency of 46% in all products (Fenn, 1989).

These studies have concluded that HICC is commonly distributed in consumer–available products at low concentrations. HICC was identified in 10 to 80% of the consumer products analyzed. Concentration levels varied from low ppm-levels (0.0036%) up to 0.63%. In one product 3.8% was detected.

High frequency of sensitization in humans

A continued effort has been made by the scientific community to explore the level of HICC allergy in Europe in the last two decades. The prevalence of HICC sensitization in Europe is more common than in other continents, such as North America or the Middle East.

The average incidence in Europe was 2.7% while the national incidences in different studies ranged between 1.2-17%; in North America the incidence was 0.4% and in Iran 0.7% (Bruze, *et al*, 2002; Baxter, *et al*, 2003; Belsito, *et al*, 2006; Braendstrup, *et al*, 2008; Firooz, *et al*, 2010). The high rate in Europe has been ascribed to its more frequent presence in products and wider consumption of products containing HICC in Europe than in other continents.

A recent population-based evaluation of 9 years' epidemiological data by Heisterberg clearly demonstrated that there is no decline in the national rate of skin allergy due to HICC in Denmark over 2003 to 2011 since new cases continued to be added. The national rate remains at 2.5% (Heisterberg, *et al*, 2012). This has also been shown by the multicenter surveillance made in Germany which found an unchanged 2.1% incidence rate over the last decade (Schnuch, *et al*, 2012a). Another European time trend study of HICC sensitization prevalence (between 2002 and 2011) has recently suggested a slight decrease, but not conclusive, in central Europe (Schnuch, *et al*, 2012b). Thus there is still wide public exposure to the potent allergen HICC particularly in the European Community, and the prevalence of allergic contact dermatitis to HICC is persistent, an issue that calls for immediate attention.

Furthermore, patch test studies have demonstrated that HICC has strong additive/synergistic effects when it is found in a mixture with other sensitizing ingredients which is a real and common scenario to which the public is exposed to (Bonefeld, *et al*, 2011).

Animal data

LLNA studies have demonstrated the sensitizing properties of HICC where it had an EC3 value of 17.1% (Patlewicz, *et al*, 2002).

Many countries are affected

Several national and multicenter studies in Europe on HICC sensitization have found it as a leading single fragrance allergen causing most cases of sensitization and paced it among the "top 6" allergens (Frosch, *et al*, 1999; Geier, *et al*, 2002; Baxter, *et al*, 2003; Johansen, *et al*, 2003; Frosch, *et al*, 2005a,b; Heras, *et al*, 2006; Schnuch, *et al*, 2007; An, *et al*, 2007; Braendstrup, *et al*, 2008; Bruze, *et al*, 2008; Schnuch, *et al*, 2009; van Oosten, *et al*, 2009; Cuesta, *et al*, 2010; Heisterberg, *et al*, 2010; Krautheim, *et al*, 2010; Carvalho, *et al*, 2011).Thus the outcomes of these studies led to recommendations of further regulatory measures, from limiting the concentration allowed to be used in products to banning the substance (Uter, *et al*, 2007; SCCS, 2012). Based on the findings and recommendations, therefore, this allergen has been initially included in the FM II series and as well been suggested for inclusion in national series, for example in Germany, in 2002, and later in the UK, in 2006. In 2008 it was recommended for inclusion in the European base line series for diagnostic patch testing by the European Environmental Contact Dermatitis Group (EECDRG) and the European Society of Dermatitis (ESCD) (Geiger, *et al*, 2002; Bruze, *et al*, 2008; Davies, *et al*, 2011). It can therefore be concluded that HICC has been identified to give clinically relevant sensitization in dermatitis patients in many countries in Europe.

Costs of allergy

Allergic contact dermatitis is a major cause of absence from work and causes social and economic impacts on the individual, families and the community at large. ACD diagnosis, care and treatment

is very difficult and in many cases unsuccessful where the sole option available is avoidance of the allergen in question.

Self-classification not satisfactory

According to the C&L Inventory notifiers have self-classified HICC as Skin Sens. 1 or Skin Sens. 1B. However, due to the high frequency of human sensitization to HICC across Europe and due to the relatively low concentrations of HICC in consumer products a classification as a strong skin sensitizer in sub-category 1A with a specific concentration limit (SCL) of 0.01% is proposed. This means that products containing at least 0.01% HICC will be classified as sensitizing and that products containing at least 0.001% HICC will be assigned specific labelling for sensitizers (according to CLP, Annex VI, Table 3.4.6, Note 1).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1. IDENTITY OF THE SUBSTANCE

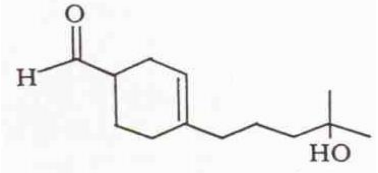
1.1 Name and other identifiers of the substance

Table 6: Substance identity

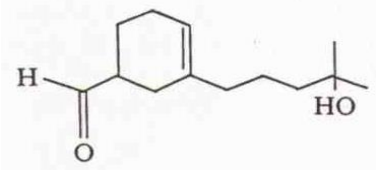
EC number:	- [1]; 250-863-4[2]; 257-187-9 [3]
Substance name:	Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
CAS number (EC inventory):	-[1]; 31906-04-4 [2]; 51414-25-6 [3]
CAS number:	-[1]; 31906-04-4 [2]; 51414-25-6 [3]
CAS name:	Not available
IUPAC name:	Reaction mass of 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde and 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [1]; 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [2]; 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde [3]
CLP Annex VI Index number:	Not listed in CLP, Annex VI.
Molecular formula:	$C_{13}H_{22}O_2$
Molecular weight range:	210.31

Structural formula:

Major isomer



Minor isomer



1.2. Composition of the substance

Table 7: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
4-(4-Hydroxy-4-methylpentyl) cyclohex-3-ene-1-carboxaldehyde	70%	Range values not found	Major constituent (Refer to section 2.1. in part B)
3-(4-Hydroxy-4-methylpentyl) cyclohex-3-ene-1-carboxaldehyde	30%	Range values not found	Minor constituent (Refer to section 2.1. in part B)

Current Annex VI entry: *Not listed in Annex VI of the CLP.*

Table 8: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Information not available			

Table 9: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
Information not available				

1.3. Physico-chemical properties

Table 10: Summary of physico-chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Clear viscous liquid	SCCP (2011)	-
Melting/freezing point	-	-	-
Boiling point	318.7 °C at 760 mmHg	SCCP (2011)	-
Relative density	-	-	-
Vapour pressure	<0.001 mmHg at 20 °C	IFFI* (2006), SCCP (2011)	-
Surface tension	42.62 dyne/cm		-
Water solubility	184.6 mg/l at 25 ^{0}C	SCCP (2011)	-
Partition coefficient n-octanol/water (Log P_{ow})	3.32 1.85 1.5	SCCP (2011) Patlewicz, *et al*, (2002) IFFI	Calculated Calculated Measured
Flash point	135.1 °C	SCCP (2011)	-
Flammability	Highly flammable above 100 ^{0}C	INCI	-
Explosive properties	-	-	-
Self-ignition temperature	-	-	-
Oxidising properties	-	-	-
Granulometry	-	-	-
Stability in organic solvents and identity of relevant degradation products	-	-	-
Dissociation constant	-	-	-
Viscosity	-	-	-

* International Flavours & Fragrances Inc., Takasago International Corp.

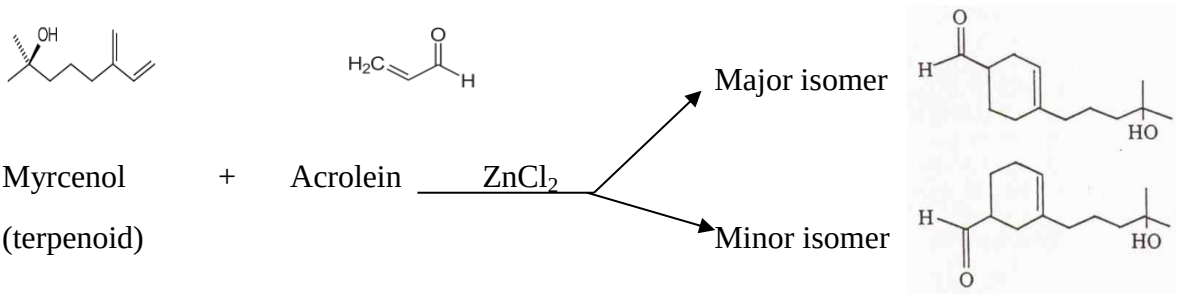
2. MANUFACTURE AND USES

2.1. Manufacture

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) or hydroxymethyl pentylcyclohexene carboxaldehyde (HMPCC) is usually known by the trade name Lyral® in Europe and the US. It is manufactured by the International Flavours and Fragrances Inc. (New York, NY, U.S.A.) since 1960. It is also available from Japan by the commercial name Kovanol® produced by Takasago International Corp., Tokyo (Japan). Its other trade names are Cyclohexal, Muganol, Landolal, Leerall, Lydoucal and HydroxyEmpetal (IFRA, 2008). HICC is supplied in Europe by companies in France, Germany and the UK and produced in China and India under the same name, Lyral. The amount currently produced or imported to Europe is not clear. However, the global consumption in 1996 was 520 tons, and in 2004 it was 1000 tons (SCCP, 2004), while the production was estimated

to have been about 2000 tons in the year 2000 (Rieger, 2001). An increase in the production volume would be expected as the consumption has escalated.

This synthetic aldehyde has a sweet odour reminiscent of lily of the valley. The chemical synthesis process entails a Diels-Alder reaction of myrcenol and acrolein catalyzed by Lewis catalysts, such as zinc chloride, that gives a mixture of two aldehydes as well described by Bauer, *et al.* (1988; 2001), Rieger (2001) and Frosch, *et al* (2001). The preferential synthesis of this reaction is depicted below. HICC, usually called by its common trade name Lyral®, is a mixture of the two isoforms 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carboxaldehyde) with CAS No. 31906-04-4 (major isomer) and EC no. 250-863-4 and 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carboxaldehyde) with CAS No. 51414-25-6 and EC No. 257-187-9 (minor isomer). The mixture contains these two compounds in a ratio of 70:30, major isomer:minor isomer (Bauer, *et al*, 2001; SCCS, 2011). The two isomers also have different odours (Rieger, 2001).



4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carboxaldehyde (Major isomer) is the dominant constituent while the second isomer (3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carboxaldehyde) is a minor constituent occurring in a ratio of 70:30, respectively (Bauer, et al, 2001;Frosch, et al, 2001; SCCP, 2011).

2.2. Identified uses

HICC is known to be used as a fragrance and masking agent in personal care products, household cleaners, air fresheners and detergents including surface cleaners.

3. CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1. Relevant physic-chemical properties

Not evaluated.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

No toxicokinetic studies are available for HICC. However from structural and chemical considerations as well as mechanistic studies on related skin sensitizer terpenes and inherent toxicological properties of HICC the following evaluation is suggested. HICC is a small lipophilic turpentine-based aldehyde as it is a reaction product of a diene molecule (Myrcenol) and dienophilic Acrolein. It is a substituted cyclohexene with a functional group –CHO similar to Citral while its –OH group can act similar to that of Hydroxicitronellal. Both citral and hydroxicitronellal are skin sensitizers via a mechanism of metabolic activation in the skin and share the same property during skin penetration and metabolic activation in the cutaneous environment (Roberts, *et al*, 2007). Citral is a highly electrophilic monoterpene that reacts very actively in the nucleophilic cutaneous environment which is naturally rich in water and with electron rich nucleophilic proteins that have active functional groups. Thus in the skin the thiol groups (-SH) in cysteine, the primary amines ($-NH_2$) in lysine, or other nucleophilic amino acids such as histidine (=N-), methionine (-S-) and thyrosine (-OH) are known to interact with the -CHO functional groups of the fragrances (Karlberg, *et al*, 2008). The attacking nucleophile is an amine for the mechanistic domain on the fragrance forming Schiff base (Chipinda, *et al*, 2011). From its structural properties HICC is a class 1 Schiff base aldehyde with a tertiary hydroxyl group susceptible to acid catalysis to form an unsaturated bond upon dehydration. HICC is therefore potentially capable of binding through the Schiff's base mechanism to the ε-group of lysine or the α-amino N-terminal amine group (Gimenez-Arnau, *et al*, 2002; Patlewicz, *et al*, 2002).

Citral has a molecular weight of 152.2 and a partition coefficient (logP value, lipophilicity) of 2.54 whereas HICC has a molecular weight of 210 and logP of 2.89 (Gerberick *et al*, 2005). Both of these two substances are low molecular weight compounds and have close logP values. Citral is a very fast skin penetrant, and since HICC has a similar lipophilic activity and low molecular weight as citral, HICC is assumed to be able to penetrate the cutaneous membrane likewise. Thus HICC readily gets absorbed into the stratum corneum where it may further bind to cutaneous proteins after metabolic activation. Recent studies have found that Cytochromes (CYPs) including Cyp 1A1, 1B1, 2B6, 2E1 and 3A5 are expressed in the skin, and could be involved in the bioactivation of HICC. Thus prohaptens or weak allergens could be activated to highly potent forms upon entry into the skin. Therefore, metabolic modifications could give rise to unsaturation where activated oxygen species will be trapped so that epoxide and hydroxide intermediates could be generated. These intermediates are known to alkylate DNAs, proteins and other biomolecules (Chipinda, *et al*, 2011). Compounds such as citral are also known to deteriorate when exposed to light, heat, oxygen and acids. Subsequently therefore, it is subject to react with nucleophilic cutaneous proteins. HICC has been found to be phototoxic *in vitro* via an oxygen-dependent mechanism leading to membrane damage (Eun, *et al*, 2004) and it is likely that such factors as light, heat and oxygen facilitate the formation of a hapten-protein complex that further initiates activation of dendritic cells to process HICC and further present it to the nearest lymph nodes. As a result, the dendritic cells recruit naïve Th1cells and memory will be developed by a portion of these Th1 cells. Upon re-exposure through the dermal route from skin applications of the substance, these memory cells will be activated to migrate to the site of exposure which eventually leads to an inflammatory response expressed as skin allergy and allergic contact dermatitis. This proposed mode of action indicates that HICC will unlikely be available to the circulation and therefore not will be available to be transported for elimination. It will instead be bound to proteins in the skin and gets modified to be a potent antigen. Furthermore, as a result of frequent exposure to such allergens as HICC, continuous induction of Phase II detoxification enzymes together with the build-up of reactive oxygen species (ROS) may

lead to depletion of these enzymes and the antioxidant pool which aggravates the reaction on the skin of ACD patients (Redeby, *et al*, 2010).

4.2 Acute toxicity

Not evaluated.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated.

4.4 Irritation

Not evaluated.

4.5 Corrosivity

Not evaluated.

4.6 Sensitization

4.6.1 Skin sensitization

Table 16: Summary table of relevant skin sensitization studies

Table 16 a Human Studies

(i) Population studies

Table 16 a (i) summarizes over 40 patch test studies on HICC involving several thousand dermatitis patients from various countries in Europe and elsewhere. Most of the studies are diagnostic patch test studies. Diagnostic patch testing is conducted in order to diagnose contact allergy to a substance and is performed according to international standards by dermatologists. Such tests do not say anything about the clinical relevance, i.e. if the identified positive patch test result relates to the ACD a patient suffers from. Therefore a use test with the patient's products may be performed or a Repeated Open Application Test (ROAT) in order to verify a contact allergy which has been diagnosed by patch testing. Studies of diagnostic patch testing is usually reported as positive patch test frequencies, e.g. number of patients having a positive patch test result in relation to the total number of patients tested, as well as the percentage of positives. It is important to note how patients or individuals have been selected for patch testing; if all patients at a clinic with suspected ACD are patch tested they are often called *consecutive* patients at the clinic. Sometimes more *aimed* patch testing is performed among patients from a certain work environment or where exposure to certain groups of allergens, such as preservatives, fragrances or pigments, is suspected. In aimed patch testing the frequency of positive patch test results is usually higher than among consecutively tested patients at a clinic. This needs to be considered when evaluating the results.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE

Most of the studies below are key studies. The selection of patients in the studies has been reported which is necessary to know for the evaluation of the given positive patch test frequencies. The information which is considered to be relevant for the evaluation of human patch test studies for classification purposes has been summarized for each study.

Method	Results	Remarks	Reference
Patch test: Retrospective and descriptive study on 37 860 eczema patients patch tested with HICC 5% in petrolatum in the base line series.	928/37 860 (2.5%) patients were positive with a minimum of 2.1% in 2003 and a maximum of 2.8% in 2008. No change in prevalence over the last 9 years was found despite restrictions by IFRA[1]. HICC still remains a very relevant and frequent cause of allergy.	Retrospective descriptive analysis of a patch test study from the clinical database of the Danish Contact Dermatitis Group (DCDG[2]), Denmark (2003-2011).	Heisterberg, *et al*, 2012 (Key study)
Patch test: Retrospective and descriptive study of 25 181 dermatitis patients in 10 European countries using HICC 5% in petrolatum.	Rates vary between 0.8-2.9% with the exception of Poland which had a lower value (0-1.4% in 256 patients).	Data from Austria, Denmark, Finland, Germany, Italy, Lithuania, Poland, Spain, Switzerland and the UK were used (2007-2008). HICC is the most important single allergen in the FM II[3] series which is included in the European baseline series.	Uter, *et al*, 2012 (Key study)
Patch test: Retrospective and descriptive time trend analysis of previous patch test data with HICC 5% in petrolatum.	Sensitization to HICC remains frequent in European patch test populations. In Germany, Austria and Switzerland increase in frequencies between 1.8% and 2.5% observed. Until 2008 no decrease in frequencies seen. In 2009 the first sign of decrease to 1.8% observed but not conclusive.	Time trend analysis of registered data under the Information Network of Departments of Dermatology (IVDK[3] network (Austria, Germany and Switzerland) (2000-2009).	Thyssen, *et, al* 2012 (Key study)
Patch test: Retrospective and descriptive study on 84 733 eczema patients patch tested with HICC 5% in petrolatum as part of the base line series.	227/10 097(2.1%) patients for 2010 and 223/10 794 (2.12%) patients for 2011 had positive reactions to HICC; annual crude prevalence did not show decline.	Time trend analysis of registered data under the IVDK network (Austria, Germany and Switzerland) (2002-2011). Further continuous surveillance recommended.	Schnuch, *et al*, 2012b (Key study)
Patch test: Retrospective and descriptive study on1508 eczema patients patch tested with HICC 5% in petrolatum.	34/1508 (2.5%) had clear positive reactions and 27 were clinically relevant.	Retrospective descriptive analysis of a patch test study at Gentofte Hospital, Denmark (2008-2010).	Heisterberg, *et al*, 2011a (Key study)
Patch test: Retrospective and descriptive study on 74 611foot/hand allergic dermatitis patients patch tested with HICC 5% in petrolatum.	1716/74 611 (2.3%) of patients had positive reactions.	Cross-sectional study of registered data by 59 departments within the IVDK network (Austria, Germany and Switzerland) (2001-2010).	Landeck, *et al*, 2011 (Key study)
Patch test: Retrospective and descriptive study on 629	17/629 (2.7%) patients were positive.	A retrospective study on patch test data at Lisbon Hospital,	Carvalho, *et al*, 2011(Key

consecutive dermatitis patients patch tested with HICC 5% in petrolatum.		Portugal (2007- 2009). Inclusion of HICC in the European base line series proposed.	study)
Patch test: 816 consecutive patients suspected with allergic contact dermatitis tested with HICC 5% in petrolatum in the European baseline series.	7/816 (0.9%) patients were positive.	A retrospective analysis of IVDK data in Lithuania, 2006-2008. The low rate was associated with the low exposure.	Beliauskiene, *et al*, 2011
Patch test: Retrospective and descriptive study on 12 302 consecutive fragrance allergic patients patch tested with HICC 5% in petrolatum.	292/12 032 (2.4%) patients were positive; 224 (76.7%) of these were clinically relevant.	Retrospective study on the DCDG database in Denmark (2005-2008).	Heisterberg, *et al*, 2010 (Key study)
Patch test: Retrospective and descriptive study on 37 270 dermatitis patients tested with HICC 5% in petrolatum.	880/37 270 (2.4%) patients had positive responses.	Retrospective analysis of IVDK data in Germany (2005-2008).	Uter, *et al*, 2010 (Key study)
Patch test: Retrospective and descriptive study on 35 633 dermatitis patients tested with HICC 5% in petrolatum.	836/35 633 (2.4%) reacted positive; HICC was identified as the most important allergen in FM II[4].	Retrospective study on patch test results of the German IVDK surveillance (2005-2008).	Krautheim, *et al.*, 2010 (Key study)
Patch test: Retrospective and descriptive study on 1253 dermatitis patients tested with HICC 5% in petrolatum.	9/1253 (1.1%) patients were positive. Fragrances in total had 117/1253 (9.3%) positive reactions.	Retrospective analysis of patch test data in Spain (2004-2008).	Cuesta, *et al*, 2010 (Key study)
Patch test: Retrospective and descriptive study on 1137 dermatitis patients patch tested with HICC 5% in petrolatum.	8/1137 (0.7%) of patients were positive.	Retrospective analysis of patch test data in Iran (2004- 2008).	Firooz, *et al*, 2010
Patch test: 320 patients patch tested with HICC 5% in petrolatum.	10/320 (3.1%) patients were positive; 61% of the reactions were clinically relevant. HICC was the leading cause of allergy in the Fragrance Mix series.	Groningen, the Netherlands (2005-2007).	van Oosten, *et al*, 2009 (Key study)
Patch test and ROAT[5]: 15 HICC-allergic patients were tested with a dilution series of HICC in patch test and a ROAT.	The ROAT threshold in dose per area per application was lower than the patch test threshold.	Clinical study at Gentofte and Rødovre, Denmark. Year not stated.	Fischer, *et al*, 2009 (Key study)
Patch test, ROAT and predictive model using the CE-DUR model[6]: Patch testing was made using 2.5% and 5% HICC in petrolatum applied for two weeks. Use test ROAT was done with 64 dermatitis patients in two preparations (a) with 0.005%, 0.01%, 0.1%, 0.5% and 2.5% in 15% glycerol stearate in water (cream) and (b) with the same grades of concentration but in 96% ethanol (perfume).	ROAT concentration and patch test strength were inversely related. Of the 52 ROAT-positive subjects with cream 50 (96%) were patch test confirmed and of the 49 perfume-positive subjects 48 (98.0%) were patch test confirmed. Based on clinical sensitization frequency, 2.5% for HICC, the prevalence of HICC allergy in the general population in Germany in 10 years was estimated as 233 209 people (0.28% of the German	IVDK study in Austria, Germany, Spain and Switzerland (2005-2007). Confirmatory patch test employed for Lyral® 2.5% and 5% gave clear results for the later concentration to be preferred. ROAT was found useful for evaluation and identification of optimal patch test preparations. The elicitation thresholds for cream were lower than the minimum limits set by IFRA in 2003 and in 2009. For perfume no clear limit was recommended. Readjustment of IFRA's standard	Schnuch, *et al*, 2009 (Key study)

	population). At a threshold of 90 ppm (0.009%) HICC in cream and 270 ppm (0.027%) in perfume a theoretical risk was calculated that 2300 (0.028%) persons would get relapse of dermatitis. With a threshold of < 1791 ppm (0.18%) for cream and < 3420 ppm (0.34%) for perfume, the number was 116 605 (0.14%).	requested.	
Patch test and use test: 15 patients positive to 5% HICC in petrolatum were patch tested with 0.0006%-6% HICC in ethanol. Use test was conducted on products with 200 ppm, 600 ppm and 1800 ppm HICC.	In patch tests thresholds for elicitation was 25 ppm for 10 % of HICC allergies and 610 ppm for 50% of HICC allergies. In use test 9/14 patients (64%) reacted to deodorants containing 200 ppm HICC. Common usage concentration and the 1.5% cut-off value recommended by IFRA (2003) were found far from safe. A concentration limit of 200 ppm in deodorants will not provide adequate protection against allergic contact dermatitis.	Gentofte and Odense Hospitals (Denmark) (Year not stated). Only trace amounts may be used in deodorants.	Jørgensen, *et al*, 2007 (Key study)
Patch test and use test (ROAT): 1701 consecutive hand eczema patients tested with 0.5%, 2.5% and 5% lyral[7] (HICC) in petrolatum; 11 of the HICC positive patients performed use test.	Lyral was the predominant individual fragrance with a positive rate of 1/22 (4.5%) of patients to 0.5%, 18/49 (36.7%) to 2.5% and 26/70 (37.1%) to 5%. Both at 2.5% and 5% HICC clear positive reactions predominated in all centres. 5/11 patients had positive use test; 19/24 (79.2%) of patient products had 0.017-3.832% HICC content.	Multicenter study at 4 centers in Europe (Copenhagen, Leuven, London and Dortmund). Year not stated. In all centers the main site of eczema was on hands. Routine testing with HICC in diagnostic patch test strengthened with more evidence.	Frosch, *et al*, 2004; 2005b (Key studies)
Patch test: 2901 consecutive dermatitis patients were patch tested with HICC 5% in petrolatum.	62/2901 (2.1%) patients were positive to HICC.	Leuven, Belgium as part of the base line series (1999-2005).	Nardelli, *et al*, 2008 (Key study)
Patch test: Retrospective evaluation of data on 53 anogenital allergic patients patch tested with HICC in petrolatum.	2/53 (3.77%) patients were positive and one of them had a clinically relevant reaction. Avoidance of products containing HICC brought relief.	Retrospective evaluation; Amsterdam, The Netherlands (2002-2006). Concentration and vehicle is not mentioned.	Vermaat, *et al*, 2008 (Key study)
Patch test: 18 789 ACD patients tested with Lyral® (HICC) 5% in petrolatum.	An increase from 2.1% in year 2003 to 2.8% in year 2007 in rate of positive reactions to HICC recorded; 49% of cases were clinically relevant.	Gentofte hospital, Denmark (2003-2007). No absolute numbers of positive cases mentioned. No effect observed due to IFRA's recommended concentration limit in 2003.	Braendstrup and Johansen, 2008 (Key study)
Patch test: Retrospective evaluation of patch test data using HICC 5% in petrolatum.	HICC was found as the most common sensitizer in Europe at a rate of 1.5-3%.	Patch test data from the European multicenter studies between1998 and 2007 were used to draw conclusions on the effects of	Bruze, *et al*, 2008 (Key study)

		HICC by the ESCD[8] and EECDRG[9].	
Patch test: Retrospective study on 21 325 patients patch tested with 5% HICC in petrolatum.	502/21 325 (2.4%) positive reactions were observed to HICC. It was found as an important allergen.	Multicenter study in 40 dermatology departments in Austria, Germany & Switzerland (2003-2004). Further studies in other European countries were recommended along with restriction/banning of HICC.	Schnuch, *et al*, 2007 (Key study)
Patch test: Retrospective and descriptive study on 701 dermatitis patients tested with their own products and 5% HICC in petrolatum.	33/701 (4.7%) patients reacted positive to HICC; 82.8% of those positive to consumer products were also positive in patch test.	Part of the IVDK study in 40 centers in Germany (1998-2002).	Uter, *et al*, 2007 (Key study)
Patch test: 1314 consecutive dermatitis patients patch tested with HMPCC[10] (HICC) 5% in petrolatum).	27/1314 (2.1%) patients were positive. HMPCC (HICC) was identified as a necessary addition to be tested for in a broad-spectrum diagnosis of contact allergy to fragrances.	Study performed at the Ludwig-Maximilians University dermatology and allergology clinic, Munich, Germany (2004-2005)	Oppel, *et al*, 2006 (Key study)
Patch test: 170 dermatitis patients were patch tested with Lyral® (HICC).	2/170 (1.2%) patients were positive.	Patch tested in Spain (2005). Confirmed as an important allergen and recommended for inclusion in the GEIDC[11] despite the small sample size. Concentration and vehicle not mentioned.	Heras, *et al*, 2006 (Key Study)
Patch test: 3 558 atopic dermatitis and 5947 non-atopic dermatitis patients were tested with HICC 5% in petrolatum.	99/3558 (2.8%) atopic and 117/5947 (2%) non-atopic patients were positive. The pattern and frequencies of observed sensitizations did not differ between the two groups.	Retrospective analyses of IVDK data (1998-2003) collected from Austria, Germany and Switzerland.	Heine, *et al*, 2006 (Key study)
Patch test: 422 patients suspected with contact allergy tested with HICC 5% in petrolatum.	7/422 (1.7 %) patients were positive.	Multicenter study in 9 departments in Korea (2002-2003).	Eun, *et al*, 2004; An, *et al*, 2005 (Key studies)
Patch test: HICC was tested on 99 geriatric nurses with occupational hand dermatitis.	1 nurse reacted positive.	At the University of Osnabrük, Germany (2003). Dose and vehicle not specified.	Schüerer and Schwanitz, 2004
Patch test: Retrospective and descriptive study on 220 female hairdressers and 303 dermatitis patients patch tested with HICC.	8/220 (3.7%) of the hairdressers and 5/303 (1.6%) of the patients were positive.	IVDK study in Austria, Germany and Switzerland during the period 2001-2002. Dose not mentioned. Hairdressers had occupational hand dermatitis while the clients had dermatitis on the head.	Uter, *et al*, 2003 (Key study)
Patch test on 766 consecutive dermatitis patients with 5% HMPCHC[9], HICC).	16/766 (2%) patients reacted positive; 62% of the positive reactions were of current relevance while 19% of possible relevance in the past. 38% had eczema on face and neck, 31% on hands , 19% on axillae and 6% on trunk.	A 12 months' study in Leeds, the UK; year not stated.	Baxter, *et al*, 2003 (Key study)

Patch test and use test: 18 eczema patients with positive response in a previous study to 5% HICC were patch tested with 0.5% and 3% w/v HICC in pure ethanol.	16/18 (89%) patients had positive use test; 11 reacted to 0.5% and 5 to 3% HICC. All controls were negative. Dose-response curve from patch testing gave 29 ppm (0.9µg/cm^2) to cause elicitation in 10% of the group and 662 ppm (20µg/cm^2) in 50%.	Multicenter study (Dortmund, Gentofte, Malmö and Odense); year not stated. 1-10µg/cm^2 (0.02-0.3% depending on the type of product) suggested as safe level of exposure. Need to reduce use concentration underlined.	Johansen, *et al*, 2003 (Key study)
Patch test on 658 consecutive eczema patients using 5% Lyral® (HICC) in petrolatum.	14/658 (2.1%) patients reacted positive.	Multicenter study (Gentofte, Malmö and Odense) (2001-2002).	Heydorn, *et al*, 2003a (Key study)
Repeated skin exposure using hand immersion: 15 hand eczema patients patch test positive to HICC were hand-immersed as in real-life exposure in 10-250 ppm HICC solutions.	Patients were negative.	Malmö, Odense and Gentofte (2001-2002).	Heydorn, *et al*, 2003b
Patch test: 315 ACD patients tested with 5% lyral (HICC) in petrolatum.	4/315 (1.3%) patients were positive.	Consecutive patients in Gentofte, Malmö and Odense (2001-2002).	Heydorn, *et al*, 2002 (Key study)
Patch test: 1% and 5% lyral® (HICC) in petrolatum were tested enrolling 1855 consecutive dermatitis patients.	Lyral had rates from 1.2-17%; Overall or on the average 50/1855 (2.7%) patients were positive to 5% HICC. Found to be the highest in incidence among all individual sensitizers.	First European Multicenter study (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998). 5% lyral in petrolatum recommended as most appropriate for diagnostic patch testing.	Frosch, *et al*, 1999; 2002 (Key studies)
Patch test: 3245 consecutive patients tested with lyral (HICC) 5% in petrolatum.	62/3245 (1.9%) patients showed a positive reaction. Only slight concordance between positive reactions to lyral and FM II series observed.	20 dermatology clinics in Germany (2000-2001). Lower frequency than a former multicentre European study but closer to the previous German centre. As a result HIC 5% in petrolatum was included in the standard series in Germany.	Geier, *et al*, 2002 (Key study)
Patch test: 1% and 5% Lyral® (HICC) in petrolatum tested on 106 consecutive patients.	1/106 (0.9%) patient reacted to 1% lyral in petrolatum; 3 (2.8%) patients showed clinically important positive reactions to 5% lyral in petrolatum.	Part of the first inter-European group multicenter study. Year not stated. Barcelona was the only center that patch tested with 1% and 5% lyral in petrolatum; 5% in petrolatum was identified as convenient.	Frosch, *et al*, 1995 (Key study)
Patch test: 119 dermatitis patients were tested with 2% HICC.	1/119 (0.84%) patient reacted positive	Multicenter study, the Netherlands (1987).	de Groot, *et al*, 1988 (Key study)
Patch test, ROAT: 75 dermatitis patients allergic to cosmetics were tested with HICC and cosmetics products.	1/75 (1.3%) patient reacted positive.	The Netherlands, (1981-1986). Dose not mentioned.	de Groot, *et al*, 1987 (Key study)
Patch test: 16 dermatitis patients, 27 eczema patients, and 10 control subjects were tested with 5% HICC.	No positive reaction was observed.	Japan, 1978-1980. Vehicle not described.	Ishihara, *et al*, 1981

Patch test: 55 dermatitis patients and 9 control subjects were tested with HICC 5% in petrolatum.	No positive reaction was observed in all.	Japan. Year and vehicle not described.	Ishihara, *et al*, 1979

[1]IFRA, International Fragrance Association; [2]DCDG, Danish Contact Dermatitis Group; [3]IVDK, Information Network of Departments of Dermatology; [4]FM II, Fragrance Mix II series; [5]ROAT, Repeated Open Application Testing; [6]CE-DUR, Clinical Epidemiology-Drug Utilization Research. [7]Lyral®, trade name for HICC [8]ESCD, European Society of Contact Dermatitis; [9]EECDRG, European Environmental Contact Dermatitis Research Group; [10]HMPCC or HMPCHC, Hexyl methylpentyl cyclohexylcarboxaldehyde, other name for HICC; [11]GEIDC, Spanish Contact Dermatitis Group.

(ii) Case studies

Table 16 a (ii) below shows case reports with ACD in different clinics in Europe and the US where HICC has been found as a causative agent. Two of the studies have great relevance and have been considered as key studies. One of them was associated to patch test and use test and described the structure activity relationship of HICC to its intrinsic allergenic potential (Giemenez-Arnau, *et al*, 2002). The second study has clearly shown its sensitizing effect at low concentrations together with the use of controls (Handley and Burrows, 1994).

Method	Results	Remarks/Comments	Reference
Patch test: A 76-year old female with chronic neck and face dermatitis was patch tested with 5% HICC in petrolatum.	Positive reaction was observed.	Case study, USA (2007).	Zirwas and Bechtel, 2008
Patch test: A 65-year old male with axillary ACD patch tested with 5% HICC in petrolatum.	Positive reaction was observed.	Case study, USA (2008).	Jacob, 2008
Patch test and use test: A 50-year old female dermatitis patient with eczema was tested with her own eau de toilette product and its components including 2% HICC in petrolatum.	Patient was positive to her own product, its components and HICC.	Case study, Spain (2001).	Giemenez-Arnau, *et al*, 2002 (Key study)
Patch test: A 37 year-old female with ACD to cosmetics was tested with 5% HICC in petrolatum.	Positive reaction was observed.	Case study, France (1999).	LeCoz and Goldberg, 2002
Patch test: A 22-year old female with severe axillae dermatitis tested with 10% HICC in petrolatum.	Positive reaction was observed	Case study, The Netherlands (1998).	Hendriks, *et al*, 1999
Patch test: A 28-year old male who had ACD and 20 controls were tested with 0.075%, 0.125% and 0.25% HICC in petrolatum.	Patient reacted positive to all concentrations. None of the controls reacted positive.	Case study, UK (1993).	Handley and Burrows, 1994 (Key study)
Patch test: A 22-year-old man with axillary dermatitis was tested with 5% HICC.	No positive reaction was observed.	Case study; Year and vehicle not stated.	Larsen, *et al*, 1983

Table 16 b Animal studies:

Table 16 b has documented two animal studies with LLNA. In one of them an EC3 value of 17.1 was demonstrated. It was shown that HICC has a concentration-dependent activity. The QSAR data show that HICC is a Class 1 Schiff base aldehyde with high protein affinity related to its moderate Log P value.

Method	Results	Remarks/Comments	Reference
LLNA[12], according to OECD[13] test guideline 429. 2.8%, 8.3%, and 25% HICC and in mixture with other fragrances.	HICC caused a clear concentration-dependent induction of local inflammation. The mixture had two- to three-fold increase in the SI values of both CD8+ and CD4+ cells over the individual fragrance. Testing with a mixture of fragrances including HICC had the highest effect where the highest concentration induced 130% ear thickness as compared to the control group.	The final concentration of the mix was the same as the respective HICC concentration. Concentration-dependent increase in infiltration of immune cells and same effect on the stimulation index (SI) observed.	Bonefeld, *et al*, 2011
LLNA, according to OECD test guideline 429. Lyral®(HICC) was evaluated in LLNA and for structure activity relationships to develop a QSAR[14] model.	Lyral® was shown to have an EC3 value of 17.1 in the LLNA and is therefore a sensitizer.	It was classified as a Class 1 Schiff base aldehyde with hydrophobic activity of 1.85-2.89 (log P). Lyral was found capable of reacting with proteins.	Patlewicz, *et al*, 2002 (Key study)

[12] LLNA, Local Lymph Node Assay; [13]OECD, Organization for Economic Cooperation and Development; [14]QSAR, Quantitative Structure Activity Relationship.

Table 16 c *In vitro* studies:

Table 16 c contains *in vitro* studies on the phototoxic properties of HICC. In the first study HICC and 18 other fragrance ingredients were tested employing two phtotoxicity assays. HICC has been identified to have photohemolytic properties using oxygen-dependent membrane damage whereas all the other tested fragrance ingredients were negative to the tests. The second study has demonstrated the phototoxic effect of HICC along with the strength of its activity being dependent on the closeness of the UVA source and use of sunscreens or protective substances. These two studies have identified the phototoxic nature of HICC and explained the mechanism of cell damage which contributes to photoallergic contact dermatitis. The other two studies have shown supportive results making HICC phototoxic.

Method	Results	Remarks/Comments	Reference
In vitro Phototoxicity study of lyral and 18 other fragrance ingredients with Photohemolysis Test and 3T3NRU phototoxicity Test.	Lyral was positive to Photohemolysis test therefore it is phototoxic. All the other fragrance ingredients were negative in both tests.	The mechanism of phototoxicity is through oxygen-dependent membrane damage.	Eun, *et al*, 2004
In vitro phototoxicity test using *Saccharomyces cerevisiae* with 5% HICC in methanol on agar overlay inhibition assay. Plates were exposed to UVA[15] at 10.5 cm for 7 hours and compared with a reference phototoxic substance 8-methoxylsoralen (8-MOP)	Phototoxic activity of HICC observed was 0.01% of the reference material 8-MOP. Addition of benzophenones or sunscreen agents reduced or eliminated the phototoxic activity of HICC.		DiNardo, *et al*, 1985
In vitro phototoxicity test using *Saccharomyces cerevisiae* with 5% HICC in methanol on agar overlay inhibition assay. Plates were exposed to UVA at 31cm for 18 hours and compared with a reference phototoxic substance 8-methoxylsoralen (8-MOP).	Phototoxic activity of HICC was 0.004% of the reference material 8-MOP.		Tenenbaum, *et al*, 1984
In vitro phototoxicity test using Fleischman's Baker's yeast with 0.1%, 1% and 10% HICC.	0.1% and 1% HICC did not have phototoxic effect while the 10% did.		Weinberg, *et al*, 1981

[15]*UVA, Ultraviolet-A has a wavelength of 315-400nm.*

4.6.1.1 Non-human information

The aldehyde HICC is a lipophilic turpentine-based molecule with a high capability of penetrating the skin. Structurally, HICC is a simple aliphatic aldehyde (class 1 aldehyde) in which the carbonyl group is a hard electrophile, and is expected to react with the amino groups of lysine residues on proteins via Schiff base formation (Patlewicz, *et al*, 2003). HICC possesses a tertiary hydroxyl group likely to be susceptible to acid catalyzed dehydration *in vivo* resulting in loss of water and so forms an unsaturated bond (Patlewicz, *et al*, 2002; Chipinda, *et al*, 2007). This property, thus,

makes it to be more reactive in the skin rendering further subsequent interactions with proteins provoking allergic reactions.

Structure Activity Relationship (SAR) analysis and LLNA studies have shown that, beside the capacity to react with proteins in the skin, the hydrophobic properties of a molecule are important as they enable skin penetration (Karlberg, *et al*, 2008). Thus, HICC, as a low molecular weight compound, has been found to have a hydrophobic activity (partition coefficient) between 1.5 and 3.5 (log P) (Patlewicz, *et al*, 2002; Gerberick, *et al*, 2005; IFFI, 2006; SCCS, 2011). The EC3 value in LLNA was 17.1% thereby demonstrating its sensitizing properties (Patlewicz, *et al*, 2002).

In addition to this HICC has been found *in vitro* to be a phototoxic fragrance when studied by both 3TS NRU phototoxicity test and photohemolysis test (Eun, *et al*, 2004). Thus this fragrance compound has been suggested to have a potential to cause DNA or cellular damage and oxygen-dependent membrane damage upon skin exposure which may contribute to photoallergic contact dermatitis.

Besides, it could be noted that the absence of high potency for HICC in the LLNA test doesn't mean low sensitizing properties in humans similar to what has been observed in nickel allergy. Where there was no significant response from mice on the LLNA test for nickel it still showed severe sensitizing effects on humans. The reason behind this difference has been clarified recently as the response to be dependent on the presence or absence of a specific receptor to trigger activation of the allergen as observed by Schmidt, *et al*, (2010) while defining the immunological bases of this species difference. The relevance of such a critical observation during characterization of skin sensitizing chemicals has been well stressed by Kimber, *et al* (2011).

Likewise, although the EC3 value of 17.1% in the LLNA assay categorises HICC as a moderate skin allergen (Patlewicz, *et al*, 2002; Gerberick, *et al*, 2005) the high frequency of sensitization in patch tests should make it an important sensitizer in humans.

4.6.1.2 Human information

As shown in Table 16, since 1987 clinical reports have documented that HICC is a skin sensitizer. Much information from multicentre patch test studies in Europe began to accumulate in late 1990s and in the last decade. To date plenty of information has been compiled and analyzed to elucidate its role in induction and elicitation of contact dermatitis in humans. As a result of its clinical relevance in skin sensitization it has been tested in several centres in Europe and elsewhere involving several thousand patients (Frosch, *et al*, 1999; 2002; 2004; 2005a,b; Geier, *et al*, 2002; Johansen, 2003; An, *et al*, 2007; Schnuch, *et al*, 2007; Uter, *et al*, 2003; 2007; Bruze, *et al*, 2008; Nardelli, *et al*, 2008; Heisterberg, *et al*, 2010; Krautheim, *et al*, 2010; Heisterberg, *et al*, 2012; Schnuch, *et al*, 2012b). The results show that it is a major fragrance allergen, the positive patch test frequencies usually vary between 2 and 3% in dermatitis patients (2.7% on the average for Europe). Frequencies up to 17% have been documented.

A risk assessment model on induction thresholds has been used to determine a sensitization reference dose for various products. For fine fragrances this threshold dose was 10µg/cm^2 (0.02%) indicating that exposures exceeding this limit would likely induce sensitization (Gerberick, *et al*, 2001). And in a related view the SCCNFP in 2003 suggested a risk assessment model based on Johansen, *et al*, (2003), indicating that the safe level of exposure for the consumer is in the range of 0.9µg/cm^2 to 10µg/cm^2 (18 ppm to 200 ppm, respectively).

Patch tests and use tests (repeated open application test, ROAT) using products containing HICC have been conducted with the aim to identify the minimum elicitation doses or concentrations on patients. With patch testing elicitation thresholds around 25 ppm have been found on 10% of patients sensitized to HICC: 50% of patients reacted to around 650 ppm (Johansen, *et al*, 2003;

Jørgensen, *et al*, 2007). Use tests, with skin applications resembling normal use conditions, have indicated that 200 ppm is not a safe use concentration (Johansen, *et al*, 2003; Jørgensen, *et al*, 2007).

As an outcome of the extensive studies in early 2000, HICC was recommended for inclusion in national and European fragrance mix series for diagnostic patch testing, which was first made effective in Germany followed by other European countries (Baxter, 2003; Heras, *et al*, 2006; Braendstrup and Johansen, 2008; Schnuch, *et al*, 2009; van Oosten, *et al*, 2009; Cuesta, *et al*, 2010; Carvalho, *et al*, 2011). In 2008 it was recommended for inclusion in the European base line series for patch testing by the European Environmental Contact Dermatitis Group (EECDRG) and the European Society of Dermatitis (ESCD) (Geiger, *et al*, 2002; Bruze, *et al*, 2008; Davies, *et al*, 2011). Most of the aforementioned studies have also suggested its inclusion in the national series. The inclusion had also been extended to North America (Belsito, 2006).

IFRA (IFRA, 2003; 2009) and SCCNFP (2003) have made recommendations on maximum levels of HICC in consumer products. Recent comprehensive reviews (Heisterberg, *et al*, 2012; Schnuch, *et al*, 2012b; Thyssen, *et al*, 2012) have underlined that sensitization to HICC still remains frequent in Europe irrespective of existing measures. As a result the SCCS (2012) has proposed that it should not be used at all in consumer products.

Thus HICC skin allergy is currently a serious health issue. A recent comment by Basketter and White (2012) emphasized that as HICC is an entirely synthetic fragrance the only exposure is from its use as a fragrance in consumer products. Previous reviews have also concluded that it was through its wide use that it developed to be a leading cause of contact allergy (Bruze, *et al*, 2008; Heisterberg, *et al*, 2011b).

4.6.1.3 Summary and discussion of skin sensitization

HICC being an active protein-binder and lipophilic in nature, was shown to have a great potential to interact with skin proteins (Patlewicz, *et al*, 2002; Gerberick, *et al*, 2005). These properties are alerts for the potential to induce skin sensitization which have been demonstrated in both humans and animals.

Human data

Currently there is a lot of clinical evidence from diagnostic patch testing carried out in the last decade in several thousands of dermatitis patients in Europe and elsewhere demonstrating high frequencies of positive patch test reactions to HICC. This has rendered HICC to be a major sensitizer in Europe, positive patch test frequencies varying between 2 and 3% (average 2.7% with a top notation of 17%) and it was recommended for inclusion in the European baseline series for diagnostic patch testing in 2008 (Bruze, *et al*, 2008). Despite recommendations to limit the concentration of HICC in consumer products by IFRA and the SCCNFP (IFRA, 2003; SCCNFP, 2003) evaluations have revealed that its rate is still persistent as shown in recent national and European multicentre data bases (Heisterberg, *et al*, 2012; Schnuch, *et al*, 2012b; Thysssen, *et al*, 2012).

In the most recent recommendation by IFRA and the SCCNFP a maximum level of 200 ppm was recommended for different consumer products (IFRA, 2009; SCCNFP, 2003). However, use tests have demonstrated that this level is not safe for HICC allergic patients. Patch testing in patients has identified 25 ppm as an elicitation threshold among 10% of HICC allergics and 650 ppm as a threshold for 50% of the same group (Johansen, *et al*, 2003; Jørgensen, *et al*, 2007.

The Scientific Committee on Consumer Safety, SCCS (2012) has very recently stressed on the lack of safety at or even below the aforementioned recommended threshold value by IFRA and further recommended that HICC should not be used in consumer products at all.

The SCCS (2012) has concluded HICC to be an established contact allergen in humans. It belongs to fragrances of special concern due to the high number of published cases of allergy in scientific literature, more than 1500 cases since 1999. The SCCS considered the number of cases reported over the last decade to be exceptionally high; HICC was outstanding as compared to other fragrances. It was emphasized by the SCCS that the number of published cases is a severe underestimation of the real number of cases in the population as all cases are not published and all sensitized individuals don't attend a dermatological clinic.

Animal data

In the Local Lymph Node Assay (LLNA) the calculated EC3 value was 17.1%, which could be interpreted as moderate sensitising potency. The discrepancy between the animal data on HICC and the abundant human data demonstrating HICC to be a major sensitizer in humans could be attributed to the widespread use of HICC in consumer products making it possible to be repeatedly exposed to HICC from different kinds of products. However, considering the low levels of HICC in consumer products as found in market surveys, usually varying from ppm-level up to 0.6%, the high frequencies of human sensitization to HICC is remarkable.

Exposure to low concentrations

In 2009 IFRA recommended 200 ppm of HICC as a maximum level in finished products in order to prevent sensitisation. However, since then the incidences of contact allergy to HICC are still high in European clinics as shown in different studies (Heisterberg, *et al*, 2011a; Landeck, *et al*, 2011; Heisterberg, *et al*, 2012; Schnuch, *et al*, 2012b). Apparently the IFRA recommendations are not sufficiently protective. According to the CLP criteria the generic concentration limit that triggers classification as a skin sensitizer in subcategory 1A is ≥ 0.1%. This limit is higher than concentrations of HICC in consumer products causing sensitization. Therefore an SCL below the GCL is proposed. Lowering the GCL by a factor of 10, which will be below the 200 ppm recommended by IFRA, will give an SCL of 100 ppm or 0.01%.

4.6.1.4 Comparison with criteria

i) HICC is a widely used fragrance ingredient that causes skin sensitization. Several European clinical studies have documented its skin sensitizing properties. Therefore, HICC should be classified as a skin sensitizer according to Annex I of the CLP Regulation (1272/2008/EC) section 3.4.2.2. The criteria in Table 3.4.2 states that "evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons or if there are positive results from an appropriate animal test"**,** then the substance shall be classified as a skin sensitizer.

<u>The following human and animal data meet the criteria for classification of HICC as a skin sensitizer:</u>

Human data

- There are clear and abundant positive data from patch testing on HICC obtained from various multicenter studies involving several dermatology clinics and several thousands of patients over the last decade (Frosch, *et al*, 1999; 2002; 2004; 2005a,b; Geier, *et al*, 2002; Heydorn, *et al*, 2003a; Johansen, 2003; Uter, *et al*, 2003; 2007;

Schnuch, *et al*, 2007; Bruze, *et al*, 2008) and other independent clinical studies in Europe and abroad (Baxter, 2003; Heras, *et al*, 2006; An, *et al*, 2007; Braendstrup and Johansen, 2008; Vermaat, *et al*, 2008; Schnuch, *et al*, 2009; van Oosten, *et al*, 2009; Cuesta, *et al*, 2010; Heisterberg, *et al*, 2010; 2011; Carvalho, *et al*, 2011). The frequency of positive patch test reaction in tested consecutive dermatitis patients was usually between 2 and 3% but up to 17% has been reported.

- Epidemiological evidences have been well compiled along with the aforementioned studies demonstrating the high rate of sensitization caused by HICC (Uter, *et al*, 2007; Bruze, *et al*, 2008; Cuesta, *et al*, 2010; Krautheim, *et al*, 2010; Landeck, *et al*, 2011; Heisterberg, *et al*, 2012; Schnuch, *et al*, 2012a,b; Thyssen, *et al*, 2012).

Animal data

- An EC3 value of 17.1 was demonstrated in an LLNA study (Patlewicz, *et al*, 2002).

ii) According to table 3.4.2 "substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitization in humans". Such substances shall be classified in sub-category 1A.

<u>The following human data meet the criteria for classification of HICC in sub-category 1A:</u>

- Diagnostic patch test data from different European clinics with reported frequencies of sensitization to HICC between 1.2% and 17% with an average of 2.7. These frequencies are so high that in 2008 the European Society of Contact Dermatitis (ESCD) has recommended to include HICC in the European baseline series for diagnostic patch testing.

- More than 1500 cases of sensitization to HICC have been published in scientific literature. The number of cases was considered to be exceptionally high, though being subject to a severe underestimation of the real number of cases in the population (SCCS, 2012). For instance, based on the clinical sensitization frequency of HICC, 2.5% in Germany, Schnuch, *et al*, (2009) have estimated the prevalence of HICC sensitization in the general population in Germany in 10 years to be 0.28% of the population (233 209 persons).

iii) According to 3.4.2.2.2.1 human evidence for sub-category 1A can include "diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure; other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure"**.**

<u>The following data on exposure to HICC are evidence for the relatively low exposure of consumers to HICC:</u>

- HICC is a synthetic chemical substance. Therefore the only source of exposure is from fragrances added to consumer products. Available studies have suggested that HICC is capable of sensitization over a reference dose of 10µg/cm^2 (0.02%) (Gerebrick, *et al*, 2001) a threshold which was demonstrated to be not tolerated for

elicitation (Handley and Burrows, 1994; Johansen, *et al*, 2003; Frosch, *et al*, 2004; 2005b; Jörgensen, *et al*, 2007; Schnuch, *et al*, 2009) and

- Various studies on products from different markets across EU have demonstrated the concentration of HICC in consumer products to vary commonly between approximately 100 and 6300 ppm (0.01% and 0.63%). The concentration limit for classification in sub-category 1A is 0.1% and for 1B 1%. In the light of this the HICC concentration in consumer products must be regarded as low. Subsequently the exposure of consumers to HICC is low.

iv) The current general concentration limit for classification in sub-category 1A, 0.1%, is not sufficiently protective for induction of sensitisation to HICC. Therefore, lowering the GCL with a factor of 10 is proposed, resulting in an SCL of 0.01% (100 ppm).

The following evidence supports an SCL of 0.01% (100 ppm):

- IFRA (2009) recommended to lower the maximum concentration of HICC in finished products to 200 ppm in order to prevent sensitisation. However, it has not lead to decreased frequencies of positive patch tests to HICC in Europen clinics (Heisterberg, *et al*, 2011a; Landeck, *et al*, 2011; Heisterberg, *et al*, 2012; Schnuch, *et al*, 2012b). Therefore an SCL for classification below 200 ppm is warranted.

4.6.1.5 Conclusions on classification and labelling

During the last decades HICC has become a major human sensitizer in Europe; since 2008 it is included in the European baseline series for diagnostic patch testing in dermatological clinics. Based on its high frequency of sensitization in humans, despite the low concentration of HICC in consumer products, a classification of HICC as a skin sensitizer in sub-category 1A is proposed. Further, a specific concentration limit of 0.01% for classification of products containing HICC is proposed.

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) is a multi-constituent substance (reaction mass of 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde and 3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde) [1] composed of two isomers, i.e.: 70% of the major isomer (4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde) [2] and 30% of the minor isomer (3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde) [3]; differing in the 4- and 3-position of the aldehyde group, respectively. Classification as Skin Sens. 1A was proposed for both isomers, [2] and [3], as well as for the reaction mass [1]. Data on skin sensitisation is only available for the multi-constituent form [1] and for the major isomer [2], although the proposal also applies to isomer [3]. This is due to the fact that HICC is expected to bind proteins via a Schiff-base formation mechanism, which is independent of the position of the aldehyde group. Furthermore, the Dossier Submitter (DS) reported that there were no data to indicate any difference in potency between the two isomers.

The skin sensitisation properties of HICC have been demonstrated in both humans and animals. The DS summarised data from several thousand dermatitis or eczema patients showing positive patch test reactions to HICC, reported in Europe and elsewhere in retrospective descriptive analyses, patch tests and use tests published during the past decade

The EC3 value (effective concentration inducing a stimulation index of 3) resulting from the Local Lymph Node Assay (LLNA) was 17.1%, which indicates that HICC has moderate sensitising properties. However, the evidence from humans was sufficient for the DS to propose HICC as a skin sensitizer (Category 1A).

Despite the generally low concentrations of HICC in consumer products, varying approximately between 100 ppm (0.01%) and 6300 ppm (0.63%), the high number of positive human cases from patch testing could be attributed to the wide-spread use of HICC in different products, resulting in consumers being repeatedly exposed to this substance.

The current recommendation by the International Fragrance Association (IFRA) (2009) and the Scientific Committee on Cosmetic Products and Non-food products intended for Consumers (SCCNFP,2003) for a maximum level of 200 ppm for HICC in consumer products was considered not protective by the Scientific Committee on Consumer Safety (SCCS, 2012) and the DS. In particular, the DS stated that the generic concentration limit (GCL) for Skin Sens. 1A (≥ 0.1%) is higher than the usual concentration of HICC in consumer products which resulted in sensitisation, and that a high number of allergic reactions to HICC also occurred after the 200 ppm threshold was set by IFRA.Therefore, the DS proposes a specific concentration limit (SCL) of 100 ppm (0.01%) for HICC in consumer products.

Based on the high frequency of sensitisation by HICC shown to occur in humans, classification of HICC as Skin Sens. 1A is proposed by the DS, with an SCL of 0.01%.

A REACH registration dossier was not available at the time the CLH proposal was submitted to ECHA (see RCOM).

Comments received during public consultation

Comments from four Member State Competent Authorities (MSCAs) who supported the classification proposal for category 1A for skin sensitisation. In their detailed comments one MSCA argued that the results from patch testing of HICC fulfil the Skin Sens. 1A criteria based on both high frequency and low exposure, and that the additive exposure index (based on CLP Guidance) score = 4 when considering the use/exposure. Another MSCA found the proposal appropriate as the SCCS has already proposed the addition of HICC to Annex II to Regulation (EC) no. 1223/2009. One MSCA submitted a reference to a report from a Consumer Exposure Skin Effects and Surveillance (CESES) project reporting an 8 % positive response to HICC in patch testing performed during 2009-2012. One MSCA disagreed with an SCL of 0.01 %, as they claimed that it has not been documented that induction takes place at HICC concentrations < 0.1 %. They also questioned that an effect of the IFRA recommendation to limit the content of HICC to 200 ppm can be seen already, as products still contain 100-6300 ppm HICC. One MSCA critised the use of two risk assessment models (Johansen *et al.*, 2003; Gerberick *et al*., 2001), as the first model does not present any experimental data on HICC, and the other only provides data on elicitation, not induction. Another MSCA called for a justification on why an even lower SCL is not proposed (0.001 %), if the SCL should be based on extreme potency, but expressed doubt about whether the short time that has elapsed since the recommended concentration limit of 200 ppm has been implemented would be sufficient to already significantly influence the incidence of HICC sensitisation. One MSCA understood the concern that the GCL probably would not be sufficiently safe, but still

questioned the justification for the proposed SCL.

International Flavors & Fragrances Inc. (IFF) (on behalf of the SIEF for REACH registration) claimed that HICC should be classified as a skin sensitiser in sub-category 1B based on key animal studies, and that this key animal study (LLNA) was described in the REACH registration dossier. In addition IFF stated that the human repeat insult patch test (HRIPT), also provided in the REACH registration dossier, indicated absence of skin sensitisation up to 15 % (equivalent to 8000 μg/cm^2, i.e. > 500 μg/cm^2). With reference to Basketter *et al*. (2005) IFF, stated that the prevalence in diagnostic patch testing does not necessarily equate to the potency of the allergen, as they are indicative of elicitation and not induction of sensitisation. IFF further stated that the concentration of HICC applied in the reported diagnostic patch testing exceeds the concentration in consumer cosmetic products by far (1-5 % vs. maximum 0.63 %), and that the DS should discuss this in relation to the severity of the effect. IFF questioned the relevance of the CLP and REACH Regulations for cosmetics, and suggested that the DS distinguish between cosmetic and non-cosmetic products and that it should not use the SCCS opinion as the basis for a CLH proposal. They stated that SCL should normally be based on animal data according to the CLP Regulation, and not on human studies of elicitation. They noted that cosmetic products are complex mixtures and that there is uncertainty as to the causative ingredient(s) eliciting the reaction, and opposed the use of these mixtures as a basis for setting an SCL. Finally, IFF stated that the CLH proposal fails to meet the quality requirements for a CLH dossier, as robust study summaries are missing, and transparency and justifications are not well addressed. IFF questioned whether the REACH Endpoint specific guidance (Chapter R.7a), section R.7.3.4.2 (Human data on skin sensitisation) has been applied to address the uncertainty of the information in the clinical trials, and they considered that the validity of the diagnostic patch test has not been adequately described. Further, they provided their rationale for classification in sub-category 1B, based on animal data (the same study as reported by the DS, with an EC3 value > 2 %). IFF finds support for this sub-category in Human Repeat Insult Patch Test (HRIPT). In IFFs view, data from diagnostic patch testing can only be used for risk characterisation, and not for CLP because of the limited guidance. IFF questioned the DS justification that action is needed at community level. IFF denoted the data on HICC and phototoxicity as outside the scope of the CLP Regulation. IFF claimed that the timeline between IFRA (2009) recommending lower HICC concentration in consumer products and the latest publications (2012) is too short to show an increase or decrease in skin sensitisation from HICC. IFF agreed that the individual isomers of HICC as well as the reaction mass should be classified consistently for skin sensitisation, but in sub-category 1B and not in 1A. IFF agrees that HICC has a structural alert for skin sensitisation.

IFRA disagreed with the use of the SCCS opinion (SCCS/1459/11) as a basis for deriving conclusions under the the CLP Regulation, as CLP addresses hazards while SCCS addresses risks, and because cosmetic products are excempted from CLP. They stated that cosmetic products have different exposure scenarios from consumer products such as household and detergent products which are covered by the CLP Regulation. They also stated that the IFRA standard on HICC includes elements of elicitation, and not only induction, and thus is inadequate for concluding on an SCL to prevent induction. Finally they stated that according to CLP Guidance (3.4.2.5, ECHA 2012), an SCL should normally be based on animal studies. AISE (the International Association for Soaps, Detergents and Maintenance Products) fully supported IFRA.

Additional key elements

A summary of two unpublished studies with volunteers were available from the REACH registration of HICC via the ECHA dissemination website (checked in January 2014). The studies were assessed as supporting and reliable without restriction by the registrant.

In study 1 (1999, GLP compliant) patch tests with a dose of 0.2 ml of a 15 % solution of HICC in 75 % alcohol/25 % DEP was applied under occlusion to the back of 119

volunteers (18-77 years old) for 24 hours for a total of 9 applications. Challenge was performed 2 weeks after the last application, and rechallenge was performed twice in positive cases. The treatment resulted in zero subjects with positive reactions, 107 subjects with negative reactions, 4 with irritation reactions and the rest had equivocal reactions.

In study 2 (2003, not GLP compliant) patch tests with a dose of 0.3 ml of a 15 % solution of HICC in 25 % alcohol/75 % DEP was applied under occlusion to the back of 235 volunteers (18-70 years old) for 24 hours for a total of 9 applications. Challenge was performed 2 weeks after the last application. The treatment resulted in zero subjects with positive reactions, 201 subjects with negative reactions and zero subjects with equivocal reactions.

Assessment and comparison with the classification criteria

Relevant information from human data with respect to skin sensitisation is available for HICC from multiple population studies (mostly diagnostic patch test studies, several multicentre patch test studies), and case studies of allergic contact dermatitis. Relevant information from animal data with respect to skin sensitisation is available for HICC from a key LLNA study. *In vitro* studies of the phototoxic properties of HICC were also submitted, but RAC considered that the relevance of these studies for classification was low.

Structural alert data/SAR: As stated by the DS, the aldehyde HICC is a lipophilic turpentine-based molecule with a high capability of penetrating the skin. Structurally, HICC is a simple aliphatic aldehyde (class 1 aldehyde) in which the carbonyl group is a hard electrophile, and is expected to react with the amino groups of lysine residues on proteins via Schiff base formation (Patlewicz *et al*., 2003). RAC agreed with the DS that this property makes HICC more reactive in the skin resulting in further subsequent interactions with proteins and provoking allergic reactions.

According to the CLP Regulation, Annex I, Table 3.4.2, skin sensitisers shall be assigned to Category 1 if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or if there are positive results from an appropriate animal test. Based on the frequency of occurrence in humans and/or the potency in animals as well as severity of reaction substances may be assigned to sub-category 1A or 1B, if data are sufficient.

For HICC, the DS presented over 40 patch test studies involving several thousand dermatitis patients from various countries in Europe and other parts of the world. Most of the studies were diagnostic patch test studies. Due to its strong sensitising properties, HICC has been included in the base line series of such patch test studies. There are also four ROAT studies (Repeated Open Application Tests, use tests), in which the patch test results were confirmed, i.e. the contact allergy which has been diagnosed by patch testing was verified to be caused by HICC. In the ROAT tests, an increased positive response at increasing HICC concentrations was observed (dose-response studies). In use tests with the patients' own products and with various concentrations of HICC, the threshold for elicitation was found to be lower than the recommended content limit from industry.

The CLP Regulation, Annex I, section 3.4.2.2.2.1 states that human evidence for sub-category 1A can include diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure. In the CLP Guidance (Table 3.4.2-b), the frequency of occurrence of skin sensitisation in human diagnostic patch test data from dermatitis patients (unselected, consecutive) is described as high if it is ≥ 1.0 %, and as low/moderate if it is < 1.0 %.

The frequency is also described as high if the number of published cases of skin sensitisation is ≥ 100 cases, and as low/moderate if it is < 100 cases.

In most of the diagnostic patch test studies ≥ 2 % had positive reactions to HICC. The number of cases are clearly > 100. In one study, HICC is even described as the most common sensitiser in Europe at a rate of 1.5 - 3 %. Seven case studies of allergic contact dermatitis are reported by the DS, with positive reactions to HICC in six studies. In one of the two key studies a positive reaction was seen when the patient was tested with 0.075 % HICC.

Furthermore, in the CLP Guidance (Table 3.4.2-c), criteria for low and high exposure are given, and the sub-categorisation decision (CLP Guidance, Table 3.4.2-d) provides a matrix of exposure vs. frequency.

In consumer products HICC varies from 0.0036 to 0.63 %, with two exceptions (3.8 and 6.2%, respectively). Thus the score would be 0 for concentration/dose. HICC is used in consumer products that may be used more than once per day (score for repeated exposure = 2) and the number of exposures may be assumed to be ≥ 100 (score for number of exposures = 2). Thus the additive exposure index would be 0+2+2 = 4, which is described as low according to the CLP Guidance (Table 3.4.2-c and text below the table).

According to the CLP Regulation (Annex I, Table 3.4.3), a substance may be assigned to sub-category 1A if the EC3 value ≤ 2 % in a LLNA (the EC3 value is the percentage of test chemical required to elicit a stimulation index of 3 in the standard LLNA). As the EC3 value was 17.1 % in the LLNA key study for HICC (Patlewicz *et al*., 2002), sub-category 1A is not warranted based on the animal test, but fulfils the criteria for sub-category 1B, since the EC3 value was > 2 %.

Summary of the weight of evidence determination
Positive data from patch testing is available for HICC from several dermatology clinics indicating that ≥ 2 % of the tested patients (and up to 17 %) had a positive reaction to HICC. In addition to this there are four ROAT studies and six case studies where HICC was identified as the causative agent of allergic contact dermatitis. Positive data are also available from a key LLNA study, where the EC3 value was 17.1. The molecular structure of the aldehyde HICC is an alert for sensitising properties. The DS denoted the key studies in the tables and explained in the RCOM that these were considered to be key studies in the CLH proposal, as the selection of individuals tested as well as the number of individuals tested is clarified in the study reports.

Based on all available information submitted by the DS, RAC concluded that HICC should be classified as a skin sensitiser in sub-category 1A.

Specific concentration limits (SCL)
In the CLP Guidance (Table 3.4.2-f), substances are considered to have moderate, strong or extreme skin sensitisation potency, based on the LLNA. The CLP Guidance (Table 3.4.2-i) recommends that SCLs are established for substances of extreme potency and that the GCL should apply to substances of strong and moderate potency. According to the result from the key LLNA study with an EC3 value of 17.1, no SCL is warranted. However, the DS argues that a SCL is necessary because of the continued high frequency of sensitised people in the last decade, persisting even after an IND recommended maximum level of HICC in consumer products of 200 ppm (0.02 %). The DS proposed that this SCL be set at 100 ppm (0.01 %). The GCL is 0.1 % for sub-category 1A substances.

RAC agreed with the DS that HICC is a potent sensitiser. However, although the CLP

Regulation offers the possibility to assign a SCL based on human studies, RAC considered that the human data on HICC did not provide adequate and reliable scientific justification to set an SCL. The animal study indicates HICC to be a moderate sensitiser.

Conclusion: RAC recommended that HICC should be classified as a skin sensitiser in sub-category 1A, with the general concentration limit 0.1%.

RAC also noted that because HICC is classified as Skin Sens. 1A, the supplemental label element EUH208 is obligatory on the packaging of mixtures not classified as a skin sensitiser but containing HICC at a concentration ≥ 0.01 % (CLP Annex II, section 2.8), to protect already sensitised individuals.

4.7 Repeated dose toxicity

Not evaluated.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not evaluated.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated.

4.10 Carcinogenicity

Not evaluated.

4.11 Toxicity for reproduction

Not evaluated.

4.12 Other effects

Not evaluated.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Not evaluated.

5.2 Environmental distribution

Not evaluated.

5.3 Aquatic Bioaccumulation

Not evaluated.

5.4 Aquatic toxicity

Not evaluated.

6 OTHER INFORMATION

Not evaluated.

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8 ANNEXES