Committee for Risk Assessment

RAC

Annex 1

Background document

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

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

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

CLH-O-0000006960-70-01/F

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

18 March 2021

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1]

(2*E*)-3-phenylprop-2-enal [2]

EC Number:	**203-213-9 [1]**
	604-377-8 [2]
CAS Number:	**104-55-2 [1]**
	14371-10-9 [2]
Index Number:	**Not available**

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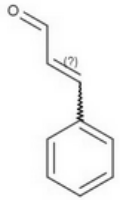
Version number: 2 **Date: 12. February 2020**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1] (2*E*)-3-phenylprop-2-enal [2]
Other names (usual name, trade name, abbreviation)	
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	203-213-9 [1] 604-377-8 [2]
EC name (if available and appropriate)	Cinnamaldehyde
CAS number (if available)	104-55-2 [1] 14371-10-9 [2]
Other identity code (if available)	
Molecular formula	C_9H_8O
Structural formula	
SMILES notation (if available)	O=C\C=C\c1ccccc1
Molecular weight or molecular weight range	132.1592
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable

Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	> 99.1 — < 99.9 % (w/w)

Cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal; (2E)-3-phenylprop-2-enal, hereafter referred to as "Cinnamaldehyde", is a viscous liquid that occurs naturally in the bark of cinnamon trees and other species of the genus *Cinnamomum*. The essential oil of cinnamon bark consists of approximately 98% cinnamaldehyde. Cinnamaldehyde is commonly used as flavouring in chewing gum, ice cream, candy and beverages. It is also used in cosmetics, cleaning agents, polishes and wax blends, air care products and pharmaceuticals. Cinnamaldehyde is also used in biocidal products.

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
Cinnamaldehyde, CAS 104-55-2	> 99.1 — < 99.9 % (w/w)	None	Acute Tox. 4; H312 Skin sens 1; H317 Skin irrit. 2; H315 Eye irrit. 2; H319
(E)-3-phenylprop-2-enal, CAS 14371-10-9	No information available	None	STOT SE; H335 Skin sens 1; H317 Skin irrit. 2; H315 Eye irrit. 2; H319

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
Not applicable				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not applicable					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	-	cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1] (2*E*)-3-phenylprop-2-enal [2]	203-213-9 [1] 604-377-8 [2]	104-55-2 [1] 14371-10-9 [2]	Skin sens 1A	H317	GHS07 Wng	H317		Skin Sens. 1; H317: C ≥ 0,02 %	-
Resulting Annex VI entry if agreed by RAC and COM	-	cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1] (2*E*)-3-phenylprop-2-enal [2]	203-213-9 [1] 604-377-8 [2]	104-55-2 [1] 14371-10-9 [2]	Skin sens 1A	H317	GHS07 Wng	H317		Skin Sens. 1; H317: C ≥ 0,02 %	-

Table 6: Reason for not proposing harmonised classification and status under public consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Cinnamaldehyde has no classification and labelling history under Directive 67/548/EEC or Regulation (EC) No 1272/2008.

Cinnamaldehyde is one of the 26 fragrance substances for which individual labelling is required under the Cosmetics Regulation (EC no. 1223/2009) and the Detergents Regulation (EC no 648/2004). Of these 26 fragrance substances cinnamaldehyde is among the 13 most frequently reported and well recognised consumer allergens (SCCS p. 11).

In 2012 the Scientific Committee on Consumer Safety (SCCS) published an opinion on fragrance allergens in cosmetic products. In this opinion cinnamaldehyde has been categorised as an established contact allergen in humans which has given rise to a significant number (more than 100) of published cases on contact allergy (SCCS 2012 p. 115).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Differences in self-classification
Disagreement by DS with current self-classification

Further detail on need of action at Community level

New classification criteria and difference in self-classification
With the 2nd ATP to CLP new classification criteria were introduced for skin sensitisation allowing sub-categorisation of skin sensitisers into Category 1A (strong sensitisers) and Category 1B (other sensitisers, corresponding to the existing Category 1. A classification in Cat. 1A will lead to more stringent labelling requirements for mixtures containing the substance and is currently regarded as the most important risk management measure for such substances. Correct identification of Category 1A skin sensitisers is thus expected to increase the human protection level for strong sensitisers due to the requirement of labelling of mixtures containing Cat 1A sensitisers ≥0.01%, with EUH208: "Contains Cinnamaldehyde. May produce an allergic reaction".

In the publicly available part of the REACH registration dossier the applicants has classified cinnamaldehyde as a Category 1 skin sensitiser. The same is true for 1702 of 1783 (95.5 %) of the notifiers in the C&L Inventory. Only 66 of 1783 (3.7 %) of the notifiers has notified cinnamaldehyde as a skin sensitiser in Category 1A.

Widespread use in low concentrations
Cinnamaldehyde is a substance that is manufactured in or imported to the EU in amounts of 1000-10.000 tonnes/year and is widely used in products on the EU market. The registered uses of cinnamaldehyde for consumers include: cosmetics, cleaning agents, polishes and wax blends, air care products, biocidal products and pharmaceuticals. Registered uses for professionals include: cosmetics, cleaning agents and polishes, and wax blends. Besides this Cinnamaldehyde is used as a biocide and as flavouring in chewing gum, ice cream, candy and beverages. As cinnamaldehyde is widely used in many different types of products the general population can be exposed from many different sources.

Cinnamaldehyde is generally present in low concentrations in individual consumer products. The International Fragrance Association (IFRA) recommends maximum limits of Cinnamaldehyde in leave-on cosmetic products between 0.02 - 0.05 % depending on the product category. The recommended limits for rinse-off cosmetic products is between 0.05 - 0.4 % depending on the product category and 0.05% for cleaning products (see Table 11 in section 10.7.4) (IFRA 2013, IFRA 2015).

The SCCS opinion refers to a number of surveys on the presence and content of various allergenic fragrances in various consumer products. Cinnamaldehyde has i.e. been found to be present in 1 - 6 % of consumer products investigated in different surveys based on labelling information alone. It was concluded that taking the total exposure into account, exposure to all 26 allergenic fragrances is foreseeable in daily life (SCCS 2012). The Danish EPA has conducted surveys and assessments of a broad range of consumer products over the last decades. Generally cinnamaldehyde is found in low concentrations (>0 - <0.02 %) in the investigated products with few exceptions (≤ 1.7 %) (DK EPA database, search June 2016).

Human exposure to cinnamaldehyde seems to be low based on the IFRA recommendations and reported contents in various consumer products. However, the exposure is assessed to be frequent due to the widespread uses and the high tonnage level of cinnamaldehyde. It is thus difficult for consumers to avoid exposure.

Human data confirm strong potency of cinnamaldehyde

Positive patch test frequencies from 46 human patch test studies range from 0.14-34% and frequencies exceeding 2% for selected dermatitis and patients 1% for consecutive (unselected) dermatitis patients are reported in a number of studies. The total number of positive reactions in published cases is > 100 (more than 2300). Overall the human data confirm strong potency of cinnamaldehyde.

5 IDENTIFIED USES

Registered uses of cinnamaldehyde for consumers include: cosmetics, cleaning agents, polishes and wax blends, air care products, biocidal products and pharmaceuticals. Registered uses for professionals include: cosmetics, cleaning agents and polishes and wax blends. Cinnamaldehyde is also used as flavouring in chewing gum, ice cream, candy and beverages. Besides this cinnamaldehyde can be used as a biocide. The biocidal active substance, cinnamic aldehyde (3-phenyl-propen-2-al), CAS number 104-55-2, is included in the Biocides Review Programme for PT2.

6 DATA SOURCES

One of the primary information sources for this CLH report is the SCCS opinion on fragrance allergens from 2012 which contains the most recent and comprehensive assessment of available information on cinnamaldehyde as well as other fragrance allergens up to year 2011 (SCCS 2012). Data cited in this opinion for cinnamaldehyde have been collected when possible.

A supplementary search in the open literature has been done for the period from January 2009 and until November 2016 to ensure that potentially relevant studies published after the SCCS opinion is taken into account. The searches have included literature databases such as SciFinder, PubMed and Scopus as well as searches in sources such as OECD SIDS, International Program on Chemical Safety INCHEM database (IPCS INCHEM) and also Google searches.

Data in the publicly available part of the REACH registration dossier for cinnamaldehyde have been assessed as well, latest at December 6th, 2019.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	liquid	REACH registration dossier	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
Melting/freezing point	< -18° C at 969.9 hPa	REACH registration dossier	Measured
Boiling point	>250° C at 969.9 hPa 252.4 at 960 hPa	REACH registration dossier	Measured
Relative density	1.041 g/cm³ at 20° C	REACH registration dossier	Measured
Vapour pressure	0.039 hPa at 25° C	REACH registration dossier	Measured
Surface tension	38.962 mN/m at 25°C	REACH registration dossier	Calculated
Water solubility	2110.4 mg/L at 22° C 10000 mg/L at 27° C	REACH registration dossier	Measured
Partition coefficient n-octanol/water	2.107 at 25° C 1.83 at 27° C	REACH registration dossier	Measured
Flash point	125 °C at 966 hPa 105 °C at 968.3 hPa	REACH registration dossier	Measured
Flammability	Non-flammable (950 °C)	REACH registration dossier	Measured
Explosive properties	No data		
Self-ignition temperature	Not flammable at 27 °C	REACH registration dossier	Measured
Oxidising properties	Mild oxidising properties	REACH registration dossier	Measured
Granulometry	No data/not applicable		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	0.476 x 10^{-7} at 27 °C	REACH registration dossier	Measured
Viscosity (dynamic)	22.12 mPa*s at 20°C 18.00 mPa*s at 40°C	REACH registration dossier	Measured

8 EVALUATION OF PHYSICAL HAZARDS

Physical hazards have not been assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
No guideline, GLP compliance not reported. Rat (Fischer 344), male Acute study: single dose, oral (gavage) of 5, 50 or 500 mg/kg bw Multiple dosing study: oral pre-treatment (gavage) for 7 days with unlabelled cinnamaldehyde at a dose of 5, 50 or 500 mg/kg bw followed by single oral dose of 5, 50 or 500 mg/kg bw mg/kg [3-^{14}C]-cinnamaldehyde after 24 hours	Absorption: Cinnamaldehyde have shown to be rapidly absorbed from the gut. Distribution: Radioactive cinnamaldehyde is distributed primarily to the gastrointestinal tract, kidneys, and liver, after single- or multiple-dose oral administration. At all dose levels, a small amount of the dose is distributed to the fat. Metabolism: Except for the high dose pre-treatment group, the major urinary metabolite is hippuric acid, accompanied by small amounts of cinnamic and benzoic acid. In the high dose pre-treatment group, benzoic acid was the major 4 metabolite, suggesting that saturation of the glycine conjugation pathway occurs at repeated high dose levels of cinnamaldehyde. Excretion: After 24 hr, >80% of the radioactivity is recovered in the urine and <7% in the feces from all groups of rats, regardless of dose level. Regardless of the dose level, species, or sex, > 85% of the radiolabel is recovered in the urine and feces.	2 (reliable with restrictions) **Test material (EC name): cinnamaldehyde** Dosed partly as ^{14}C labelled cinnamaldehyde (Key study)	Adams et al., 2004 Sapienza et al., 1993 Cited from the publicly available part of REACH reg.
No guideline, GLP compliance not reported. Metabolites identified by *Radio*-HPLC Rat (Fischer 344), male and female (4/group) Mice (CD1), male and female (6/group) Single dose, oral (gavage) and ip injection Dose: gavage: 250 mg/kg bw; ip.: 2 and 250 mg/kg bw	In both species and via both routes of administration, the major urinary metabolites form from oxidation of cinnamaldehyde to cinnamic acid, which is subsequently oxidized in the β-oxidation pathway. The major urinary metabolite is hippuric acid (71–75% in mice and 73–87% in rats), accompanied by small amounts of 3-hydroxy-3-phenylpropionic acid (0.4–4%), benzoic acid (0.4–3%), and benzoyl glucuronide (0.8–7.0%). The glycine conjugate of cinnamic acid is formed to a considerable extent only in the mouse (4–13%). To a small extent, glutathione conjugation of cinnamaldehyde competes with	2 (reliable with restrictions) **Test material (EC name): (E)-3-phenylprop-2-enal (trans-cinnamaldehyde)** Dosed as trans-^{14}C-cinnamaldehyde (Supporting study)	Peters and Caldwell, 1994 Cited from the publicly available part of REACH reg.

Method	Results	Remarks	Reference
	the oxidation pathway. Approximately 6–9% of either dose is excreted in 24 h as glutathione conjugates of cinnamaldehyde.		
Guideline and GLP compliance not reported Rat (Fischer 344), male and female (3/group) Single dose, oral (gavage) and intravenous (iv) administration[1] Vehicle: oral: corn oil; iv: ethanol-emulphor EL-620-water Dose: gavage: 50, 150, 500, 1000, and 2000 mg/kg bw; gavage microcapsulated: 50, 250, and 500 mg/kg bw; iv: 5, 15 or 24 mg/ kg bw	After iv administration a large fraction of cinnamaldehyde was immediately oxidized to cinnamic acid (estimated to be between 37 and 60 % by the authors) within the first 30 minutes. The biological half-life of cinnamaldehyde after iv administration was found to be 1.7 hours in the rat. After oral administration at 250 or 500 mg/kg bw the maximum blood concentrations were in the order of 1 µg/ml. At 50 mg/kg bw no cinnamaldehyde could be detected in the blood (< 1 µg/ml). The majority of cinnamaldehyde administered orally was excreted in urine as hippuric acid within 24 hours. The maximum excretion rate occurred at 8 hours after gavage.	2 (reliable with restrictions) **Test material:** Details not given by the regristant Purity: 98% (Supporting study)	Yuan J et al., 1992 Yuan et al., 1993 Cited from the publicly available part of REACH reg.
Guideline and GLP compliance not reported Rat (Sprague-Dawley), male (5/group) Single dose, oral and iv administration Vehicle: oral: corn oil Dose: oral: 500, 250, or 125 mg/kg bw cinnamaldehyde diluted in corn oil, iv: 20 mg/kg bw	Absorption: The GC-MS technique used in the experiment found the areas under the plasma concentration–time curve (AUC) from 0 min to terminal time of cinnamaldehyde were 1984 ± 531 and 355 ± 53 ng h/ml for oral (500 mg/kg) and iv (20 mg/kg) administration, respectively. From dosage 125 to 500 mg, maximum plasma concentration (Cmax) and area under the curve to termination time (AUC0–t) were proportional to the dose; time at maximum plasma concentration (Tmax) and mean residence time (MRT) did not change following dose escalation. The elimination half-lives of cinnamaldehyde were 6.7 ± 1.5 and 1.7 ± 0.3 hours for oral and iv administration, respectively. An excretion experiment was also performed. The group of rats (n = 5, each group) used for the urinary and fecal excretion study received a single oral dose of 500 mg/kg bw. Lower accumulative	2 (reliable with restrictions) **Test material:** Details not given by the regristant Purity: 99% (Supporting study)	Zhao H et al., 2014 Cited from the publicly available part of REACH reg.

[1] Indicated as both intraperitoneal (ip) and iv administration administration in REACH reg. The published article by Yuan et. al., 1992, however states intravenious administration.

Method	Results	Remarks	Reference
	ratio of cinnamaldehyde was found after 24 hours, with the numbers reaching at 0.3% and 0.8% in feces and urine. Metabolism: Metabolites found in blood were cinnamyl alcohol and methyl cinnamate.		
Principles of method: Skin absorption model with human skin or diffusion cell technique with excised human abdominal skin and rat skin Excised human abdominal skin and rat skin model Type of coverage: open and occlusive Duration of exposure: 72 hours	In vitro/ex vivo study on dermal absorption. Using a skin absorption model system with human skin for cinnamaldehyde it was reported that 24% and 52% cinnamaldehyde (non-occluded and occluded, respectively) were absorbed by 72 hours. Using a skin absorption model system with excised rat skin, 34% and 42% cinnamaldehyde (non-occluded and occluded, respectively) have been reported to be absorbed within 48–72 hours (Hotchkiss, 1998).	2 (reliable with restrictions) **Test material:** Details not given by the regristant Purity: 99% (Supportive study)	Bickers et al., 2005 Hotchkiss, 1998 Cited from the publicly available part of REACH reg.

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

In a study male rat radioactive cannamaldehyde was distributed primarily to the gastrointestinal tract, kidneys, and liver, after single oral dose and multiple oral administrations (Adams et al., 2004, Sapienza et al., 1993).

After 24 hours, more than 80% of the radioactivity was recovered in the urine and less than 7% in the feces from all groups of rats, regardless of dose level. At all dose levels, a small amount of the dose was distributed to the fat. At 50 and 500 mg/kg bw, radioactivity could be measured in animals terminated 3 days after dosing. Except for the high dose pretreatment group, the major urinary metabolite was hippuric acid, accompanied by small amounts of cinnamic and benzoic acid. In the high dose pretreatment group, benzoic acid was the major 4 metabolite, suggesting that saturation of the glycine conjugation pathway occurs at repeated high dose levels of cinnamaldehyde (Adams et al., 2004, Sapienza et al., 1993).

In a supporting study by Peters and Caldwell, 1994, where the metabolism of radioactive trans-cinnamaldehyde was investigated in male and female Fischer 344 rats and CD1 mice at doses of 2 and 250 mg/kg body weight given by ip injection and in males at 250 mg/kg by oral gavage. Some 94% of the administered dose was recovered in the excreta in 72 hours in both species with most (75-81%) present in the 0-24 hr urine. Less than 2% of the administered dose was found in the carcasses at 72 hours after dosing. In both species the major urinary metabolite was hippuric acid (71–75% in mice and 73–87% in rats) accompanied by 3-hydroxy-3-phenylpropionic acid (0.4–4%), benzoic acid (0.4–3%) and benzoyl glucuronide (0.8–7.0%). The glycine conjugate of cinnamic acid was formed to a considerable extent only in the mouse (4–13%). The oxidative metabolism of cinnamaldehyde essentially follows that of cinnamic acid, by beta-oxidation analogous to that of fatty acids. Apart from the metabolites common to cinnamic acid and cinnamaldehyde, 7% of 0-24-hour urinary radioactive trans-cinnamaldehyde was accounted for by two new metabolites in the rat and three in the mouse, which have been shown in other work to arise from a second pathway of cinnamaldehyde metabolism involving conjugation with glutathione.

In a supporting study by Yuan J. et al., 1992, cinnamaldehyde was immediately oxidized to cinnamic acid within the first 30 minutes in Fisher 344 rats after iv administration. The biological half-life of cinnamaldehyde after iv administration was found to be 1.7 hours in the rat. After oral administration, the majority of cinnamaldehyde was excreted in urine as hippuric acid within 24 hours. The maximum excretion rate occurred at 8 hours after gavage.

A supporting study by Zhao H. et al., 2014, also found the elimination half-life of cinnamaldehyde after iv administration to be 1.7 ± 0.3 hours and the half-life after oral administration was found to be 6.7 ± 1.5 hours by a selective and sensitive method utilizing gas chromatography-mass spectrometry. After a single oral dose of 500 mg/kg bw, a lower accumulative ratio of cinnamaldehyde was found after 24 hours, with the numbers reaching at 0.3% and 0.8% in feces and urine. Metabolites found in blood were cinnamyl alcohol and methyl cinnamate.

In a supporting in vitro/ex vivo study on dermal absorption, Bickers et al. 2005, found, using a skin absorption model system with human skin for cinnamaldehyde, that 24% and 52% cinnamaldehyde (non-occluded and occluded, respectively) were absorbed by 72 hours. Using a skin absorption model system with excised rat skin, 34% and 42% cinnamaldehyde (non-occluded and occluded, respectively) have been reported to be absorbed within 48–72 hours (Hotchkiss, 1998).

The excretion pattern and metabolic profile of cinnamaldehyde in rats and mice are not systematically affected by sex, dose size and route of administration.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier.

10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier.

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

10.7 Skin sensitisation

Table 9 summarises relevant animal studies with cinnamaldehyde which include 22 LLNAs, 2 LLNA BrdU-ELISA tests, 2 *ex vivo* LLNA: BrdU-ELISA and 3 GPMTs.

Table 9: Summary table of animal studies on skin sensitisation (chronological order)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
LLNA					
LLNA: BrdU-ELISA - Comparable to OECD 442B GLP – not stated	Mice (BALB/c), female n = 6/dose	Cinnamaldehyde (in AOO)	1, 5 and 10% Exp.: 3 days, duration 7 days (instead of 6 days as in OECD 442B)	EC2: 6.1% in the *in vivo* LLNA:BrdU-ELISA test, sensitising	Williams et al., 2015
ex vivo LLNA:BrdU-ELISA GLP – not stated	Mice (BALB/c), female n = 6/dose	Cinnamaldehyde (in AOO)	1, 5 and 10% Exp.: 3 days, duration 6 days	EC2: 6.9% in the *ex vivo* LLNA:BrdU test, sensitising	Williams et al., 2015
LLNA - Comparable to OECD 429 GLP – not stated	Mice (CBA/Ca), female n = 3/dose (in OECD 429 a minimum of 4/dose is required)	Cinnamaldehyde (in AOO)	0.1, 0.99, 3.3, 9.9 and 19.8% Exp: 3 days, duration 6 days	EC3: 0.57%, sensitising	Niklasson et al., 2013
ex vivo LLNA:BrdU-ELISA GLP – not stated	Mice (BALB/c), female n = 4/dose	Cinnamaldehyde (in AOO)	0.5, 1, 5 and 10% Exp: 3 days, duration 5 days	EC3: 1.91%, sensitising	Ulker et al, 2013
LLNA: BrdU-ELISA In accordance with OECD 442B Not in full accordance with GLP	Mice (CBA/JN), female n = 4/dose	trans-cinnamaldehyde (in AOO)	1, 3 and 10% Exp: 3 days, duration 6 days	EC2: 2.2% in the *in vivo* LLNA:BrdU-ELISA test, sensitising	Kojima et al., 2011
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 1:3 EtOH:DEP)	0.1, 0.3, 1, 3 and 10%	EC3: 0.2%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003a)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 0.1% α-tocopherol in 3:1 EtOH:DEP)	0.1, 0.3, 1, 3 and 10%	EC3: 0.2%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results**	**Reference**
					2012 (as RIFM 2003b)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 2% α-tocopherol in 3:1 EtOH:DEP)	0.1, 0.3, 1, 3 and 10%	EC3: 0.6%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003c)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 0.3% antioxidant mix* in 3:1 EtOH:DEP) * 1:1:1 α-tocopherol, BHT and eugenol	0.1, 0.3, 1, 3 and 10%	EC3: 0.7%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003d)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 0.1% Trolox C in 3:1 EtOH:DEP)	0.1, 0.3, 1, 3 and 10%	EC3: 0.7%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003e)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 2% α-tocopherol in 3:1 EtOH:DEP)	0.1, 0.3, 1, 3 and 10%	EC3: 0.8%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003f)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 3:1 EtOH:DEP)	0.1, 0.3, 1, 3 and 10%	EC3: 0.9%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003g)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 0.1% α-tocopherol in 3:1 EtOH:DEP)	0.1, 0.3, 1, 3 and 10%	EC3: 1.1%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003h)
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 0.3% antioxidant mix* in 3:1 EtOH:DEP) *1:1:1 BHT, tocopherol and eugenol	0.1, 0.3, 1, 3 and 10%	EC3: 1.3%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003i)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results**	**Reference**
LLNA (no reported deviations from OECD 429)	Mice (no further info)	Cinnamaldehyde (in 0.1% Trolox C in 3:1 EtOH:DEP)	0.1, 0.3, 1, 3 and 10%	EC3: 1.4%, sensitising	Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM 2003j)
LLNA In accordance with OECD 429 GLP – not stated	Mice (CBA/Ca) n = 4/dose	*trans*-cinnamaldehyde (in AOO)	1, 2.5, 5, 10 and 25% Exp.: 3 days, duration 6 days	EC3: 1.3%, sensitising	Elahi et al., 2004 Also cited in SCCS 2012
LLNA - Comparable to OECD 429 GLP not stated in the publication; GLP personal communication between the author and ECHA	Mice (CBA/Ca), female n = 4/dose	Cinnamaldehyde (in AOO)	0.5, 1, 2.5, 5 and 10% Exp.: 3 days, duration 6 days	EC3: 3.1%, sensitising	Basketter et al., 2001 Also cited in SCCS 2012
LLNA (too few concentrations were tested in order to comply with OECD 429)	Mice (no further info)	Cinnamaldehyde (in AOO)	1 and 2.5%	EC3: 1.4%, sensitising	Smith and Hotchkiss, 2001 cited in SCCS 2012
LLNA - Comparable to OECD 429 GLP – not stated	Mice (CBA/Ca), female n = 4/dose	Cinnamaldehyde (in 50:50 EtOH:water)	1, 2.5, 5, 10 and 25% Exp.: 3 days, duration 6 days	EC3: 1.2%, sensitising	Wright et al., 2001 Also cited in SCCS 2012
LLNA - Comparable to OECD 429 GLP – not stated	Mice (CBA/Ca), female n = 4/dose	Cinnamaldehyde (in 90:10 EtOH:water)	1, 2.5, 5, 10 and 25% Exp.: 3 days, duration 6 days	EC3: 1.6%, sensitising	Wright et al., 2001 Also cited in SCCS 2012
LLNA - Comparable to OECD 429 GLP – not stated	Mice (CBA/Ca), female n = 4/dose	Cinnamaldehyde (in DMSO)	0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 25% Exp.: 3 days, duration 6 days	EC3: 0.9%, sensitising	Wright et al., 2001 Also cited in SCCS 2012
LLNA - Comparable to	Mice (CBA/Ca),	Cinnamaldehyde (in propylene	1, 2.5, 5, 10 and 25%	EC3: 1.4%, sensitising	Wright et al., 2001

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD 429 GLP – not stated	female n = 4/dose	glycol)	Exp.: 3 days, duration 6 days		Also cited in SCCS 2012
LLNA - Comparable to OECD 429 GLP – not stated	Mice (CBA/Ca), female n = 4/dose	Cinnamaldehyde (in DMF)	0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 25% Exp.: 3 days, duration 6 days	EC3: 0.5%, sensitising	Wright et al., 2001 Also cited in SCCS 2012
LLNA - Comparable to OECD 429 GLP – not stated	Mice (CBA/Ca), female n = 4/dose	Cinnamaldehyde (in MEK)	1, 2.5, 5, 10 and 25% Exp.: 3 days, duration 6 days	EC3: 1.1%, sensitising	Wright et al., 2001 Also cited in SCCS 2012
LLNA - Comparable to OECD 429 GLP – not stated	Mice (CBA/Ca), female n = 4/dose	Cinnamaldehyde (in AOO)	1, 2.5, 5, 10 and 25% Exp.: 3 days, duration 6 days	EC3: 1.7%, sensitising	Wright et al., 2001 Also cited in SCCS 2012
LLNA - Comparable to OECD 429 GLP not stated in the publication; GLP personal communication between the author and ECHA	Mice (CBA/Ca), single sex per experiment although animals of both sexes were used throughout the study n = 4/dose	Cinnamaldehyde (in AOO)	5, 10 and 25% Exp.: 3 days, duration 6 days	Sensitising (EC3 not calculated)	Basketter and Scholes, 1992 Also cited in Bickers et al., 2005
GPMT					
GPMT Comparable to OECD 406 (Maximisation Test) GLP not stated in the publication; GLP personal communication between the author and ECHA	Guinea pig (Albino Dunkin-Hartley) Number of animals and sex not specified.	Cinnamaldehyde (vehicle 70/30 acetone/PEG 400)	Induction concentrations of 0.2% (injection) and 2.5% (patch). Challenge concentration of 0.75% (maximum non-irritant dose)	Sensitisation observed. Positive reactions seen in 100% of the animals (24, 48 hours after challenge)	Basketter and Scholes, 1992 Also cited in Bickers et al., 2005
GPMT Comparable to OECD 406 (Maximisation	Guinea pig (Albino Dunkin-Hartley, sex not	Trans-cinnamaldehyde (2 samples) (vehicle not	Induction concentrations of 0.2% (injection) and 2.5%	Sensitisation observed. Positive reactions seen in 90% (9/10) and in 100% (10/10) of the animals (24,	Basketter, 1992[1]. Also cited in Bickers et al.,

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Test) GLP not stated in the publication; GLP personal communication between the author and ECHA	specified) N = 10	reported)	(patch). Challenge concentration of 0.75% (maximum non-irritant dose)	48 hours after challenge)	2005
GPMT	Guinea pig Number and sex not specified.	Cinnamaldehyde (vehicle not reported)	3% (Not clear from Bickers et al., 2005 if this was the intradermal induction dose or challenge concentration)	Strong sensitisation effect reported (no further details)	Ishihara et al., 1986 cited in Bickers et al., 2005

[1]The Basketter 1992 publication refers to two individual GPMTs, one of which is also cited in Basketter and Scholes, 1992. Thus they count as two studies with a total of 3 GPMTs in the final summary of animal studies.

Table 10 summarises relevant human studies with cinnamaldehyde which include 46 patch test studies, 2 Human repeated open application tests (ROATs), 14 Human Repeat Insult Patch Tests (HRIPTs), 2 Human Maximation Tests (HMTs) and 3 case studies. The studies involve thousands of dermatitis patients from different EU countries, North America, Australia and Asia. The majority of the references cited below are not included in the REACH registration dossier.

Table 10: Summary table of human data on skin sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Patch tests, selected patients				
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 2798 selected Fragrance mix (FM) I positive patients patch tested with cinnamaldehyde. Data from IVDK multicentre project (IVDK: Information Network of Departments of Dermatology in Germany, Austria and Switzerland). Data obtained 1998-2013.	**10.6%** were tested positive (n = 2798)	Geier et al., 2015
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 940 selected patients patch tested with cinnamaldehyde, data from Department of Dermatology, University Hospital St Rafael, Belgium. Data obtained 1990-2011.	**7%** were tested positive (n = 940)	Nardelli et al., 2013
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 164 hairdressers and hairdressing apprentices with dermatitis tested with cinnamaldehyde. Data from	**1%** were tested positive (n = 164)	Lyons et al., 2013

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		Department of Occupational Dermatology Research and Education Centre, Australia (1993-2010).		
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 23 selected patients with chronic idiopathic urticarial patch tested with cinnamaldehyde. Data from Tufts Medical Center, USA. Year not stated.	**13%** were tested positive (n = 23)	Hession and Scheinman, 2012
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with cinnamaldehyde. Data from the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	**24.2%** were tested positive (n= 157)	Turcic et al., 2011
Patch test data, selected patients	Cinnamaldehyde, 2% (in pet.)	Study of 86 selected patients patch tested with cinnamaldehyde, data from the Department of Dermatology, Hospital General Universitario, Alicante, Spain. Data obtained 2004-2008.	**8.1%** were tested positive (n=86)	Cuesta et al., 2010
Patch test data, consecutive patients	Cinnamaldehyde, 1% (in pet.)	Study of 4527 selected patients patch tested with cinnamaldehyde. Data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	**2.64%** were tested positive (n = 4527)	Uter et al., 2010
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Retrospective study of 774 dermatitis patients with a positive patch test to fragrance mix and tested with cinnamaldehyde. Data from Odense University Hospital, Denmark (1995-2007).	**8.5%** patients were tested positive (n = 744)	Andersen et al., 2009
Patch test data, selected patients	Cinnamaldehyde, 2% (in pet.)	Study of 18 selected cinnamon-sensitive patients patch tested with cinnamaldehyde. Data from the Department of Dermatology of the VU University Medical Centre, The Netherlands (year not stated).	**22%** were tested positive (n=18)	Pentinga et al., 2009
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 37065 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with cinnamaldehyde. Data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	**0.98%** with were tested positive (n = 37065)	White, 2009
Patch test data, selected patients	Cinnamaldehyde (Concentration and vehicle not reported)	Study of 30 patients allergic to their own perfumed product, 19 of these patch tested with cinnamaldehyde.	**20%** were tested positive (n = 19)	Vocanson et al., 2006

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 422 selected patients patch tested with cinnamaldehyde, data from multicenter study, Korea. Data obtained 2002-2003.	**1.7%** were tested positive (n = 422)	An et al., 2005
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet. and 1% SSO)	Study of 747 selected patients with suspected fragrance allergy patch tested with cinnamaldehyde. Data from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	**1.9%** were tested positive (n = 747)	Wohrl et al., 2001
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 226 selected patients sensitive to FM patch tested with cinnamaldehyde. Data from Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999)	**13.3%** were tested positive (n = 226)	Brites et al., 2000
Patch test data, selected patients	Cinnamaldehyde, 2% (in SSO)	Study of 50 patients sensitive to FM patch tested with cinnamaldehyde. University Hospital Utrecht, The Netherlands (1994-1998).	**20%** were tested positive (n = 50)	Hendriks and van Ginkel, 1999
Patch test data, selected patients	Cinnamaldehyde, concentration not reported (in pet.)	Study of 40 patients sensitive to FM patch tested with cinnamaldehyde	**12.5%** were tested positive (n = 40)	Katsarma and Gawkrodger, 1999
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 167 fragrance sensitive volunteers patch tested with cinnamaldehyde. Data from seven centres located in Japan, Northern Ireland, United States, England, Switzerland and Sweden.	**14.4%** were tested positive (n = 167)	Larsen et al., 1996
Patch test data, selected patients	Cinnamaldehyde, 2% (in pet.)	Study of 105 selected patients from three age groups patch tested between 1979-1983 with 2% cinnamaldehyde in pet. Data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	**30.8-32.5%** were tested positive (n = 105);	Johansen and Menne, 1995
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 160 selected patients from three age groups patch tested between 1988-1992 with 1% cinnamaldehyde in pet. Data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	**9.1-12.8%** were tested positive (n = 160)	Johansen and Menne, 1995
Patch test data, selected patients	Cinnamaldehyde, 2% (in pet.)	Study of 61 selected patients sensitive to FM patch tested with cinnamaldehyde. Data from University of Amsterdam and University of Leiden, The Netherlands (1987).	**34%** were tested positive (n = 61)	De Groot et al., 1993
Patch test data, selected patients	Cinnamaldehyde, 1% (vehicle not reported)	Study of 162 selected patients positive to a fragrance mix patch tested with cinnamaldehyde. Data from Dermatologische Klinik und	**21%** were tested positive (n = 162)	Enders et al., 1989

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		Poliklinik, Germany (1987).		
Patch test data, selected patients	Cinnamaldehyde, 1% (vehicle not reported)	Study of 78 selected patients positive to a fragrance mix patch tested with cinnamaldehyde. Multicentre study involving 6 countries. Year not stated.	**12.8%** were tested positive (n = 78)	Wilkinson et al., 1989 cited from SCCNFP, 1999
Patch test data, selected patients	Cinnamaldehyde, 2% (in pet.)	Study of 63 selected patients with dermatitis patch tested between 1983 and 1984 with fragrance mix and cinnamaldehyde 2% in pet. Data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	**14.3%** were tested positive (n = 63)	Santucci et al., 1987
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 54 selected patients with dermatitis patch tested between 1984 and 1985 with fragrance mix. and cinnamaldehyde 1% in pet. Data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	**5.6%** were tested positive (n = 54)	Santucci et al., 1987
Patch test data, selected patients	Cinnamaldehyde (concentration and vehicle not reported)	Study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with cinnamaldehyde. It is unclear from the reference exactly how many patients were tested.	**1.5%** were tested positive (n = 403)	Adams and Maibach, 1985
Patch test data, selected patients	Cinnamaldehyde, 0.5% (in pet.)	Study of 182 selected patients suspected of contact allergy to cosmetics patch tested with cinnamaldehyde. Data from the Netherlands. Data obtained 1977.	**3.7%** were tested positive (n = 182)	Malten et al., 1984
Patch test data, selected patients	Cinnamaldehyde, 1% (in pet.)	Study of 20 selected perfume allergic patients patch tested with cinnamaldehyde	**30%** were tested positive (n = 20)	Larsen et al., 1977
Patch tests, consecutive (unselected) patients				
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of 1951 unselected dermatitis patients patch tested with cinnamaldehyde, data from St Johns Institute of Dermatology at St Thomas Hospital, UK. Data obtained 2011-2012.	**1.4%** were tested positive (n = 1951)	Mann et al., 2014
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of 41 unselected children age 0-5 years tested with cinnamaldehyde.Data collected by the North American Contact Dermatitis Group (NACDG) (2005-2012).	**4.9%** were tested positive (n = 41)	Zug et al., 2014
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of 838 children age 6-18 years tested with cinnamaldehyde. Data collected by the North American Contact Dermatitis Group (NACDG) (2005-2012).	**1.2%** were tested positive (n = 838)	Zug et al., 2014
Patch test data, unselected	Cinnamaldehyde, 1% (in pet.)	Study of 17213 unselected adults > 18 years tested with cinnamaldehyde. Data collected by the North American	**3%** were tested positive (n = 17213)	Zug et al., 2014

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
patients		Contact Dermatitis Group (NACDG) (2005-2012).		
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of 1503 unselected patients patch tested with cinnamaldehyde, data from Department of Dermato-Allergology, Copenhagen University Hospital, Gentofte. Data obtained 2008-2010.	**1.3%** were tested positive (n = 1503)	Heisterberg et al., 2011
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of 320 unselected dermatitis patients patch tested with cinnamaldehyde, data from the University Medical Centre in Groningen, the Netherlands. Data obtained 2005-2007.	**1.6%** were tested positive (n = 320)	Van Oosten et al., 2009
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of selected ACD patients patch tested with cinnamaldehyde 1% in pet. between year 2003-2004: 5138 patients Pooled patch test data from patients collected by the North American Contact Dermatitis Group (NACDG) .	**2.4%** were tested positive (n = 5138)	Zug et al., 2009
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of unselected ACD patients patch tested with cinnamaldehyde over two decades. Year 1984-1985: 1199 patients. Data from patients collected by the North American Contact Dermatitis Group (NACDG) (1970-2002).	**5.9%** were tested positive (n = 1199)	Nguyen et al., 2008
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of unselected ACD patients patch tested with cinnamaldehyde over two decades. Year 1985-1989: 3964 patients. Data from patients collected by the North American Contact Dermatitis Group (NACDG) (1970-2002).	**3.1%** were tested positive (n = 3964)	Nguyen et al., 2008
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of unselected ACD patients patch tested with cinnamaldehyde over two decades. Year 1992-1994: 3528 patients. Data from patients collected by the North American Contact Dermatitis Group (NACDG) (1970-2002).	**2.7%** were tested positive (n = 3528)	Nguyen et al., 2008
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of unselected ACD patients patch tested with cinnamaldehyde over two decades. Year 1994-1996: 3112 patients. Data from patients collected by the North American Contact Dermatitis Group (NACDG) (1970-2002).	**2.4%** were tested positive (n = 3112)	Nguyen et al., 2008
Patch test data, unselected	Cinnamaldehyde, 1% (in pet.)	Study of unselected ACD patients patch tested with cinnamaldehyde over two decades. Year 1996-1998: 3443 patients. Data from patients	**2.8%** were tested positive (n = 3443)	Nguyen et al., 2008

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
patients		collected by the North American Contact Dermatitis Group (NACDG) (1970-2002).		
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of unselected ACD patients patch tested with cinnamaldehyde over two decades. Year 1998-2000: 4735 patients. Data from patients collected by the North American Contact Dermatitis Group (NACDG) (1970-2002).	**3.7%** were tested positive (n = 4735)	Nguyen et al., 2008
Patch test data, consecutive patients	Cinnamaldehyde, 1% (in pet.)	Study on 2063 unselected patients patch tested with cinnamaldehyde, data from IVDK multicentre project (IVDK: Information Network of Departments of Dermatology in Germany, Austria and Switzerland). Data obtained 2003-2004.	**1.0%** were tested positive (n = 2063)	Schnuch et al., 2007
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of 1603 unselected patients with eczematous dermatitis patch tested with cinnamaldehyde. Data from five US sites and one Canadian site (year not reported)	**1.7%** were tested positive (n = 1603)	Belsito et al., 2006
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of 4900 unselected patients patch tested with cinnamaldehyde. Data from multicentre project IVDK (1996-1999).	**1.9%** were tested positive (n = 4900)	Schnuch et al., 2002
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet.)	Study of 702 unselected patients patch tested with cinnamaldehyde. Data from a multicentre study involving 7 European centres. Year not stated.	**0.14%** were tested positive (n = 702).	Frosch et al., 1995a
Patch test data, unselected patients	Cinnamaldehyde, 1% (in pet. with SSO (1%))	Study of 702 unselected patients patch tested with cinnamaldehyde. Data from a multicentre study involving 7 European centres. Year not stated.	**0.85%** were tested positive (n = 702).	Frosch et al., 1995a
Study of patch test data, unselected patients	Cinnamaldehyde, 1% (in pet. with SSO (1%))	Study of 1072 unselected patients patch tested with cinnamaldehyde. Multicentre study involving 9 European centres. Year not stated.	**0.93%** were tested positive (n = 1072)	Frosch et al., 1995b
Human repeated open application tests (ROATs)				
Patch test data and ROAT	Dilution series of cinnamaldehyde. Patch test: 0.00006% to 2% ROAT: 0.01% to 0.32%	17 cinnamaldehyde-allergic patients (20 controls) were tested with a dilution series of cinnamaldehyde in a patch test and a ROAT in order to investigate the development of axillary dermatitis. Copenhagen, Denmark and Malmö, Sweden. Year not stated.	The ROAT minimum effect level was 0.01% and the patch test minimum effect level was 0.002%.	Bruze et al., 2003
Patch test data and ROAT	Dilution series of cinnamaldehyde. Patch test:	22 cinnamaldehyde-allergic patients (20 controls) were tested with a dilution series of cinnamaldehyde in a	The ROAT minimum effect level was 0.1% and the patch test	Johansen et al., 1996

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	0.01% to 2% ROAT: 0.02%, 0.1% and 0.8%	patch test and a ROAT. Clinical study at Gentofte Hospital and Odense University Hospital, Denmark. Year not stated.	minimum effect level was 0.02%.	
Human Repeat Insult Patch Tests (HRIPT's)				
HRIPT	Cinnamaldehyde concentration: 0.5% Vehicle: 3:1 diethyl phthalate:ethanol (DEP:EtOH)	94 volunteers (25 male and 69 female) were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**0%** were tested positive (n = 94)	Unpublished report (RIFM 2004) cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 3% Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol	28 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**14%** were tested positive (n = 28)	Unpublished report (RIFM 2003a) cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 0.5% Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol	22 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**0%** were tested positive (n = 22)	Unpublished report (RIFM 2002) cited from Cocchiara et al., 2005.
HRIPT	Cinnamaldehyde concentration: 0.5% Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol	19 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**0%** were tested positive (n = 19)	Unpublished report (RIFM 2002) cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 0.1% Vehicle: EtOH	41 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**0%** were tested positive (n=41)	Danneman et al., 1983 cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 0.5% Vehicle: EtOH	38 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**0%** were tested positive (n=38)	Danneman et al., 1983 cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 1% Vehicle: EtOH	41 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**5%** were tested positive (n=41)	Danneman et al., 1983 cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 1.25%	10 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited	**50%** were tested positive (n=10)	Danneman et al., 1983 cited from Cocchiara et al.,

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	Vehicle: EtOH	reference.		2005
HRIPT	Cinnamaldehyde concentration: 1% Vehicle: EtOH	55 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**2%** were tested positive (n = 55)	Marzulli and Maibach 1976 and 1980 cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 1% Vehicle: pet	53 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**0%** were tested positive (n = 53)	Marzulli and Maibach 1976 and 1980 cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 1% Vehicle: alcohol SDA 39C	41 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**12%** were tested positive (n = 41)	Unpublished report (RIFM 1973b) cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 0.5% Vehicle: EtOH	38 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**0%** were tested positive (n = 38)	Unpublished report (RIFM 1965) cited from Cocchiara et al., 2005
HRIPT	Cinnamaldehyde concentration: 1.25% Vehicle: EtOH	10 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**50%** were tested positive (n = 10)	Unpublished report (RIFM 1964a) cited from Cocchiara et al.,2005
HRIPT	Cinnamaldehyde concentration: 0.125% Vehicle: EtOH	41 volunteers were tested with cinnamaldehyde in HRIPT's. No further information available in cited reference.	**0%** were tested positive (n = 41)	Unpublished report (RIFM 1964b) cited from Cocchiara et al., 2005
Human Maximation Tests (HMT's)				
HMT	Cinnamaldehyde concentration: 3% Vehicle: butylene glycol	25 volunteers were tested with cinnamaldehyde in HTM's. No further information available in cited reference.	**12%** tests were positive (n=25)	Unpublished report (RIFM 1974a) cited from Cocchiara et al., 2005
HMT	Cinnamaldehyde concentration: 2% Vehicle: pet.	25 volunteers were tested with cinnamaldehyde in HTM's. No further information available in cited reference.	**44%** tests were positive (n=25)	Unpublished report (RIFM 1973c) cited from Cocchiara et al., 2005
Case studies				
Patch test, one patient with itching eczematous lesions	Cinnamaldehyde. Concentration and vehicle not reported	A 33-year old man with itching eczematous lesions was patch tested with cinnamaldehyde. Case study, Italy (year not reported).	Positive reaction on day 2 and day 4 was observed	Guarneri, 2010
Patch test, one patient	Cinnamaldehyde. Concentration	A 47-year old man with dermatitis was patch tested with	Positive reaction on day	Decapite and

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
with dermatitis	and vehicle not reported	cinnamaldehyde. Case study, USA (year not reported)	2 was observed	Anderson, 2004
Patch test, one patient with rash on her arms	Cinnamaldehyde. Concentration and vehicle not reported	A 42-year old woman with rash on her arms was patch tested with cinnamaldehyde. Case study, UK (year not reported)	Positive reaction after 20 min (anaphylaxis) was observed	Diba and Statham, 2003

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

The sensitising properties of cinnamaldehyde have been intensively studied in both animals and humans. The mechanism of skin sensitisation by cinnamaldehyde has been suggested to involve the formation of Schiff bases of cinnamaldehyde on protein sidechains (Suskind and Majeti 1976). Numerous animal studies confirming the sensitising properties of cinnamaldehyde are available. The animal studies reported in Table 9 represent guideline studies as well as other studies based on testing principles that are equivalent to current test guidelines for skin sensitisation. According to the CLP criteria the results of LLNA (OECD 429), GPMT and Buehler tests (OECD 406) are directly applicable for classification and sub-categorisation of skin sensitisation. No Buehler tests are reported in Table 9.

Furthermore, a large number of publications are available on the sensitising properties of cinnamaldehyde seen in human patch tests. For diagnostic testing of contact allergy to fragrances in humans, standardised fragrance mixtures (FM I and FM II) are used in the European baseline series used for standardised patch testing in dermatological clinics. Cinnamaldehyde is a component of FM I, which is routinely been used for diagnostic patch testing in Europe (and elsewhere). FM I contains 1% cinnamaldehyde and a total of 8% fragrance allergens (SCCS 2012). Follow-up testing of the single fragrance substances showing positive reactions in patch tests with FM I and FM II is routinely done in many dermatological clinics and the sensitising properties of cinnamaldehyde are well documented in humans. Patch test studies with cinnamaldehyde involving several thousand dermatitis patients from dermatological clinics in various countries in Europe, North America, Australia and Asia are thus available. Diagnostic patch test data are generally seen as the primary source of clinical information on the occurrence of skin sensitisation and are considered to represent the most important human data in relation to this classification proposal.

10.7.2 Animal data

A total of 22 LLNAs, 2 LLNA: BrdU-ELISA test, 2 *ex vivo* LLNA: BrdU-ELISA and 3 GPMTs were identified for cinnamaldehyde (Table 9).

The reported EC3 values in the LLNAs range between 0.2% and 3.1%. In twenty studies the reported EC3 values < 2% and only one of the studies the reported EC3 values > 2% (Basketter et al. cited in SCCS, 2012). In one LLNA study no EC3 value was calculated (Basketter and Scholes et al., 1992).

In general, Lymphocyte proliferation may be influenced by choice of vehicle as some vehicles may either suppress or enhance the proliferative response of certain chemicals. This may especially be important for weak sensitisers with high EC3 values (Anderson et al., 2011). AOO (4:1) is among the recommended vehicles in OECD 429 test guideline. Other vehicles than those recommended may be used if sufficient scientific rationale is provided. Ethanol (EtOH) containing vehicle systems are apparently frequently used for assessing dermal effects of fragrance materials in both human and experimental studies, and the use of EtOH:DEP as an alternative vehicle to AOO has been investigated in a comparative study. EtOH:DEP induces a background proliferative lymph node response similar to that of AOO, and it was concluded that EtOH:DEP is a suitable alternative to AOO in the LLNA (Betts et al. 2007). Provided that the vehicle is suitable and does not elicit unwanted increases in background proliferative lymph node response, the choice of vehicle would not be expected to have a marked impact on the magnitude of the stimulation index (SI) as

it is measured as the increase in lymphocyte proliferation upon exposure to a test substances relative to that of the vehicle control (Anderson et al., 2011). However, the choice of vehicle may impact the level of passive absorption of a substance into the stratum corneum either by impacting the skin permeability or the level of precipitation of the substance on the skin (e.g. due to faster absorption or evaporation of the vehicle relative to the test substance) (Riviere and Papich 2009). Wright et al., 2001 studied the effect of seven different vehicles (50:50 EtOH:water, 90:10 EtOH:water, DMSO, propylene glycol (PG), DMF, MEK and AOO) on skin sensitizing potency of four chemicals, including cinnamaldehyde, using local lymph node assay. In this study AOO, MEK, DMSO and DMF were generally associated with the lowest EC3 values and PG and 50:50 EtOH:water gave higher EC3 values. The picture is, though, not clear and from this study it is difficult to generalise the effects of vehicles.

In the studies presented in Table 9 EtOH:DEP (with or without α-tocopherol, Trolox C or antioxidant mix) was the most used vehicle with ten studies (EC3 range 0.2%-1.4%), AOO was used as vehicle in four studies (EC3 range 0.57-3.1%), EtOH:Water was used as vehicle in two studies (EC3 range 1.2-1.6%) and DMSO (EC3 of 0.9%), DMF (EC3 of 0.5%), MEK (EC3 of 1.1) and PG (EC3 of 1.4) was used as vehicle in in one study each. From this it is possible that the dermal absorption of cinnamaldehyde varies depending on the choice of vehicle and thus the amount of substance available to cause the effect. As indicated by the relative narrow EC3 ranges of EtOH:DEP and AOO the effect vehicle choice does, though, not seem to exceed the inter laboratory or inter study variations. For all the tested vehicles EC3 values < 2% are seen.

In the LLNA: BrdU-ELISA tests EC2 values were reported to be between 2.2 and 6.1%. In the LLNA *ex vivo* BrdU tests an EC3 value of 1.91% were reported for one of the tests (Ulker et al., 2013) and an EC2 value of 6.9% were reported for the other (Williams et al., 2015).

Sensitisation was observed in 2 GPMTs with intradermal induction doses of 0.2 % cinnamaldehyde and a challenge concentration of 0.75%. In one GPMT study it is not clear from the review by Bickers et al. (2005) whether the concentration of 3% was the intradermal induction dose or the challenge concentration.

No relevant *in vitro* studies on cinnamaldehyde (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

21 of the 22 the LLNA studies and 2 of the 3 GMPT studies identified are relevant in terms of classification. The remaining 1 LLNA study, 1 GMPT study, 2 LLNA: BrdU-ELISA studies and 2 *ex vivo* LLNA: BrdU-ELISA studies confirm the sensitising properties of cinnamaldehyde. For 17 of the studies robust information is available and for 11 studies the results are cited from the SCCS 2012 review. One study is cited from the review by Bickers et al. (2005). Although the quality and reliability of all studies cannot be assessed in detail the results of the animal studies are, however, relatively consistent. Since it is not clear from the review by Bickers et al. (2005) whether the reported concentration in the GPMTs was the intradermal induction dose this study are not relevant in terms of classification.

Other animal studies on the skin sensitising properties of cinnamaldehyde are also identified. Such studies include Draize tests, Maguire tests, Open Epicutaneous Tests (OET), Freunds Complete Adjuvant Test (Bickers et al., 2005). However, such studies are not directly applicable for classification purposes and considering the large amount of other relevant information, these studies have not been included in this report.

10.7.3 Human data

A total of 46 results from diagnostic patch test studies, 2 ROATs, 14 HRIPTs, 2 HMTs and 3 case studies were identified for cinnamaldehyde (Table 10).

Diagnostic patch testing is conducted in order to diagnose contact allergy to a substance and is performed according to international standards by dermatologists[2]. The results of such patch tests are usually reported as number of patients/subjects having positive reactions in relation to the total number tested, i.e. the frequency of positive patch tests. An important factor when assessing the prevalence of positive reactions in

[2] European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice: https://www.ncbi.nlm.nih.gov/pubmed/26179009

diagnostic patch tests is how the group of patients are defined, i.e. selected patients versus consecutive (unselected) patients. Selected patients can be i.e. patients with dermatitis suspected of having contact allergy to fragrances or cosmetics or special occupational groups (aimed testing). Consecutive (unselected) patients are groups of patients for whom allergic contact dermatitis (ACD) is generally suspected.

As seen from Table 10 the positive patch test frequencies from the reported diagnostic patch test vary between 0.14 and 34% in dermatitis patients. For selected dermatitis patients positive reactions range between 0.98 and 34% (27 studies) and for unselected/consecutive dermatitis patients, positive reactions range between 0.14 and 5.9% (19 studies). Cinnamaldehyde was typically tested in concentrations of 1% (in petrolatum) in the diagnostic patch tests. The total number of published cases is > 2300. Although the observed frequencies show some variations the results confirm that positive reactions to cinnamaldehyde are commonly observed in dermatitis patients with relatively high frequencies observed in a number of tests.

Induction of sensitisation was reported in 6 of 14 HRIPT studies at cinnamaldehyde concentrations between 1 and 3%, with different vehicles: EtOH (4 positive; 4 negative), DEP:EtOH with or without α-tocopherol (1 positive; 4 negative), alcohol SDA 39C (1 positive; 0 negative) and petrolatum (0 positive). Both HMT studies reported positive reactions after 2-3% cinnamaldehyde with the vehicles butylene glycol and petrolatum, respectively.

Two ROATs with cinnamaldehyde are summarised in table 10 (Johansen et al., 1996; Bruze et al., 2003). In the study by Johansen et al., 1996, 22 cinnamaldehyde-allergic patients were tested with a dilution series of cinnamaldehyde in a patch test and a ROAT. The lowest threshold concentration (minimum effect level) was 0.02% for the patch test and 0.1% for the ROAT. In the study by Bruze et al., 2003, 17 cinnamaldehyde-allergic patients were tested with a dilution series of cinnamaldehyde in a patch test and a ROAT (exposure in the axilla to deodorants containing different concentrations of cinnamaldehyde). The lowest patch test and ROAT concentrations that gave positive reactions were 0.002% and 0.01%, respectively.

A few case studies are reported. In one study a 33-year old baker with itching eczematous hand lesions were patch tested positive to fragrance mix I and cinnamaldehyde (Guarneri, 2010). In one study a 47-year-old man who routinely handled a powder used to mask the vinyl odour from vinyl covers used for car seat upholstery suffered from dermatitis of his hands, feet, face and body. He were patch tested positive to cinnamaldehyde and North American Contact Dermatitis Group standard series It turned out that the powder contained cinnamaldehyde (Decapite and Anderson, 2004). In one study a 42-year old woman nurse had rash on her arms. After a positive reaction to fragrance mix she was patch tested to the constituents of fragrance mix. A strong urticarial reaction was seen to cinnamaldehyde and after 40 min. she developed widespread pruritus and erythema, and 5 min later, started to feel faint. It was concluded that she had immediate, as well as delayed, hypersensitivity to cinnamaldehyde and that this constituent of the fragrance mix was the most likely cause of the anaphylaxis (Diba and Statham, 2003).

The human studies identified are all relevant in terms of classification and confirm the sensitising properties of cinnamaldehyde. The comprehensive set of diagnostic patch test data covering the last 3-4 decades with several of the studies being published very recently are seen as the key information for this classification proposal. In order to use HRIPT and HMT data for classification the dose per unit area that gives a response is needed. This is not available for the HRIPT and HMT studies in Table 10 as these studies are cited from reviews (Cocchiara et al., 2005). Furthermore, no robust study information is available for the HRIPT and HMT studies in the reviews. For these reasons the HRIPT and HMT studies can only be seen as supporting evidence.

10.7.4 Human exposure

Cinnamaldehyde is a substance that is manufactured in or imported to the EU in amounts of 1000-10.000 tonnes/year and is widely used in products on the EU market. The registered categories of use for consumers are cosmetics, intermediates in the chemical industry, laboratory chemical and a variety of household and professional cleaning and maintenance products. Cinnamaldehyde is also a widely used flavoring agent, and some 180 ton of it is consumed globally each year in foods: 39 ton from the use of cinnamon and 141 ton deliberately added as a flavour (Gowder 2014).

According to SCCS (2012) cinnamaldehyde is used in volumes less than 175 ton per year in perfume formulations indicating that the use in other products (household and other products) may thus account for a substantial volume. As cinnamaldehyde is widely used in many different types of consumer products the general population can be exposed from many different sources.

Cinnamaldehyde is generally present in low concentrations in individual consumer products. The International Fragrance Association (IFRA) recommends maximum limits of cinnamaldehyde in leave-on cosmetic products between 0.02 - 0.05% depending on the product category. The recommended limits for rinse-off cosmetic products is between 0.04 - 0.4% depending on the product category and 0.05% for cleaning products as shown in Table 11 (IFRA 2013).

Table 11: The IFRA standard limits for cinnamaldehyde in IFRA QRA (Quantitative Risk Assessment) product categories (IFRA 2013):

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.02%
Category 2	Deodorants/antiperspirants	0.02%
Category 3	Hydroalcoholics for shaved skin	0.05%
Category 4	Hydroalcoholics for unshaved skin	0.05%
Category 5	Hand cream	0.05%
Category 6	Mouthwash	0.4%
Category 7	Intimate wipes	0.04%
Category 8	Hair styling aids	0.05%
Category 9	Rinse-off hair conditioners	0.05%
Category 10	Hard surface cleaners	0.05%
Category 11	Candles	Not restricted

The SCCS opinion (2012) refers to a number of surveys on the presence and content of various fragrances in various consumer products. It has been reported that 2.5% of a total of 516 consumer products; 6% of a total of 300 fragrance products; approx. 2% of 3000 products and 1% of children cosmetics were labelled to contain cinnamaldehyde (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 1.1% of 88 tested deodorants were labelled to contain cinnamaldehyde and the fragrance was detected in 4% (range: 5 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)). It was concluded that taking the total exposure into account, exposure to all 26 allergenic fragrances is foreseeable in daily life (survey studies cited in SCCS 2012).

The Danish EPA has conducted surveys and assessments of a broad range of consumer products over the last decades. Cinnamaldehyde has been identified in different types of products including day-to-day cosmetic products such as deodorants and lip products as well as e.g. massage oils, pleasure gels, animal care products and sports products (e.g. pain relief creams and gels). Cinnamaldehyde has also been found in household products such as cleaning agents and air care products and in articles such as toys/articles for children. Generally cinnamaldehyde is found in low concentrations (>0- <0.02 %) in the investigated products with few exceptions. Higher concentrations have thus been identified in massage oils (up to 1.7 %) (DK EPA database, search June 2016). Human exposure to cinnamaldehyde generally seems to be low based on the above information. The exposure is, however, assessed to be frequent due to the widespread uses and the high tonnage level of cinnamaldehyde. It is thus hard for consumers to avoid exposure. According to the data from IFRA the exposure of cinnamaldehyde when used as a fragrance in cosmetics is low with standard limits for leave-on cosmetics, rinse-off cosmetics and cleaning agents being below 1%.

10.7.5 Comparison with the CLP criteria

Cinnamaldehyde is a widely used fragrance and a well-known skin sensitiser. An assessment of the skin sensitizing properties of cinnamaldehyde has been conducted according to the current classification criteria as data are considered sufficient for sub-categorisation in this hazard class.

According to the classification criteria sub-category 1A represent "*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 sensitisation in humans. Severity of reaction may also be considered*" (CLP table 3.4.2).

According to the classification criteria sub-category 1B represent "*Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered*" (CLP table 3.4.2).

10.7.5.1 Animal data

According to the classification criteria evidence from animal studies for sub-category 1A and 1B, respectively, can include the following types of data and results (CLP tables 3.4.3 and 3.4.4):

	Animal data	
Sub-category 1A	LLNA	EC3 value ≤ 2 %
	GPMT	≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose
	Buehler	≥ 15 % responding at ≤ 0,2 % topical induction dose or ≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose
Sub-category 1B	LLNA	EC3 value > 2 %
	GPMT	≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
	Buehler	≥ 15 % to < 60 % responding at > 0,2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

Test results from the LLNA and GPMT can be used directly for classification. They may also be used for potency evaluation.

The skin sensitisation potency in LLNA (OECD 429) is determined according to table 3.6 in the guidance on the application of the CLP criteria as shown below (ECHA 2017).

Table 3.6 Skin Sensitisation Potency in Mouse Local Lymph Node Assay (copied from ECHA 2017)

EC3-value (% w/v)	**Potency**	**Predicted Sub-category**
≤ 0.2	Extreme	1A
> 0.2 - ≤2	Strong	1A
> 2	Moderate	1B

The skin sensitisation potency in GPMT (OECD 406) is determined according to table 3.7 in the guidance on the application of the CLP criteria as shown below (ECHA 2017).

Table 3.7 Potency on basis of the Guinea Pig Maximisation Test (copied from ECHA 2017)

Concentration for intradermal induction (% w/v)	Incidence sensitised guinea pigs (%)	Potency	Predicted sub-category
≤ 0.1	≥ 60	Extreme	1A
≤ 0.1	≥ 30 - <60	Strong	1A
>0.1 - ≤ 1.0	≥ 60	Strong	1A
>0.1 - ≤ 1.0	≥ 30 - <60	Moderate	1B
>1.0	≥ 30	Moderate	1B

In total 21 LLNA studies were suitable for sub-classification. Of these 20 studies showed cinnamaldehyde to be of extreme (n=2) or strong (n=18) potency i.e. equivalent to Category 1A. In 2 out of 22 LLNAs a (borderline) extreme potency of cinnamaldehyde was demonstrated with EC3 values equal to 0.2% (RIMF 2003a and 2003b cited in SCCS, 2012), i.e. equivalent to Category 1A. In 18 out of 22 LLNAs a strong potency of cinnamaldehyde was demonstrated with EC3 values between 0.2% and 2%, i.e. equivalent to Category 1A. In one LLNA a moderate potency of cinnamaldehyde was demonstrated with an EC3 value of 3.1%, i.e. equivalent to Category 1B. One LLNA study (Basketter and Scholes, 1992) cannot be used for classification as no EC3 value was calculated. With Stimulation Index > 3 the study, though, confirms a significant skin sensitising effect form cinnamaldehyde.

In 2 out of 3 GPMT studies an intradermal induction dose of 0.2% were used. In these 2 studies positive responses were seen in 90% and 100% of the animals, indicating a strong potency i.e. equivalent to classification in Category 1A. In the third GPMT study it is not clear from the review by Bickers et al. (2005) whether the reported concentration was the intradermal induction dose. Therefore this study is not relevant in terms of classification.

The significant skin sensitising effect from cinnamaldehyde is also confirmed by other studies including the two LLNA: BrdU-ELISA presented in Table 9.

As described in section 10.8.1 it is possible that the dermal absorption of cinnamaldehyde varies depending on the choice of vehicle. The EC3 ranges of the vehicles most frequently reported according to Table 9 EtOH:DEP (EC3 range 0.2%-1.4%) and AOO (EC3 range 0.57-3.1%) are relative narrow. The effect of the vehicle choice does not seem to exceed the inter laboratory or inter study variations. For all the tested vehicles EC3 values < 2% are seen which confirms the strong potency of cinnamaldehyde independently of the vehicle used.

Robust study information is available for 13 of 23 (21 LLNA and 2 GPMT) studies relevant for classification. For 9 of these 13 studies the quality was also assessed by SCCS (SCCS, 2012). Besides these 9 studies SCCS further assessed 11 unpublished LLNA studies that are included in Table 9. SCCS, 2012 is considered a reliable source. Collectively, the results of the animal studies confirm the strong sensitizing properties of cinnamaldehyde in a consistent manner.

10.7.5.2 Human data

According to the classification criteria human evidence for sub-category 1A and 1B, respectively, can include the following types of data (CLP section 3.4.2.2.3):

	Human data
Sub-category 1A	(a) positive responses at ≤ 500 μg/cm^2 (HRIPT, HMT — induction threshold); (b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure; (c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis (ADC) in relation to relatively low exposure.

Sub-category 1B	(a) positive responses at > 500 μg/cm^2 (HRIPT, HMT — induction threshold); (b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure; (c) other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis (ADC) in relation to relatively high exposure.

The guidance on the application of the CLP criteria further outlines how high or low frequency of occurrence of skin sensitization shall be assessed. The frequency is determined according to table 3.2 in the guidance as shown below (ECHA 2017).

Table 3.2 Relatively high or low frequency of occurrence of skin sensitisation* (copied from ECHA 2017)

Human diagnostic patch test data	**High frequency**	**Low/moderate frequency**
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Work place studies: 1: all or randomly selected workers 2: selected workers with known exposure or dermatitis	 ≥ 0.4 % ≥ 1.0 %	 < 0.4 % < 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

* Only one or two types of information may be sufficient for sub-categorisation.

The key evidence for the sensitising effects of cinnamaldehyde in this classification proposal is the human data from diagnostic patch tests from several dermatological clinics in many different countries in and outside EU. In addition several animal studies demonstrate that cinnamaldehyde has a strong or extreme sensitizing potency. In the diagnostic patch tests summarized in Table 10 relatively high incidences of positive reactions are seen upon exposure to cinnamaldehyde in a high number of published cases. For selected dermatitis patients positive reactions range between 0.98 and 34% with frequencies equal to or higher than 2% in 22 of 27 tests. For consecutive (unselected) dermatitis patients positive reactions range between 0.14 and 5.9% are observed with 16 of 19 tests reporting frequencies equal to or higher than 1%. These studies represent more than 2300 published cases of positive patch test reactions to cinnamaldehyde.

The collected data from patch test studies thus show that

- a high frequency (≥1%) of occurrence of skin sensitization is also observed in a 16/19 of the patch tests with consecutive (unselected) dermatitis patients
- a high frequency (≥2%) of occurrence of skin sensitisation the majority of the patch tests (22/27) with selected dermatitis patient studies
- the number of tested dermatitis patients showing positive reactions to cinnamaldehyde is well above 100 (>2300 cases)

These findings show a high frequency of occurrence of sensitization for cinnamaldehyde in humans. For deciding on the appropriate sub-category the data from patch test studies need to be seen in conjunction with the estimated exposure (see chapter 10.7.5.3 below).

Positive responses were reported in 6 of 14 HRIPT studies at 1-3% cinnamaldehyde and in 2 of 2 HMT at cinnamaldehyde concentrations of 2 and 3%. The HRIPT and HMT studies are non-clinical studies based on healthy volunteers representing the general population and such studies are no longer conducted due to ethical reasons. Robust study information is not available for the HRIPT and HMT studies. They are considered of lower relevance for this classification proposal.

10.7.5.3 Exposure considerations

The occurrence of skin sensitization in defined groups of patch test patients needs to be seen in conjunction with the level of exposure in order to make a decision on sub-categorisation of skin sensitisers. As described

in chapter 10.7.4 the exposure to cinnamaldehyde from cosmetic products is generally considered to be low based on the current IFRA standard limits and supported by information of the actual concentration of cinnamaldehyde in various consumer products reported in different surveys.

According to the guidance on the application of the CLP criteria an additive exposure index shall be set in order to decide on the appropriate sub-category for skin sensitisers (when based on human data). An additive exposure index of 1-4 equates to relatively low exposure, whereas 5-6 reflects relatively high exposure. The exposure index is determined according to table 3.3 in the guidance as shown below (ECHA 2017).

Table 3.3 Relatively high or low exposure (adapted from ECHA 2017)

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

To achieve the exposure index a response in each row in table 3.3 above is necessary. The exposure index of cinnamaldehyde is estimated based on the following assumptions:

- **Score 0** for concentration/dose: based on expected and observed concentrations < 1.0% of cinnamaldehyde in relevant (consumer) products
- **Score 2** for repeated exposure: based on frequent occurrence of cinnamaldehyde in consumer products with estimated daily use
- **Score 2** for number of exposures: based on an anticipated exposure of sensitised individuals to cinnamaldehyde at least more than 100 times

An additive exposure index of maximum 4 (0+2+2) has been set thus indicating relatively low exposure. A decision on the appropriate sub-category for skin sensitisers based on human data is done according to table 3.4 in the guidance on the application of the CLP criteria:

Table 3.4 Sub-categorisation decision table (from ECHA 2017)

Exposure data	**Relatively low frequency of occurrence of skin sensitisation**	**Relatively high frequency of occurrence of skin sensitisation**
Relatively high exposure (score 5-6)	Sub-category 1B	Category 1 or case by case evaluation
Relatively low exposure (score 1-4)	Category 1 or case by case evaluation	**Sub-category 1A**

10.7.5.4 Specific concentration limit

Specific concentration limits (SCL) can be set for skin sensitisers when reliable and adequate information is available to support that the specific hazard is evident below (or above) the generic concentration limit (GCL). The setting of an SCL for sensitisers is based on potency. For skin sensitisers SCLs are normally set based on the results of animal studies but reliable human data were exposure is defined can also be used.

The animal data provide evidence of strong to extreme sensitising effects of cinnamaldehyde which according to Table 3.9 of the guidance on the application of the CLP criteria supports concentration limits of 0.1% (strong) and 0.001% (extreme). It is noted that the expert group assessing classification criteria for skin sensitising potency by use of existing (animal) methods stated that if EC3 values are available from several studies then the lowest value should normally be used. The expert group further concluded that if a variety of

animal data leads to different categorisation of the same substance the higher potency category should apply (Basketter et al., 2005).

Furthermore, cinnamaldehyde has been identified as a substance of special concern by the SCCS based on its sensitizing capacity and the high number of reported human cases (SCCS 2012). The high number of reported cases demonstrates the sensitizing capacity of cinnamaldehyde under normal exposure conditions. Based on the induction experiments, human and animal studies (as presented above), IFRA has calculated limits by which different exposures entails a risk of sensitization. These limits span from 0.02%-0.4%, where 0.4% is for a product type with limited skin contact (mouth wash).

For most of the product types exposures above 0.02%-0.05% are regarded to constitute a risk of sensitization. Concerning elicitation reactions have been described down to 0.002% (by patch testing) (Bruze et al., 2003).

In conclusion cinnamaldehyde should have a SCL of 0.02%.

10.7.5.5 Weight of Evidence

Both animal and human data are available documenting the skin sensitizing properties of cinnamaldehyde. These data are considered in a total weight of evidence assessment (WoE) according to the CLP criteria and guidance.

The animal data provide evidence of strong sensitising effects of cinnamaldehyde as reflected in 22 out of 25 (22 LLNAs and 3 GPMTs) (comparable) guideline studies fulfilling the criteria for a sub-category 1A classification. 20 of 22 LLNAs have EC3 values < 2% fulfilling the criteria for sub-category 1A classification. One LLNA study shows an EC3 value of 3.1% fulfilling the criteria for sub-category 1B classification and one LLNA study cannot be used for classification due to lack of information. 2 of 3 GPMT studies confirm the strong sensitisation potential of cinnamaldehyde fulfilling the criteria for a sub-category 1A classification whereas the remaining GPMT study cannot be used for classification due to lack of information. Based on the available animal studies there is clear evidence for classification in sub-category 1A.

The human data available provide substantial evidence of strong sensitising effects of cinnamaldehyde based on the results of 46 patch tests. Diagnostic patch test data obtained from dermatitis patients attending individual dermatology clinics or collected clinic data is the primary source of clinical information on the occurrence of skin sensitisation (ECHA 2017) and diagnostic patch tests are generally performed under internationally standardised conditions. Human patch test studies with cinnamaldehyde show a high frequency of occurrence of skin sensitisation according to the classification criteria. According to the guidance the following three types of human information confirm the high frequency of occurrence of skin sensitisation: Data from unselected and selected dermatitis patients as well as a high number of published cases (>100). The comprehensive set of patch test data include thousands of dermatitis patients tested in dermatological clinics in different countries.

Although frequent/daily exposure to cinnamaldehyde is anticipated the overall exposure to cinnamaldehyde is estimated to be relatively low based on information on the use in consumer products.

Based on the high frequencies of skin sensitisation observed in human patch tests (≥2.0% in 22 of 27 patch tests with selected dermatitis patients and ≥1.0% in 16 of 19 patch tests with unselected dermatitis patients) and the high number of published cases combined with the estimated low exposure, classification of cinnamaldehyde as a strong skin sensitiser in sub-category 1A is justified.

10.7.6 Conclusion on classification and labelling for skin sensitisation

The available animal and human studies confirm the sensitising properties of cinnamaldehyde. The potency of the sensitising effect is reflected in both the animal studies and the human patch test data available - both fulfil the criteria for classification of cinnamaldehyde as a strong skin sensitiser in sub-category 1A.

Cinnamaldehyde shall therefore be classified in hazard category Skin sens 1A with the hazard statement H317 (May cause an allergic skin reaction). Cinnamaldehyde should have a SCL of 0.02%.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

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

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

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

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

Comments received during consultation

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

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

- the IFRA standards cannot be used to derive a SCL
- the two LLNA studies with EC_3 values at the border for extreme potency are unpublished RIFM studies which should not be used for classification, leading to the position that only "strong potency" can be concluded based on the LLNA studies.
- human diagnostic patch test data cannot be used to establish the proposed SCL of 0.02%.

Assessment and comparison with the classification criteria

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

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

Human data

The following human studies with cinnamaldehyde have been assessed:

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

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

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

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

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

Patch testing with serial dilutions and ROAT are performed on sensitised individuals to assess the degree of sensitivity and safe limits of exposure (CLP guidance, Table 3.1).

Two human ROAT were included in the CLH report. In the study by Johansen *et al.* (1996) the lowest threshold concentration (minimum effect level) were 0.02 % for the patch test and 0.1% for the ROAT when 22 cinnamaldehyde allergic patients were tested on the upper back and upper arm respectively with a dilution series of cinnamaldehyde. In the patch test 18/22 had at least 1 positive reaction to cinnamaldehyde and 4/22 had doubtful reactions. In the ROAT use test 8 patients reacted to 0.1% and 5 to 0.8% cinnamaldehyde in ethanol. None reacted to 0.02% cinnamaldehyde in ethanol. Further a total of 13/18 of the patients with a clearly positive patch test reaction to cinnamaldehyde (2% in petrolatum.) also developed a positive reaction in the ROAT test. The 4 patients with doubtful patch test responses to cinnamaldehyde (2% in petrolatum.) were all negative in the ROAT test. The study by Bruze *et al.* (2003) showed that the lowest patch test and ROAT concentrations that gave positive reactions were 0.002% and 0.01% respectively in the 17 cinnamaldehyde allergic patients exposed in the axilla to deodorants containing different concentrations of cinnamaldehyde. In the patch test all 17 patients had at least 1 positive reaction to cinnamaldehyde. In the ROAT test 8/8 patients in the first part of the study and 8/9 patients in the second part of the study gave positive reactions in the axilla when tested with cinnamaldehyde in deodorants. It was concluded in this study that deodorants containing cinnamaldehyde in the concentration range of 0.01–0.32% used 2 times daily on healthy skin can elicit axillary dermatitis within a few weeks. However, it is noted that patch testing with serial dilutions and ROAT are performed solely on sensitised individuals in order to estimate the elicitation threshold of an allergen. This is not a standardised protocol and only provides an indication on the degree on sensitivity (CLP guidance, table 3.1).

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

The DS also included 3 case studies. In the study by Guarneri (2010) a 33-year old baker with itching eczematous hand lesions was positively patch tested to Fragrance Mix I (FM I) and cinnamaldehyde. It is noted that standardised fragrance mixtures (FM I and FM II) contained in the European baseline series used for patch testing in dermatological clinics. FM I contains 1% cinnamaldehyde and a total of 8% fragrance allergens (SCCS 2012). The study by Decapite and Anderson (2004) described a 47-year-old man who routinely handled a powder, containing cinnamaldehyde, to mask the vinyl odour from covers used

for car seat upholstery suffering from dermatitis of his hands, feet, face and body. He was patch tested positive to cinnamaldehyde and North American Contact Dermatitis Group standard series. In the study by Diba and Statham (2003) a 42-year old woman nurse with rash on her arms showed a positive reaction to fragrance mix containing cinnamaldehyde. A strong urticarial reaction was seen to cinnamaldehyde. After 40 min she developed widespread pruritus and erythema, and 5 min later, started to feel faint. It was concluded that she had immediate, as well as delayed, hypersensitivity to cinnamaldehyde and that this constituent of the fragrance mix was the most likely cause of the anaphylaxis.

Exposure considerations

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

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

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

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

	Relatively low frequency of occurrence of skin sensitisation	Relatively high frequency of occurrence of skin sensitisation
Relatively high exposure (score 5-6)	Sub-category 1B	Category 1 or case by case evaluation
Relatively low exposure (score 1-4)	Category 1 or case by case evaluation	Sub-category 1A

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

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

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

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

Animal data

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

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

Two out of three GPMTs showed sensitisation with intradermal induction concentrations of 0.2% cinnamaldehyde and a challenge concentration of 0.75%. In the study by Basketter and Scholes (1992) positive reactions were seen in 100% of the animals 24 and 48 hours after challenge, while the study by Basketter (1992) similarly showed positive reactions in 90% and 100% of the animals at 24 and 48 hours after challenge, respectively. Both studies indicate a strong potency for cinnamaldehyde and support a classification in sub-category 1A. It is however noted that concentrations for intradermal induction lower than 0.2% have not been tested, and it cannot be concluded if cinnamaldehyde could be an extreme sensitiser based on these studies. The third study by Ishihara *et al.* (1986) is not clearly reported and cannot be used for classification.

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

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

Overall, the criteria for the Skin Sens. 1A classification of cinnamaldehyde are fulfilled in

several LLNA test and two GPMTs.

Conclusion by RAC

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

Setting of a specific concentration limit

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

Two human studies (Patch testing with serial dilutions and ROAT) indicate that a SCL lower than the GCL should be applied based on elicitation thresholds. In the study by Johansen *et al.* (1996) the lowest threshold concentration (minimum effect level) were 0.02% for the patch test and 0.1% for the ROAT. The study by Bruze *et al.* (2005) showed the lowest patch test and ROAT concentrations that gave positive reactions were 0.002% and 0.01% respectively.

Overall, based on the two human studies showing elicitation reaction at concentrations as low as 0.002% and supported by the LLNA studies with EC_3 values as low as 0.2% RAC is of the opinion that, in a weight of evidence assessment, **an SCL of 0.01% (being intermediate between 0.1% and 0.001% in terms of order of magnitude) is justified for cinnamaldehyde.[3]**

10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

10.9 Carcinogenicity

Hazard class not assessed in this dossier.

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

10.10 Reproductive toxicity

Hazard class not assessed in this dossier.

10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier.

10.13 Aspiration hazard

Hazard class not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Environmental hazards have not been assessed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Additional hazards have not been assessed in this dossier.

13 ADDITIONAL LABELLING

For mixtures not classified as sensitising but containing at least one sensitising substance, the CLP criteria allow for the setting of concentration limits for elicitation of components of a mixture.

Given that cinnamaldehyde is classified as a skin sensitiser in Category 1A with a specific concentration limit of 0.02%, the concentration limit for elicitation should be set at one tenth of the specific concentration limit, to protect already sensitised individuals (CLP Annex I, Table 3.4.6, Note 1). Hence, the concentration limit for elicitation should be 0.002%, and therefore labelling with EUH208 will apply when cinnamaldehyde is present in mixtures in concentrations ≥ 0.002%.

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15 ANNEXES

Annex I: detailed study summaries

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1]

(2*E*)-3-phenylprop-2-enal [2]

EC Number:	**203-213-9 [1]**
	604-377-8 [2]
CAS Number:	**104-55-2 [1]**
	14371-10-9 [2]
Index Number:	**Not available**

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Version number: 2 **Date: 12. February 2020**

CONTENTS

1 PHYSICAL HAZARDS

Classification for physical hazards is not a part of the CLH proposal for cinnamaldehyde.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The information below on toxicokinetics has largely been copied from the public part of the registration dossier.

2.1.1 STUDY 1

Reference:

Adams T.B., Cohen S.M., Doull J., Feron V.J., Goodman J.I., Marnett L.J., Munro I.C., Portoghese P.S., Smith R.L., Waddell W.J., Wagner B.M.: The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. Food and Chem Toxicology 42: 157-185, 2004

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Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: non-guideline study.

Method: Tissue distribution and excretion of [3-^{14}C]-labelled cinnamic aldehyde following single and multiple oral administration in male Fischer 344 rats.

Test substance:

Cinnamaldehyde, Aldrich Chemical Co. and [3-^{14}C]-cinnamaldehyde, Amersham Corporation

Purity of non-radiolabelled Cinnamaldehyde >95% and purity of [3-^{14}C]-cinnamaldehyde 97% (both measured with TLC)

No data available on impurities

Radiolabelling, specific activity: 10.5 mCi/mmol

Trioctanoin, National Centre for Toxicological Research, purity >95% was used as vehicle for oral dosing

Test animals:

Rat (Fischer 344), male (8/group)

- Source: Charles River Breeding Laboratories, Wilmington, MA, USA
- Age at study initiation: No data
- Weight at study initiation: 179±24 g
- Fasting period before study: For the acute study groups of rats were fasted overnight
- Individual metabolism cages: Yes, in both single an multiple dosing study
- Diet: Ad libitum (Rodent Chow Diet No. 5002, Ralston Purina Co., St. Louis, MO, USA)
- Water: Ad libitum
- Acclimation period: No data

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 ±3
- Humidity (%): No data
- Air changes (per hr): No data
- Photoperiod (hrs dark / hrs light): 12/12

Dosing:

Acute dosing study: Groups of male rats (8/group) were fasted overnight and given a single dose by gavage at levels of 5, 50, or 500 mg/kg bw of [3-^{14}C]-cinnamaldehyde. After administration of the radioactive dose, the animals were killed at the following time periods for each dose level: 5 mg/kg bw, 0.5, 2.5 or 24 hours; 50 mg/kg bw, 0.5, 3.5, 24 or 72 hours; 500 mg/kg bw 1, 6.5, 24 or 72 hours.

Multiple dosing study: Groups of male rats (8/group) were pre-treated with single daily oral dose levels of 5, 50, or 500 mg/kg bw of cinnamaldehyde by gavage for seven days at 24 hours interval. Twenty-four (24) hours later, animals in each group received a single oral dose of [3-^{14}C]-cinnamaldehyde equivalent to the pre-treatment level. The rats were killed 1, 2.5 or 24 hours after the radioactive dose for the 5- and 50 mg/kg bw dose levels, and at 1, 2.5, 24 or 72 hours after the 500 mg/kg bw dose.

After treatment with [3-^{14}C]-cinnamaldehyde the rats in both the acute and multiple dosing study were placed in individual stainless-steel metabolism cages fitted with a bottom pan which had a screen to separate faeces from urine.

Sampling:

Tissues and body fluids sampled: Urine, faeces, blood, liver, kidneys, spleen, brain, heart, lungs, muscle, gastrointestinal tract, subcutaneous fat and carcass

Time and frequency of sampling:

- Urine and faeces were collected at the end of each experimental period. If the experiment was longer than 24 hours, samples were collected at 24hour intervals

- Tissue samples were collected at the end of each experimental period.

Detailed study summary and results:

Radioactive cinnamaldehyde was distributed primarily to the gastrointestinal tract, kidneys, and liver, after single oral dose and multiple oral administrations.

After 24 hours, more than 80% of the radioactivity was recovered in the urine and less than 7% in the feces from all groups of rats, regardless of dose level. At all dose levels, a small amount of the dose was distributed to the fat. At 50 and 500 mg/kg bw, radioactivity could be measured in animals terminated 3 days after dosing. Except for the high dose pre-treatment group, the major urinary metabolite was hippuric acid, accompanied by small amounts of cinnamic and benzoic acid. In the high dose pre-treatment group, benzoic acid was the major 4 metabolite, suggesting that saturation of the glycine conjugation pathway occurs at repeated high dose levels of cinnamaldehyde.

2.1.2 STUDY 2

Reference:

Peters M.M., Caldwell J.: Studies on trans-cinnamaldehyde. 1. The influence of dose size and sex on its disposition in the rat and mouse. Food and Chemical Toxicology 32 (10): 869-76, 1994

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Metabolism

Test guideline: non-guideline study.

Principles of method: To test the influence of dose size and sex on its disposition in the rat and mouse

Test substance:

trans-[3-^{14}C]Cinnamaldehyde (CAS 14371-10-9; EC 604-377-8); purity 96.8%

No data available on impurities

Radiolabelling, specific activity: 4.1 mCi/mmol

Test animals:

Rat (Fischer 344), male and female (4/group)

Mice (CD1), male and female (6/group)

- Source: Fischer 344 rats, Harlan-OLAC, Bicester Oxon, UK and CD1 mice, Charles River Breeding Laboratories, Manston, Kent, UK
- Age at study initiation: No information available
- Weight at study initiation: Fischer 344 rats 200±10g; CD1 mice 27±2g
- Housing: individual
- Individual metabolism cages: yes
- Diet: ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS

- Temperature (°C): No information available
- Humidity (%): No information available
- Photoperiod (hrs dark / hrs light): No information available

Dosing:

Single dose, oral (gavage) and intraperitoneal injection.

Concentrations: gavage: 250 mg/kg bw; ip.: 2 and 250 mg/kg bw

No. of animals per dose: male and female F344 rats (4/group); male and female CD1 mice (6/group)

Sampling - metabolite characterisation studies:

- Urine and faeces collected on the day before experiment and 3 days after dosing
- From how many animals: No information available
- Method type(s) for identification: *Radio*-HPLC

Detailed study summary and results:

The metabolism of trans-[3-^{14}C]cinnamaldehyde was investigated in male and female Fischer 344 rats and CD1 mice at doses of 2 and 250 mg/kg body weight given by ip injection and in males at 250 mg/kg by oral gavage. Some 94% of the administered dose was recovered in the excreta in 72 hr in both species with most (75-81%) present in the 0-24 hr urine. Less than 2% of the administered dose was found in the carcasses at 72 hr after dosing. Urinary metabolites were identified by their chromatographic characteristics. In both species the major urinary metabolite was hippuric acid (71–75% in mice and 73–87% in rats) accompanied by 3-hydroxy-3-phenylpropionic acid (0.4–4%), benzoic acid (0.4–3%) and benzoyl glucuronide (0.8–7.0%). The glycine conjugate of cinnamic acid was formed to a considerable extent only in the mouse (4–13%). The oxidative metabolism of cinnamaldehyde essentially follows that of cinnamic acid, by beta-oxidation analogous to that of fatty acids. Apart from the metabolites common to cinnamic acid and cinnamaldehyde, 7% of 0-24-hr urinary ^{14}C was accounted for by two new metabolites in the rat and three in the mouse, which have been shown in other work to arise from a second pathway of cinnamaldehyde metabolism involving

conjugation with glutathione. The excretion pattern and metabolic profile of cinnamaldehyde in rats and mice are not systematically affected by sex, dose size and route of administration. The data are discussed in terms of their relevance to the safety evaluation of trans-cinnamaldehyde, particularly the validity or otherwise of extrapolation of toxicity data from high to low dose.

Based upon the metabolism and rapid excretion of the metabolites formed in rats and mice (24 hr), it can be concluded that the chemical trans-Cinnamaldehyde is expected to exhibit low bio-accumulation potential upon entry within the body of animals.

2.1.3 STUDY 3

Reference:

Yuan, J. et al. 1992. Toxicokinetis of Cinnamaldehyde in F344 rats. Fd. Chem. Toxic. 30, 997-1004, 1992.

Yuan, et al. 1993. Application of microencapsulation for toxicology studies. Fundamental and Applied Toxicology 20, 83-87, 1993.

Cited from the publicly available part of REACH registration.

Test type

No information on guideline or GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: No data

Method: Toxicokinetic study by single dose oral (gavage) and intravenous (iv) administration[1]

Test substance:

No details on test substance given by the registrant

Purity of cinnamaldehyde 98%

No data available on impurities

Test animals:

Rat (Fischer 344), male and female (3/group)

No additional data in publicly available part of REACH reg.

[1] Indicated as both intraperitoneal (ip) and iv administration administration in REACH reg. The published article by Yuan et. al., 1992, however states intravenious administration.

Dosing:

Single dose, oral (gavage) and intravenous (iv) administration

Vehicle: oral: corn oil; iv: ethanol-emulphor EL-620-water

Dose: gavage: 50, 150, 500, 1000, and 2000 mg/kg bw; gavage microcapsulated: 50, 250, and 500 mg/kg bw; iv: 5, 15 or 24 mg/ kg bw.

Sampling:

No data in publicly available part of REACH reg.

Detailed study summary and results:

After iv administration a large fraction of cinnamaldehyde was immediately oxidized to cinnamic acid (estimated to be between 37 and 60 % by the authors) within the first 30 minutes. The biological half-life of cinnamaldehyde after iv administration was found to be 1.7 hours in the rat.

After oral administration at 250 or 500 mg/kg bw the maximum blood concentrations were in the order of 1 µg/ml. At 50 mg/kg bw no cinnamaldehyde could be detected in the blood (< 1 µg/ml). The majority of cinnamaldehyde administered orally was excreted in urine as hippuric acid within 24 hours. The maximum excretion rate occurred at 8 hours after gavage.

2.1.4 STUDY 4

Reference:

Zhao H, et al. 2014. Pharmacokinetic study of cinnamaldehyde in rats by GC-MS after oral and intravenous administration. Journal of Pharmaceutical and Biomedical Analysis 89, 150-157, 2014.

Cited from the publicly available part of REACH registration.

Test type

No information on guideline or GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: No data

Method: GC-MS study on toxicokinetics (absorption, metabolism and excretion)

Test substance:

No details on test substance given by the registrant

Purity of cinnamaldehyde 99%

No data available on impurities

Test animals:

Rat (Sprague-Dawley), male (5/group)

- Source: No data
- Age at study initiation: No data in publicly part of REACH reg.
- Weight at study initiation: No data in publicly part of REACH reg.
- Fasting period before study: No data in publicly part of REACH reg.
- Individual metabolism cages: Stainless-steel metabolic cages – no data on indivual cages
- Diet: Free access to food
- Water: Free access to water
- Acclimation period: No data in publicly part of REACH reg.

Dosing:

Single dose, oral (gavage) and intravenous (iv) administration

Vehicle: oral: corn oil

Dose: oral: 500, 250, or 125 mg/kg bw; iv: 20 mg/ kg bw.

Three groups of rats (n = 5) received a single oral dose of 500 mg/kg, 250 mg/kg, or 125 mg/kg cinnamaldehyde (diluted in corn oil). The group of rats (n = 5) used for the urinary and fecal excretion study received a single oral dose of 500 mg/kg cinnamaldehyde. One group of rats (n = 5) were dosed with 20 mg/kg by iv administration.

Sampling:

Blood was collected at 10, 30, 60, 120, 180, 240, 360, 480, 720, 1080, and 1440 min post-administration. For the group with iv administration, blood was collected at 2, 5, 10, 15, 30, 60, 90, 120, and 180 min after iv administration. The blood samples were processed similarly to the blank sample. Urine and feces were collected at 0–4, 4–8, 8–12, 12-18, and 18–24 h post-dosing. The feces were dried at room temperature.

Detailed study summary and results:

The GC–MS technique was used to separate and determine cinnamaldehyde and its metabolites in rat plasma after oral and intraveneous administration. The areas under the plasma concentration–time curve (AUC) from 0 min to terminal time of cinnamaldehyde were 1984 ± 531 and 355 ± 53 ng h/ml for oral (500 mg/kg) and iv (20 mg/kg) administration, respectively. The elimination half-lives of cinnamaldehyde were 6.7 ± 1.5 and 1.7 ± 0.3 h for oral and iv administration, respectively. From dosage 125 to 500 mg, maximum plasma concentration (Cmax) and area under the curve to termination time (AUC0–t) were proportional to the dose;

time at maximum plasma concentration (Tmax) and mean residence time (MRT) did not change following dose escalation. The metabolites in blood were cinnamyl alcohol and methyl cinnamate.

An excretion experiment was also performed. A lower accumulative ratio of cinnamaldehyde was found after 24 hours, with the numbers reaching at 0.3% and 0.8% in feces and urine.

A double peak was observed in the concentration-time profile of 500 mg/kg oral administration; the Cmax was 249 ± 36 ng/ml and the other peak was 130 ± 56 ng/ml. Enterohepatic circulation may be an explanation for this because the double-peak was not observed in the iv concentration-time profile; furthermore, the metabolites of cinnamaldehyde presented the same phenomenon. Half-life was about 6.5 hours independent of oral dose.

2.1.5 STUDY 5

Reference:

D. Bickers, P. Calow, H. Greim, J.M. Hanifin, A.E. Rogers, J.H. Saurat, I.G. Sipes, R.L. Smith, H. Tagami, 2005. A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients. Food and Chemical Toxicology 43 (2005) 799–836.

Hotchkiss SAM, 1998. Absorption of fragrance ingredients using in vitro models with human skin. In: Frosch, P.J., Johansen, J.D., White, I.R. (Eds.), Fragrances: Beneficial and Adverse Effects. Springer-Verlag, Berlin, pp. 125–135, 1998. Cited in Bickers (original literature not available).

Cited from the publicly available part of REACH reg.

Test type

Skin absorption model with human skin or diffusion cell technique with excised human abdominal skin and rat skin. Dermal absorption.

Material and methods

Test guideline:

Type of method: In vitro/ex vivo

Objective of study: Dermal absorption

Test guideline: No data

Method: Skin absorption model

Test substance:

No details on test substance given by the regristrant

Dosing:

Type of coverage: open and occlusive

Duration: 72 hours

Detailed study summary and results:

Using a skin absorption model system with human skin for cinnamaldehyde it was reported that 34% and 66% cinnamyl alcohol, 24% and 52% cinnamaldehyde and 18%, and 61% cinnamic acid (non-occluded and occluded, respectively) were absorbed by 72h.

Using a skin absorption model system with excised rat skin, 34% and 42% cinnamaldehyde (non-occluded and occluded, respectively) have been reported to be absorbed within 48–72h (Hotchkiss, 1998).

3 HEALTH HAZARDS

3.1 Skin sensitisation

3.1.1 Animal data

3.1.1.1 STUDY 1 and 2 (LLNA)

Study reference:

Williams W.C., Copeland C., Boykin E., Quell S.J., Lehmann D.M.: Development and utilization of an *ex vivo* bromodeoxyuridine local lymph node assay protocol for assessing potential chemical sensitizers. Journal of Applied Toxicology; 35: 29-40, 2015.

Detailed study summary and results:

Test type

ex vivo LLNA: BrdU-ELISA – No OECD guideline exists

LLNA:BrdU-ELISA (*in vivo)* according to the ICCVAM, 2010 protocol which is comparable to OECD guideline 442B

GLP: Not stated

Test substance

Cinnamaldehyde (Sigma–Aldrich)

Purity: No information on purity available

Test animals

Mice (BALB/c), female

6 animals per dose

Age: 8-9 weeks old

All mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care approved facility that provided constant environmental conditions with an ambient temperature of 21.5 ± 1.5 °C, relative humidity of 55 ± 5%, a 12 h light/dark cycle. Mice were housed (six per cage) in polycarbonate cages with hardwood chip bedding (NEPCO, Warrensburg, NY, USA) and were provided a balanced diet of mouse chow (5POO Prolab RMH3000, PMI Nutrition International, Richmond, IN, USA) and water ad libitum. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of NHEERL, US EPA.

Administration/exposure

Three groups of mice (n=6 per dose) were treated with 1, 5 and 10% cinnamaldehyde. Vehicle: acetone-olive oil (AOO) 4:1. One group was treated with vehicle alone (vehicle control). The test substance or vehicle alone was applied 25 µl to the dorsum of each ear on experimental day 1, 2 and 3. On experimental day 6, mice for *in vivo* LLNA:BrdU-ELISA was injected i.p. with 0.5 ml of pyrogen-free saline containing 5mg BrdU. Twenty-four (24) hours later, the mice were killed. Immediately following killing, the lymph nodes draining the ears were harvested and placed in PBS at room temperature. Lymph nodes were mechanically disaggregated using a disposable plastic pestle and passed through a 100 µm Celltrics filter into a sterile 15 ml collection tube. Lymph node cells were pelleted by centrifugation and re-suspended in 1ml PBS. Cells were counted using a Coulter Counter, and viability was determined by trypan blue dye exclusion. Cell suspensions were diluted to a final volume of 15 ml, and 100 µl aliquots were then plated into duplicate wells of a 96-well plate. Cells were adhered to the plate by centrifugation and then dried to the plate at 60 °C for 1 h. After drying, the plates were stored at 4 °C until assessment of BrdU incorporation by ELISA.

On experimental day 6, mice for *ex vivo* LLNA:BrdU labelling was killed. Immediately following killing, the lymph nodes draining the ears were harvested and placed in room temperature RPMI 1640 with 25mM HEPES and 2.05mML-glutamine supplemented with 10% fetal bovine serum and 2% penicillin/streptomycin. Lymph nodes were processed into single cell suspensions. After counting, 3×10^5 live cells in 100 µl volume were plated in duplicate wells of a 96-well plate. Cells were incubated in the presence of 10 µM BrdU for 8‑12 hours. BrdU-labelled cells were adhered to the plate by centrifugation (300 g for 7 min at room temperature) and then dried to the plate at 60 °C for 1 h. After drying, the plates were stored at 4 °C until assessment of BrdU incorporation by ELISA.

BrdU incorporation was quantified using the BrdU Cell Proliferation ELISA kit and protocol.

Results and discussion

The responses to test substances exposure were characterized by BrdU incorporation into the lymph node cells and the stimulation index at each dose was calculated as the ratio of the mean BrdU labelling index for each treatment group to the mean BrdU labelling index of the concurrent vehicle control group. An SI of 2 indicates a positive threshold response in the assay.

Cinnamaldehyde was shown to be sensitising with an EC2 value of 6.1% in the *in vivo* LLNA:BrdU-ELISA test and with an EC2 value of 6.9% in the *ex vivo* LLNA:BrdU test. Irritation was not observed for cinnamaldehyde (determined by ear thickness, erythema score and differentiation index (DI). Detailed information of the responses of each animal per test group is not presented in the article.

3.1.1.2 STUDY 3 (LLNA)

Study reference:

Niklasson I.B., Delaine T., Islam M.N., Karlsson R., Luthman K., Karlberg A-T.: Cinnamyl alcohol oxidizes rapidly upon air exposure. John Wiley & Sons A/S Contact Dermatitis, 68, 129–138, 2013.

Detailed study summary and results:

Test type

LLNA, comparable to the most recent version of OECD guideline 429, however, with only 3 animals used pr. dose instead of 4.

GLP: Not stated

Test substance

Cinnamaldehyde (Aldrich Chemicals, Sweden), purity > 98%

Test animals

Mice (CBA/Ca), female

3 animals per dose (two-week air-exposed cinnamyl alcohol, epoxy cinnamyl alcohol and epoxy cinnamaldehyde also tested)

Age at study initiation: 8-9 weeks

The mice were housed in HEPA-filtered air flow cages, and kept on standard laboratory diet and water ad libitum.

Administration/exposure

Groups of mice (N=3) were treated daily with 25µl the test substance in vehicle or vehicle alone on dorsum of both ears for three consecutive days (day 0-2). The concentrations used for were cinnamaldehyde 0.1, 0.99, 3.3, 9.9 and 19.8% and the vehicle was acetone-olive oil (AOO). On day 5, all mice were injected intravenously via the tail vein with [^{3}H]methylthymidine and were sacrificed after 5 hours. The draining lymph nodes were excised and pooled for each group, and single-cell suspensions of lymph node cells in PBS were prepared with cell strainers. The [^{3}H]methylthymidine incorporation into DNA was measured by β-scintillation counting on a Beckman LS 6000TA instrument.

Results and discussion

Results are expressed as mean dpm/lymph node for each experimental group and as stimulation index (SI), that is, test group/control group ratio. Cinnamaldehyde was shown to be sensitising with an EC3 value of 0.75% wt/vol (57 mM) in the LLNA assay. No information on irritation was reported. Detailed information of the responses of each animal per test group is not presented in the article.

3.1.1.3 STUDY 4 (LLNA)

Study reference:

Ulker O.C., Ates I., Atek A., Karakaya A.: Evaluation of non-radioactive endpoints of *ex vivo* local lymph node assay-BrdU to investigate select contact sensitizers. Journal of Immunotoxicology, 10(1): 1–8, 2013.

Detailed study summary and results:

Test type

ex vivo LLNA: BrdU-ELISA, no OECD guideline exists

GLP: Not stated

Test substance

Cinnamaldehyde (Sigma, St. Louis, MO)

Purity: No information on purity available

Test animals

Mice (BALB/c), female

4 animals per dose

Age at study initiation: 8-12 weeks

The animals were kept at 23 °C and relative humidity 55 % with alternating 12h light and dark. The animals had ad libitum access to water and diet.

Administration/exposure

Five groups of mice (n = 4/group) were exposed topically (on dorsum of both ears) for 3 consecutive days to 25µl of different doses of known sensitizers or vehicle (AOO) alone daily. All mice were rested on Day 4 and then euthanized by cervical dislocation on Day 5 to permit collection of their auricular lymph nodes. The excised right and left lymph nodes from each mouse were pooled and homogenized, and the released cells suspended in 15 ml physiological saline. After culture had occurred for 48 hours at 37°C, BrdU was added to the wells for a 24 hour labelling period. The cells in the wells were then recovered by aspiration and the extent of BrdU incorporation measured by ELISA.

Results and discussion

Cinnamaldehyde was shown to be sensitising with an EC3 value of 1.91%. No information on irritation reported.

Calculated stimulation index, cinnamaldehyde

Applied concentration	0.5%	1%	5%	10%
SI	1.85	2.60	4.36	9.19

3.1.1.4 STUDY 5 (LLNA)

Study reference:

Kojima H., Takeyoshi M., Sozu T., Awogi T., Arima K., Idehara K., Ikarashi Y., Kanazawa Y., Maki E., Omori T., Yuasaj A., Yoshimurak I.: Inter-laboratory validation of the modified murine local lymph node assay based on 5-bromo-2'-deoxyuridine incorporation. J. Appl. Toxicol.; 31: 63–74, 2011

Detailed study summary and results:

Test type

LLNA:BrdU-ELISA (*in vivo*) in accordance with OECD 442B

The studies were not conducted under full compliance with GLP. However, all the laboratories were equipped to perform, and competent with, GLP.

Test substance

Trans-Cinnamaldehyde (though the study refers to the CAS no. of cinnamaldehyde, 104-55-2)

Purity: No information on purity available

Test animals

Mice (CBA/JN), female

4 animals per dose

Age at study initiation: 8-12 weeks

The animals were kept at 22±3 °C and relative humidity 30-70 % with artificial light for12 hours. The animals had ad libitum access to water and diet.

Administration/exposure

A minimum of four successfully treated animals was used per dose group, with a minimum of three consecutive doses of the chemical, and one group each for the negative vehicle control (AOO) and positive control (50% hexyl cinnamaldehyde). A 25µl dose of test solution was applied to the dorsum of both ears of the mice for three consecutive days using a micro volume pipette. A single intraperitoneal injection of 0.5 ml of BrdU solution (5mg/mouse/injection) was given to the mice 48 h after the final application. Approximately 24 hours after BrdU injection, the auricular lymph nodes were removed. The lymph nodes

were carefully dissected and trimmed of fascia and fat, weighed and stored individually in a 1.5 ml centrifuge tube at -20°C until BrdU-ELISA measurement.

Results and discussion

The EC2 was defined as the estimated concentration that yielded an SI of 2 from the dose–response curve. EC2 of the weighted average was estimated and classified into the appropriate chemical category. trans-cinnamaldehyde was shown to be sensitising with an average EC2 value of 2.2% for the 3 laboratories. No information on irritation reported.

Calculated stimulation index, trans-cinnamaldehyde

Applied concentration	1%	3%	10%
SI laboratory 2	1.10	2.23	3.37
SI laboratory 4	1.57	2.94	3.49
SI laboratory 5	1.14	2.10	4.11

3.1.1.5 STUDY 6-15 (LLNA, 10 studies cited in SCCS 2012)

Study reference:

Unpublished summary reports by the Research Institute for Fragrance Materials (RIFM), cited in:

Scientific Committee on Consumer Safety SCCS OPINION on Fragrance allergens in cosmetic products. June 2012 (SCCS 2012). RIFM references: 2003a, 2003b, 2003c, 2003d, 2003e, 2003f, 2003g, 2003h, 2003i, 2003j.

Detailed study summary and results:

Test type

LLNA with no reported deviations from OECD 429 according to SCCS 2012

Test substance

Cinnamaldehyde, no information on purity.

Test animals

Mice, n=4 animals per dose.

No further information available in SCCS 2012.

Administration/exposure

In all 10 studies cinnamaldehyde was tested in concentrations of 0.1, 0.3, 1.0, 3.0 and 10.0%

Vehicles used were either:

- 3:1 ethanol:diethyl phthalate (EtOH:DEP) (2 studies)
- 0.1% α-tocopherol in 3:1 EtOH:DEP (2 studies)

- 2.0% α-tocopherol in 3:1 EtOH:DEP (2 studies)
- 0.3% antioxidant mix (1:1:1 of α-tocopherol, butylated hydroxytoluene (BHT) and eugenol,) in 3:1 EtOH:DEP (2 studies)
- 0.1% Trolox C in 3:1 EtOH:DEP (2 studies)

No further information available in SCCS 2012.

Results and discussion

Although detailed information is not available for the studies conducted by RIFM the results generally confirm the sensitising properties identified for cinnamaldehyde in other LLNA studies. The EC3 values reported by RIFM are in the range 0.2%-1.7%.

3.1.1.6 STUDY 16 (LLNA)

Study reference:

Elahi E. N., Wright Z., Hinselwood d., Hotchkiss S. A. M., Basketter D. A., Pease C. K. S.: Protein Binding and Metabolism influence the Relative Skin Sensitization Potential of Cinnamic Compounds. Chem. Res. Toxicol., 17, 301-310, 2004

Detailed study summary and results:

Test type

LLNA, in accordance with OECD 429

GLP: Not stated

Test substance

trans-Cinnamaldehyde

Purity: 96%

Impurities: Cinnamic acid 3.26% and Cinnamic alcohol 0.71%

Test animals

Mice (CBA/Ca)

4 animals per dose

Age at study initiation: 7-12 weeks (Harlan Olac, U.K.)

Administration/exposure

Groups of mice (N=4) were treated daily with 25µl the test substance in vehicle or vehicle alone (acetone-olive oil (AOO)) on dorsum of both ears for three consecutive days. The concentrations used for were cinnamaldehyde 1, 2.5, 5, 10 and 25%. On day 5 after the initiation of the exposure, all mice were injected intravenously via the tail vein with 250 µL PBS containing 20 µCi of [^{3}H]methylthymidine and were

sacrificed after 5 hours. The draining lymph nodes were excised and pooled for each group, and single-cell suspensions of lymph node cells in PBS were prepared with cell strainers. The [^{3}H]methylthymidine incorporation into DNA was measured by β-scintillation counting.

Results and discussion

Cinnamaldehyde was shown to be sensitising with an EC3 value of 1.3%.

Calculated stimulation index, cinnamaldehyde

Applied concentration	1%	2.5%	5%	10%	25%
SI*	2.5	4.9	8.8	10.2	13

*Based on figure 4 in Elahi et al., 2004

3.1.1.7 STUDY 17 (LLNA)

Study reference:

Basketter D. A., Wright Z. M., E. Warbrick V., Dearman R. J., Kimber I., Ryan C. A., Gerberick G. F., White I. R.: Human potency predictions for aldehydes using the local lymph node assay. Contact Dermatitis, 45, 89–94, 2001

Detailed study summary and results:

Test type

The local lymph node assay employed in this study predates the most recent version of OECD guideline 429 but is comparable to it

GLP: Not stated

Test substance

Cinnamaldehyde

Purity: 99%

Impurities: No information available

Test animals

Mice (CBA/Ca), female

4 animals per dose

Age at study initiation: 6-12 weeks (Harlan Olac, U.K.)

Administration/exposure

Groups of mice (N=4) were treated daily with 25µl the test substance in vehicle or vehicle alone (acetone-olive oil (AOO)) on dorsum of both ears for three consecutive days. The concentrations used for were cinnamaldehyde 0.5, 1, 2.5, 5, 10 and 25%. On day 5 after the initiation of the exposure, all mice were

injected intravenously via the tail vein with 250 µL PBS containing 20 µCi of [^{3}H]methylthymidine and were sacrificed after 5 hours. The draining lymph nodes were excised and pooled for each group, and single-cell suspensions of lymph node cells in PBS were prepared with cell strainers. The [^{3}H]methylthymidine incorporation into DNA was measured by β-scintillation counting.

Results and discussion

Cinnamaldehyde was shown to be sensitising with an EC3 value of 3.1%.

Calculated stimulation index, cinnamaldehyde

Applied concentration	0.5	1%	2.5%	5%	10%
SI	1.37	0.9	1.85	7.7	15.75

3.1.1.8 STUDY 18 (LLNA)

Study reference:

Smith C. K., Hotchkiss S. A.: Enzymes and mechanisms of xenobiotic metabolism. Allergic Contact Dermatitis Chemical and Metabolic Mechanisms. Taylor and Francis, London and New York 45-87, 2001. As *cited in:* Scientific Committee on Consumer Safety SCCS OPINION on Fragrance allergens in cosmetic products. June 2012 (SCCS 2012).

Detailed study summary and results:

Test type

LLNA with only two concentrations tested. This is the only deviation reported from with OECD 429 in SCCS 2012

Test substance

Cinnamaldehyde, no information on purity.

Test animals

Mice, n= 4 animals per dose.

No further information available in SCCS 2012.

Administration/exposure

Cinnamaldehyde was tested in concentrations of 1.0 and 2.5% and the vehicle used were 4:1 acetone-olive oil (AOO).

No further information available in SCCS 2012.

Results and discussion

Although detailed information is not available for the study conducted by RIFM the result generally confirm the sensitising properties identified for cinnamaldehyde in other LLNA studies. The EC3 values reported by RIFM are 1.4%.

3.1.1.9 STUDY 19 - 25 (LLNA)

Study reference:

Wright Z. M., Basketter D. A., Blaikie L., Cooper K. J., Warbrick E. V., Dearman R. J., Kimber I.: Vehicle effects on skin sensitizing potency of four chemicals: assessment using the local lymph node assay. International Journal of Cosmetic Science, 23, 75-83, 2001

Detailed study summary and results:

Test type

The local lymph node assay employed in this study predates the most recent version of OECD guideline 429 but is comparable to it

GLP: Not stated

Test substance

Cinnamaldehyde

Purity: 99%

Impurities: No information available

Test animals

Mice (CBA/Ca), female

4 animals per dose

Age at study initiation: 6-12 weeks (Harlan, U.K.)

Administration/exposure

Five concentrations of cinnamaldehyde were tested in seven different vehicles (50:50 EtOH:water, 90:10 EtOH:water, DMSO, propylene glycol, DMF, MEK and AOO). In order to derive EC3 cinnamaldehyde were re-tested at lower concentrations in DMF and DMSO.

Groups of mice (N=4) were treated daily with 25µl the test substance in vehicle or vehicle alone on dorsum of both ears for three consecutive days. The concentrations used for were cinnamaldehyde 1, 2.5, 5, 10 and 25% and for DMF and DMSO also 0.1, 0.25 and 0.5. On day 5 after the initiation of the exposure, all mice were injected intravenously with 250 µL PBS containing 20 µCi of [^{3}H]methylthymidine and were sacrificed after 5 hours. The draining lymph nodes were excised and pooled for each group, and single-cell suspensions of lymph node cells were prepared by gentle mechanical disaggregation through stainless steel gauze. The mesh was washed twice with PBS and the cells precipitated in 5% TCA the 4 °C overnight. Each precipitate

were pelleted and re-suspended in 5% TCA and transferred to a scintillation vial with 10 ml scintillation liquid. The [^{3}H]methylthymidine incorporation was measured by β-scintillation counting.

Results and discussion

Cinnamaldehyde was shown to be sensitising in all the tested vehicles. EC3 values depending vehicle are show in the table below.

EC3 values (%v/v) in different vehicles, cinnamaldehyde

Vehicle	AOO	MEK	DMF	PG	DMSO	EtOH:water; 90:10	EtOH:water; 50:50
EC3	1.7	1.1	0.5	1.4	0.9	1.6	1.2

3.1.1.10 STUDY 26-27 (LLNA & GPMT)

Study reference:

Basketter D. A. and Scholes E. W.: Comparison of the Local Lymph Node Assay with the Guinea-pig Maximization test for the detection of a range of contact allergens. Food and Chemical Toxicology, 30, 65-69, 1992.

Also cited in: Bickers D., Calow P., Greim H., Hanifin J.M., Rogers A.E., Saurat J.H., Sipes I.G., Smith R.L., Tagami H.: A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic

Detailed study summary and results:

Basketter and Scholes (1992) investigated the potential for cinnamic aldehyde to induce skin sensitisation in a study designed to compare the local lymph node assay with the guinea pig maximisation test for the detection of a range of contact allergens.

Test type:

GMPT: The guinea pig maximization test employed in this study predates the most recent version of OECD guideline 406 but is comparable to it.

LLNA: The local lymph node assay employed in this study predates the most recent version of OECD guideline 429 but is comparable to it.

Test substance

Cinnamaldehyde

Purity: No information available

GMPT vehicle: 70:30 acetone:PEG 400 (A/P)

LLNA vehicle: 4:1Acetone-olive oil (AOO)

Test animals

GMPT: Guinea pig, Albino Dunkin-Hartley. Weight at study initiation approximately 350 g.

LLNA: CBA/Ca mice. Age at study initiation: 8-12 weeks

Administration/exposure:

Preliminary irritation tests were carried out to determine the concentrations of cinnamaldehyde suitable for induction of sensitisation and for sensitisation challenge. Guinea pigs were then treated by a series of six 0.2% cinnamaldehyde intradermal injections in the shoulder region to induce sensitization. After 6-8 days, sensitization was boosted by a 48 hour occluded patch (2.5% cinnamaldehyde) placed over the injection site. Following a rest period of 12-14 days, the animals were challenged on one flank by a 24 hour occluded patch at the maximum non-irritant concentration (0.75% cinnamaldehyde). Challenge sites were scored for erythema (scale 0-3) and oedema 24 and/or 48 hours after removal of the patches. The study does not refer to control animals but the study did identify strong, moderate and mild sensitisers plus a number of non-sensitising chemicals.

Groups of 4 mice received daily topical applications of 5, 10 or 25% cinnamic aldehyde on the dorsal surface of each ear for 3 consecutive days. Control mice were treated with vehicle alone. On day 4 or 5 of the study all mice were injected intravenously in the tail vein with phosphate buffered saline containing tritiated thymidine and killed 5 hours later. The proliferative response of the local lymph node cells was analysed and presented as a ratio of tritiated thymidine incorporation into lymph node cells relative to controls.

Results and discussion

The results of the GMPT study revealed cinnamic aldehyde to be a potent skin sensitiser with 100% of the animals tested judged to have elicited a positive response after 24 or 48 hours.

In the LLNA study a chemical was regarded as a sensitiser if at least 1 concentration of the chemical resulted in at least a 3-fold increase in tritiated thymidine incorporation compared to controls. Cinnamic aldehyde elicited test/control ratios of 12.5, 18.4 and 15.4 for the 5, 10 and 25% concentrations tested respectively and was therefore judged to be a skin sensitiser.

3.1.1.11 STUDY 28 (GPMT)

Study reference:

Basketter D. A.: Skin Sensitization to Cinnamic Alcohol: The role of Skin Metabolism. Acta Derm Venereol, 72, 264-265, 1992.

Also cited in: Bickers D., Calow P., Greim H., Hanifin J.M., Rogers A.E., Saurat J.H., Sipes I.G., Smith R.L., Tagami H.: A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients. Food and Chemical Toxicology 43, 799–836, 2005.

The current study includes 2 GPMTs, one of which is referred to in Basketter and Scholes, 1992. Including Study 29, there is a total of 3 GPMTs.

Detailed study summary and results:

Basketter (1992) investigated the potential for trans-cinnamaldehyde, cis- and trans-cinnamic alcohol to induce skin sensitisation in a study designed to investigate the hypothesis that cinnamic alcohol (via oxidation) and cinnamaldehyde gives rise to the same allergen *in vivo*, perhaps via the combination of reactive aldehyde species with skin protein.

Test type:

The guinea pig maximization test employed in this study predates the most recent version of OECD guideline 406 but is comparable to it.

Test substance

trans-Cinnamaldehyde

Purity: No information available.

Test animals

Guinea pig, Albino Dunkin-Hartley

Administration/exposure:

Preliminary irritation tests were carried out in groups of four albino Dunkin-Hartley guinea pig to determine the concentrations of cinnamaldehyde suitable for induction of sensitisation and for sensitisation challenge. Guinea pigs were then treated in the shoulder region by a series of six intradermal injections of 0.2% trans-cinnamaldehyde in combination with Freund's complete adjuvant to induce sensitization. After 6-8 days, sensitization was boosted by a 48 hour occluded patch (2.5% trans-cinnamaldehyde) placed over the injection site. Following a rest period of 12-14 days, the animals were challenged on one flank by a 24 hour occluded patch at the maximum non-irritant concentration (0.75% trans-cinnamaldehyde). A group of four animals treated as above but without cinnamaldehyde served as controls Challenge sites were scored for erythema (scale 0-3) and oedema 24 and/or 48 hours after removal of the patches.

Results and discussion

Sensitisation caused by trans-cinnamaldehyde was observed in 90% (9/10) and in 100% (10/10) of the animals. The mean erythema scores on positive responders (scale 0-3) were 2.0 and 2.2, respectively for the two test groups. The study only found limited evidence for cross reactivity between trans-cinnamaldehyde and trans-cinnamic alcohol.

3.1.1.12 STUDY 29 (GPMT)

Study reference:

Ishihara, M., Itoh, M., Nishimura, M., Kinoshita, M., Kantoh, H., Nogami, T., Yamada, K.: Closed epicutaneous test. Skin Research 28 (Suppl 2), 230–240, 1986

cited in: Bickers D., Calow P., Greim H., Hanifin J.M., Rogers A.E., Saurat J.H., Sipes I.G., Smith R.L., Tagami H.: A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients. Food and Chemical Toxicology 43, 799–836, 2005.

Detailed study summary and results:

Test type:

Guinea pig maximation test, no further information available from Bickers et al., 2005

Test substance

Cinnamaldehyde

Purity: No information available

Test animals

Guinea pig. No information on strain, number or sex

Administration/exposure:

Only information from Bickers et al., 2005 is a concentration of 3.0%. It is expected that this concentration refers to the challenge concentration but it is not clear. No information on vehicle.

Results and discussion

Strong sensitisation effect reported (no further details)

3.1.2 Human data

3.1.2.1 STUDY 1 (Patch test, selected)

Study reference:

Geier J, Uter W, Lessmann H, Schnuch A: Fragrance mix I and II: results of breakdown tests. Flavour Fragr. J., 30, 264–247, 2015.

Detailed study summary and results:

Test type

The IVDK (a network of departments of Dermatology in Germany, Austria and Switzerland) has performed a retrospective study of patch test data on the standardised fragrance mixtures Fragrance Mix I and II (FM I and FM II) obtained in the period from 1998-2013 (FMI) and 2005-2013 (FM II). Cinnamaldehyde is a component of FM I (1% cinnamaldehyde). In cases where positive reactions were observed for FM I, testing of the full mix breakdown (and other fragrance allergens) have been done. FM I was patch tested in 141 372 patients in 1998–2013. Of these 13 074 patients (9.25%) had a positive reaction. Time trends were analysed by dividing the time span into eight 2-year periods. The FM I full mix breakdown was tested in 2 798 patients with a positive reaction to FM I. The results obtained with cinnamaldehyde alone are based on patch tests with 1% cinnamaldehyde in petrolatum.

Description of test method as cited from Geier et al. 2015: "*Diagnosing contact sensitization is done by patch testing. Briefly, during this procedure, the incriminated allergen, incorporated in a vehicle (usually petrolatum or water) in a standardized concentration, is filled into a test chamber which is applied occlusively on the patient's upper back for 1 or 2 days. After removal of the patches, reactions in the test areas are observed at least until 3 days after the application. In case of an allergen-specific sensitization, a positive reaction with erythema, infiltration and possibly papules (+), additionally vesicles (++), or even coalescing vesicles (+++) occurs, depending on the degree of sensitization. Patients, who are not sensitized, usually show no reaction at all; however, in some cases, irritant or doubtful reactions can occur, which are coded as 'ir' and '?', respectively. Within the IVDK, patch tests are performed according to international and DKG guidelines [ref]. All patch test preparations were obtained from Almirall Hermal, Reinbek, Germany*."

Patch test results at day three were evaluated (except in a few cases where no reading could be done at day 3, a day 4 reading was chosen instead). Statistical analysis and data management were done using SAS software (SAS 9.3, SAS Institute, Cary, NC, USA).

The results for cinnamaldehyde showed that during the period 1998-2013 10.6% of the 2 798 selected patients were tested positive. The results divided into time spans are listed in the table below (note that the patient counts of the single time periods to not sum up to 1058 as FM I and its single components were tested in different time periods in 66 patients):

IVDK results of retrospective analysis of patch tests with cinnamaldehyde 1% in petrolatum:

Year, patient count	1998-1999 n = 162	2000-2001 n = 139	2002-2003 n = 249	2004-2005 n = 281	2006-2007 n = 285	2008-2009 n = 469	2010-2011 n = 634	2012-2013 n = 513	1998-2013 n = 2 798
Positive reactions	8.6% (4.8-	4.3% (1.6-	10.4% (6.9-	12.1% (8.5-	14.4% (10.5-	9.6% (7.1-	9.6% (7.4-	12.5% (9.7-	**10.6% (9.5-11.8)**

(95% conf. intervals)	14.1)	9.2)	14.9)	16.5)	19.0)	12.6)	12.2)	15.7)	

3.1.2.2 STUDY 2 (Patch test, selected)

Study reference:

Nardelli A, Carbonez A, Drieghe J, Goossens A: Results of patch testing with fragrance mix 1, fragrance mix 2, and their ingredients, and Myroxylon pereirae and colophonium, over a 21-year period. Contact Dermatitis, 68, 307–313, 2013.

Detailed study summary and results:

Test type

The Department of Dermatology at University Hospital St Rafael, Belgium, has performed a retrospective study of patch test data for 13 332 patients who had been patch tested in the period from 1990-2011. A total of 13 114 patients were tested with FM I. The number of patients reacting to FM I (which includes 1% cinnamaldehyde) was 1 259. Subsequent patch testing was in done with the individual ingredients of the fragrance mixture.

Description of test method as cited from Nardelli et al., 2013: *All subjects had been tested with the European baseline series (Trolab, Hermal, Reinbeck, Germany) containing FM 1, M. pereirae (balsam of Peru), and colophonium. Since 2002, 3927 have been tested with HICC 5% pet., and from 2005, 3416 have been tested with FM 2. The patients reacting to FM 1 and FM 2 were, in most cases, tested with the individual ingredients, and some of the subjects were occasionally also tested with other fragrance components. The patch tests were administered with Van der Bend patch test chambers (Van der Bend, Brielle, The Netherlands) applied on the back with Micropore™ (3M Health Care, Borken, Germany), and fixed with Fixomull (Beiersdorf, Hamburg, Germany), and later with Mefix (Mölnlycke Health Care, Göteborg, Sweden).*

The patch test readings were performed according to the international guidelines of the International Contact Dermatitis Research Group (12) after 2 days, 3 days (exceptionally), and 4 days, and sometimes later.

Statistical analysis of the patch data were performed with SAS™ version 9.2 (SAS Institute, Cary, NC, USA).

The results showed that 7% of the selected patients (66/940) had positive reactions for cinnamaldehyde when tested at 1% in petrolatum.

3.1.2.3 STUDY 3 (Patch test, selected)

Study reference:

Lyons G., Roberts H., Palmer A., Matheson M. Nixon R.: Hairdressers presenting to an occupational dermatology clinic in Melbourne, Australia. Contact Dermatitis, 68, 300–306, 2013

Detailed study summary and results:

Test type

Department of Occupational Dermatology Research and Education Centre, Australia performed a retrospective study of 164 selected hairdressers and hairdressing apprentices diagnosed with occupational contact dermatitis between 1993 and 2010. Patients were patch tested with a number of allergens including 1% cinnamaldehyde in petrolatum.

Description of test method as cited from Lyons et al., 2013: *"The allergens used for patch testing were obtained from Chemotechnique Diagnostics (Vellinge, Sweden) and applied to the back with Finn Chambers on Scanpor tape (Epitest OY, Tuusula, Finland). Patches were removed after 48 hr, and test readings were performed at D2 and D4. Patients were generally tested with an extended European baseline series, cosmetics series, hairdressers' series, and their own samples appropriately diluted. Patients were tested with additional series, for example a rubber series, if clinically relevant. Positive patch test reactions were assessed for relevance by the occupational dermatologist.*

When there was a history of exposure to natural rubber latex, patients were also tested for latex protein allergy, usually with a screening radio-allergosorbent test. Patients were then diagnosed with allergic contact dermatitis, irritant contact dermatitis, contact urticarial (caused by natural rubber latex proteins or ammonium persulfate), endogenous eczema, mucosal atopy, or other conditions. Endogenous eczema included the diagnosis of atopic eczema and other forms of eczema, such as seborrhoeic or discoid eczema. When there were multiple contributory factors, the diagnosis thought to be most contributory to the OCD was referred to as the primary diagnosis. The severity of the skin conditions was assessed on initial presentation with use of the occupational dermatitis disease severity index (ODDI) (20). The ODDI score rates severity of OCD on a scale of 1–5, based on disease course, treatment, clinical signs, and impact on work-related activities."

The results for cinnamaldehyde showed that during the period 1990-2010 1% of 164 selected hairdressers and hairdressing apprentices were tested positive.

3.1.2.4 STUDY 4 (Patch test, selected)

Study reference:

Hession M.T., Scheinman P. L.: The Role of Contact Allergens in Chronic Idiopathic Urticaria. Dermatitis, Vol 23, No 3, 2012

Detailed study summary and results:

Test type

The Dermatology Department at Tufts Medical Center, USA, conducted a prospective study of 23 selected patients with chronic idiopathic urticarial patch tested with a number of allergens including cinnamaldehyde.

Description of test method as cited from Hession and Scheinman, 2012: "*Patch testing was performed using a modified North American Contact Dermatitis Group (NACDG) standard series, as well as cosmetic and fragrance series. Other series were tested if warranted by relevant history or occupational exposure. Textile series were placed when urticaria was in a distribution on trunk and extremities consistent with a possible textile dye or formaldehyde textile resin allergy, a rubber series in patients complaining of hives under bra elastic or waistband elastic, and a hair series for patients with scalp symptoms who had colored their hair, and so on. All allergens were purchased from Chemotechnique Diagnostics (Vellinge, Sweden), except for individual fragrance mix I (FM I) components, which were purchased from Hermal (Reinbek, Germany). Readings were performed at 48 and 72 hours and graded according to the NACDG grading system of 1+ (weak reaction; papules with erythema), 2+ (strong reaction; papules plus edema or vesiculation), or 3+ (extreme reaction; spreading papulovesicles or bullae).*".

The results showed that 13% of the selected patients with chronic idiopathic urticarial (3/23) had positive reactions for cinnamaldehyde when tested at 1% in petrolatum.

3.1.2.5 STUDY 5 (Patch test, selected)

Study reference:

Turcic P., Lipozencic J., Milavec-Puretic V., Kulisic S. M.: Contact Allergy Caused by Fragrance Mix and *Myroxylon pereirae* (Balsam Of Peru) – A Retrospective Study. Coll. Antropol. 35, 1, 83–87, 2011

Detailed study summary and results:

Test type

Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia concocted a retrospective study of 157 selected patients patch tested with cinnamaldehyde between 2001 and 2005. The 157 patients were chosen out of 509 patients tested positive to FM I.

Description of test method as cited from Turcic et al., 2011: "*Patch-test allergens were applied on the patients' upper back with 2-day occlusion. According to the International Contact Dermatitis Research Group (ICDRG) system, the tests were read 48 and 72 hours after their application 21, 22. The test results were interpreted using the following scale: negative reaction (0); macular erythema (?); erythema/in filtration and possibly papules (1+); erythematous papules and/or vesicles (2+); spreading blisters and/or*

crust with ulceration (3+); and irritant reaction (IR); whereby 1+, 2+ and 3+ were considered positive allergic reactions21. Statistical analysis was performed using the STATISTICA software, Version 7.1. (StatSoft, Inc.).".The results showed that 24.2% of the selected patients (38/157) had positive reactions for cinnamaldehyde when tested at 1% in petrolatum.

3.1.2.6 STUDY 6 (Patch test, selected)

Study reference:

Cuesta L., Silvestre J. F., Toledo F., Lucas A., Pérez-Crespo M., Ballester I.: Fragrance contact allergy: a 4-year retrospective study. Contact Dermatitis 63 (2010): 77–84.

Detailed study summary and results:

Test type

The Department of Dermatology, Hospital General Universitario in Alicante, Spain performed a 4-year retrospective study of patients tested with the Spanish baseline series and/or fragrance series. A total of 1253 patients were patch tested with the baseline Spanish Group series. A total of 86 patients were tested with the Chemotechnique® fragrance series. The objective of the study was to define the characteristics of the population allergic to perfumes, to determine the usefulness of markers of fragrance allergy in the baseline GEIDAC series, and to describe the contribution made by the fragrance series to the data obtained with the baseline series.

Description of test method as cited from Cuesta et al., 2010: *"The allergens used both in the standard series and in the fragrance series were supplied by Chemotechnique Diagnostics®. The markers of the baseline Spanish Group series used in our study to detect fragrance allergic contact dermatitis were: the 'traditional' markers (M. pereirae and FM I), hydroxyisohexyl 3-cyclohexene carboxaldehyde (included as of October 2005), and FM II (included as of January 2007)."*

"The patches were prepared using Finn Chambers® fixed with Scanpor® adhesive and removed after 2D in contact with the skin. Readings were taken at D2 and D4, with the evaluation criteria (+, ++, and +++) recommended by the ICDRG. If the result was doubtful, a late reading was taken at D7. The relevance was considered current if the clinical picture could be attributed totally or partially to the fragrance obtained, past if this positivity explained only previous dermatitis, and unknown if the clinical picture could not be attributed to the use of these fragrances. Patients who were positive to any fragrance marker in the GEIDAC baseline series (M. pereirae,FM I, hydroxyisohexyl 3-cyclohexene carboxaldehyde, or FM II) were identified, and the percentage of patients positive to each of the markers was determined."

The results showed that among the patients tested with the Chemotechnique® fragrance series 8.1% of the selected patients (7/86) had positive reactions to cinnamaldehyde when tested at 2% in petrolatum. It was concluded that the fragrance markers detect the majority of cases of fragrance contact allergy. Furthermore it

was recommended to include FM II in the Spanish baseline series, as in the European baseline series, and to use a specific fragrance series to study patients allergic to a fragrance marker.

3.1.2.7 STUDY 7 (Patch test, selected)

Study reference:

Uter, W., Geier, J., Frosch, P., Schnuch, A.: Contact allergy to fragrances: current patch test results (2005-2008) from the Information Network of Departments of Dermatology. Contact Dermatitis 63, 254-261, 2010

Detailed study summary and results:

Test type

The IVDK (a network of departments of Dermatology in Germany, Austria and Switzerland) has performed a retrospective study of patch test data from a multicentre project. During 2005-2008, the frequency of contact sensitization to fragrance allergens in patients routinely patch tested for suspected allergic contact dermatitis with the baseline series and special series (including cinnamaldehyde) was investigated in a total of 40709 patients. Cinnamaldehyde was tested as a single constituent in 4527 selected patients as part of a special breakdown series of fragrance mix (FM) I.

Description of patch test as cited from Uter et al., 2010: "*The IVDK (www.ivdk.org), a contact allergy surveillance network in Germany, Switzerland and Austria, has been described elsewhere. Briefly, results for all patients patch tested in the participating departments are electronically recorded, along with important demographic and clinical data. The diagnostic procedure follows international guidelines (9) that have been further refined by the German Contact Dermatitis Research Group (10), of which all IVDK participants are members."*

Statistical analysis of the data was performed using the statistical software package SAS (version 9.2, SAS Institute, Cary, NC, USA).

The results showed that 2.64% (95% CI: 2.16-3.13%) of the 4527 selected patients were tested positive for cinnamaldehyde (1% in pet.).

3.1.2.8 STUDY 8 (Patch test, selected)

Study reference:

Andersen, K.E., Christensen L. P., Vølund AA., Johansen J. D., Paulsen E: Association between positive patch tests to epoxy resin and fragrance mix I ingredients. Contact Dermatitis, 60, 155–157, 2009

Detailed study summary and results:

Test type

In order to investigate association between positive reactions to epoxy resin and fragrance mix has Andersen et al. conducted a retrospective study of 6115 consecutive eczema patients tested from 1995 to 2007 were

included and test results from all patients tested with fragrance mix ingredients were analysed. 774 of the selected eczema patients were tested with 1% cinnamaldehyde petrolatum.

Description of test method as cited from Andersen et al., 2009: "*Patch tests were applied for 2 days with two readings routinely on D3 and D5–D7. The maximal scoring of test reactions was used in the calculations. Readings were scored according to the International Contact Dermatitis Research Group (ICDRG) ranking scale. A homogenous infiltration was required for a + reading, and ++ to +++ reactions were regarded as positive tests.*"

The results showed that 8.5% (66/774) of the selected patients were tested positive for cinnamaldehyde (1% in pet.).

3.1.2.9 STUDY 9 (Patch test, selected)

Study reference:

Pentinga S. E, Kuik D. J., Bruynzeel D. P., Rustemeyer T.: Do 'cinnamon-sensitive' patients react to cinnamate UV filters? Contact Dermatitis, 60, 210–213, 2009.

Detailed study summary and results:

Test type

Department of Dermatology of the VU University Medical Centre, The Netherlands, conducted a prospective study of 18 selected cinnamon-sensitive patients who were patch tested with 2% cinnamaldehyde in petrolatum.

Description of test method as cited from Pentinga et al., 2009: *"Finn Chambers® (Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape (Epitest Ltd Oy, Tuusula, Finland) were applied in duplicate on the left and right side of the mid–upper back (avoiding the paravertebral groove) and removed after 2 days. The left side was covered with a light impermeable MoliNea plus D® dressing (Paul Hartmann BV, Nijmegen, the Netherlands). The right side was first exposed to 5 J/cm^2 UVA (Psoralen UVA 800 Unit; Waldmann, FRG) and then covered with MoliNea plus D dressing. Photopatch test readings were scheduled according to the recommendations of the European Taskforce for Photopatch Testing at D0 (2 days after application) before and 15 min after irradiation, D1, and D2, and patch test and photopatch test results were graded according to the scoring system of the International Contact Dermatitis Research Group (12).*

A positive photopatch test was defined as a negative patch test (−) at the non-irradiated side (left) at all readings in combination with a positive patch test (≥+) at the irradiated side (right) for at least one reading.

An 'inverse photopatch test' was defined as a negative patch test (−) at the irradiated side (right) at all readings in combination with a positive patch test (≥+) at the non-irradiated side (left) for at least one reading.".

The results showed that 22% of the selected patients (4/18) had positive reactions for cinnamaldehyde when tested at 2% in petrolatum.

3.1.2.10 STUDY 10 (Patch test, selected)

Study reference:

White J. M. L, White I. R., Kimber I., Basketter D. A., Buckley D. A., McFadden J.P.: Atopic dermatitis and allergic reactions to individual fragrance Chemicals. Journal compilation © 2009 Blackwell Munksgaard Allergy, 64, 312–316, 2009

Detailed study summary and results:

Test type

The study was performed to compare rates of atopic dermatitis between patients with allergic contact dermatitis arising out of individual fragrance chemicals with known oral/cutaneous exposure against exclusively cutaneous exposure. Between 1982 and 2007, 37065 dermatitis patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, London, were tested with Fragrance mix (FM) I. Those who were positive were tested for individual fragrance allergy. The patients were either categorised as 'current' atopic dermatitis patients or 'past' atopic dermatitis patients. Cinnamaldehyde was tested at 1% in petrolatum.

Description of patch test as cited from White et al., 2009: "*Allergens were applied to the skin on 8 mm Finn chambers® (Epitest Oy; Tuusula, Finland) under Scanpor® tape (Beiersdorf, Hamburg, Germany). Patch-test readings were performed at days 2/3 and 4/5, according to standard ICDRG criteria (6). A positive (+, ++, +++) patch-test reaction signified allergy. Wherever possible, patients who were allergic (patch-test positive) to FM1 were then patch tested to the individual ingredients of the mix, all at 1% pet.*"

The results of the study showed that 0.98% of the selected patients (364/37065) were tested positive for cinnamaldehyde at 1% in petrolatum.

3.1.2.11 STUDY 11 (Patch test, selected)

Study reference:

Vocanson, M., Goujon, C., Chabeau, G., Castelain, M., Valeyrie, M., Floc'h, F., Maliverney, C., Gard, A., Nicolas, J. F.: The skin allergenic properties of chemicals may depend on contaminants - Evidence from studies on coumarin. International Archives of Allergy and Immunology 140, 231-238, 2006.

Detailed study summary and results:

Test type

The aim of the study by Vocanson et al., was to test the importance of purity in the skin allergenic properties of a chemical exemplified by coumarin. A total of 30 patients allergic to their own perfumed product were recruited in 12 months in a multicentre study involving 7 dermatoallergology departments. The inclusion criterion was the presence of a relevant positive patch test to their own perfumed product. Nineteen of the 30

patients were patch tested with the first 8 allergens of the fragrance series (including cinnamaldehyde) in addition to coumarin.

Description of patch test as cited from Vocanson et al., 2006: "*All patients underwent patch testing. Patch testing was done on the skin on the back using Finn Chambers on Scanpor (dc 8 mm).*" ... "*Readings were done after 48/72 h and results were scored using the International Contact Dermatitis Research Group criteria [7] : – = negative; ? = doubtful; + = weak reaction (no vesicle); ++ = strong reaction (edema and vesicles); +++ = extreme reaction (ulceration, bullies); IR = irritant reaction; NT = not tested*."

The results of the study showed that 20% of the 19 patients were tested positive for cinnamaldehyde.

3.1.2.12 STUDY 12 (Patch test, selected)

Study reference:

An S, Lee A-Y, Lee CH, Kim D-W, Hahm JH, Kim K-J, Moon K-C, Won YH, Ro Y-S, Eun HC: Fragrance contact dermatitis in Korea: a joint study. Contact Dermatitis 2005: 53: 320–323.

Detailed study summary and results:

Test type

A multicentre study was performed by the Korean Society for Contact Dermatitis and Skin Allergy. Nine dermatology departments at university hospitals in Korea took part in this prospective analysis of allergic responses to fragrances where 422 patients with suspected contact allergy were patch tested. In addition to the Korean (fragrance) standard and a commercial fragrance series, 18 additional fragrances were patch tested.

Description of patch test as cited from An et al., 2005: *"Test substances: The Korean standard series, which is a variant of the European standard series, and a fragrance series were purchased from Chemotechnique Diagnostics, Malmo, Sweden. We selected additional allergens based on past relevant references and information as to usage frequency. The additionally selected 18 fragrances were prepared in batches by the Korean cosmetic company and distributed to researchers at the different hospitals. Patch test method: Finn Chambers on Scanpor tape (Epitest, Tuusula, Finland) tape was used for patch testing, and the results were evaluated according to the recommendation of the International Contact Dermatitis Research Group (15)."*

The results of the study showed that 1.7% of the selected patients (7/422) were tested positive for cinnamaldehyde at 1% in petrolatum.

3.1.2.13 STUDY 13 (Patch test, selected)

Study reference:

Wohrl, S., Hemmer, W., Focke, M., Gotz, M., Jarisch, R., 2001. The significance of fragrance mix, balsam of Peru, colophony, and propolis as screening tools in the detection of fragrance allergy. British Journal of Dermatology 145, 268-273.

Detailed study summary and results:

Test type

The aim of the study by Wohrl *et al.* was to determine the usefulness of adding propolis to the European standard series to test for fragrance allergy. For this purpose between 1997 and 2000 a total of 2660 consecutive patients were patch tested with a standard patch test series. In a prospective study 747 patients suspected of fragrance allergy were tested further with a special fragrance series (including cinnamaldehyde at 1 % in petrolatum and 1% SSO).

Description of patch test as cited from Wöhrl *et al.* 2001: "*The readings were done after 72 h and scored according to the recommendations of the International Contact Dermatitis Research Group (ICDRG).*"

The results of the study showed that 1.7% of the selected patients suspected of fragrance allergy (14/747) were tested positive for cinnamaldehyde.

3.1.2.14 STUDY 14 (Patch test, selected)

Study reference:

Brites, M.M., Goncalo, M., Figueiredo, A., 2000. Contact allergy to fragrance mix - a 10-year study. Contact Dermatitis 43, 181-182.

Detailed study summary and results:

Test type

A total of 2600 consecutive patients were patch tested with fragrance mix (FM) during a 10-year period from 1989 to 1999. A sub-group of 226 selected FM-reactive patients were also tested with the individuel FM constituents including 1% cinnamaldehyde in petrolatum.

The method of patch testing was not described by Brites et al., 2000.

The results of the study showed that 13.3% of the selected FM-reactive patients (30/226) were tested positive for cinnamaldehyde at 1 % in petrolatum.

3.1.2.15 STUDY 15 (Patch test, selected)

Study reference:

Hendriks, S.A., van Ginkel, C.J: Evaluation of the fragrance mix in the European standard series. Contact Dermatitis 41, 161-162, 1999

Detailed study summary and results:

Test type

In a retrospective evaluation of the fragrance mix in the European standard series a total of 757 patients suspected of allergy to cosmetics were patch tested between 1994 and 1998 with the European standard series, including fragrance mix (FM). The results from 50 fragrance-mix-positive/component-positive patients tested with cinnamaldehyde 2% in sorbitan sesquioleate (SSO, 1%) was reported by Hendriks & van Ginkel., 1999.

The method of patch testing was not described by Hendriks & van Ginkel., 1999.

The results of the study showed that 20% of the fragrance-mix-positive/component-positive patients (10/50) were tested positive for 2% cinnamaldehyde in 1% SSO.

3.1.2.16 STUDY 16 (Patch test, selected)

Study reference:

Katsarma, G., Gawkrodger, D.J.: Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. Contact Dermatitis 41, 193-197, 1999.

Detailed study summary and results:

Test type

This study was performed to evaluate the efficacy of fragrance mix (FM) as a screen for fragrance allergy. A total of 91 patients with positive allergic reactions to FM, to 1 of the 8 ingredients of FM, to 1 of 14 other fragrance materials, or to their own perfume were identified out of 744 consecutive unselected patients patch tested in 1994-1995. Cinnamaldehyde was tested in 40 FM-allergic patients identified among the 91 patients with positive allergic reactions to FM, to 1 of the 8 ingredients of FM, to 1 of 14 other fragrance materials, or to their own perfume.

Description of patch test as cited from Katsarma & Gawkrodger 1999: "*The materials were applied in Finn Chambers on Scanpor to the upper back, left on for 2 days (D), and read at D2 and D4, using the International Contact Dermatitis Research Group's grading system. Data were collected from each patient using a form on which were recorded demographic information (i.e., age, sex and occupation), dermatitis site and type, any personal history of atopy, the test results and the final diagnosis.*"

The results of the study showed that 12.5% of the FM-allergic patients (5/40) were tested positive for cinnamaldehyde in petrolatum (concentration not specified).

3.1.2.17 STUDY 17 (Patch test, selected)

Study reference:

Larsen, W., Nakayama, H., Lindberg, M., Fischer, T., Elsner, P., Burrows, D., Jordan, W., Shaw, S., Wilkinson, J., Marks, J., Jr., Sugawara, M., Nethercott, J.: Fragrance contact dermatitis: a worldwide multicentre investigation (Part I). American journal of contact dermatitis: official journal of the American Contact Dermatitis Society 7, 77-83, 1996

Detailed study summary and results:

Test type

The aim of the study by Larsen et al., 1996 was to determine the prevalence of responses to selected fragrance materials in patients with suspect fragrance allergy and to evaluate risk factors and associations with such responses. A total of 167 fragrance sensitive volunteers from seven centres worldwide were patch tested with fragrance mix (FM) and its constituents (including cinnamaldehyde at 1% in petrolatum).

Description of patch test as cited from Larsen et al., 1996: *"The test materials were applied to Finn chambers (Epitest Ltd, Oy, Helsinki, Finland) that were applied to the upper back.[7] The chambers were then further secured to the skin with Scanpor tape (Norgesplaster A/S, Aksjeselskap, Finland). Fifteen to 45 minutes were allowed between the initial patch test removal and the first reading to allow the pressure effect of the patch test appliance to resolve so as not to mask faint responses. The patch test sites were evaluated using the North American Contact Dermatitis Group modification [11] of the International Contact Dermatitis Research Group morphological grading systcm.[12] The patch test sites were evaluated initially at 48 or 72 hours. The test sites were re-examined in the majority of cases, usually between 48 and 120 hours after the first reading. All test site readings were made by the investigators.*"

Statistical analysis of the data was performed using the Statistical Analysis System (release 6.07, SAS Institute, Cary, NC, USA).

The results of the study showed that 14.4% of the 167 selected fragrance sensitive volunteers were tested positive for 1% cinnamaldehyde in petrolatum.

3.1.2.18 STUDY 18-19 (Patch test, selected)

Study reference:

Johansen, J.D., Menne, T.: The fragrance mix and its constituents: a 14-year material. Contact Dermatitis 32, 18-23, 1995.

Detailed study summary and results:

Test type

This study is a review of results from 14 years of patch testing with the fragrance mix (FM) and its constituents and includes 8215 consecutive patients patch tested with FM between 1979 and 1992 at the Department of Dermatology in Gentofte, Denmark. Individual FM constituents were tested in a total of 367 selected patients reacting to the fragrance mix between 1979 and 1992. Of these 105 were tested with 2% cinnamaldehyde in petrolatum, 1979-1983 and 160 were tested with 1% cinnamaldehyde in petrolatum, 1988-1992.

Description of patch test as cited from Johansen and Menné 1995: "*The patches were occluded using Finn Chambers affixed with Scanpor tape." …" The test substances were applied to the upper back for 2 days. Readings were made on the 2nd, 3rd and 5th- 7th days. In 1987, the scale of readings was adjusted to conform with ICDRG recommendations; before that, a less rigorous scale was used, defining a positive reaction as a definite erythema.*"

The results of the study showed a significant decrease in the frequency of reaction to cinnamaldehyde at the same time as the test concentration was reduced from 2% to 1% pet.

Frequency of positive reactions to cinnamaldehyde

	Patients age 15-34	Patients age 35-60	Patients age >60
2% Cinnamaldehyde in pet. (1979-1983)	32.5%	31.6	30.8
1% Cinnamaldehyde in pet. (1988-1992)	9.1%	12.8	10.4

3.1.2.19 STUDY 20 (Patch test, selected)

Study reference:

de Groot, A.C., van der Kley, A.M., Bruynzeel, D.P., Meinardi, M.M., Smeenk, G., van Joost, T., Pavel, S.: Frequency of false-negative reactions to the fragrance mix. Contact Dermatitis 28, 139-140, 1993.

Detailed study summary and results:

Test type

The purpose of the study by de Groot et al., was to determine the frequency of false-negative reactions to fragrance mix (FM). Between September 1991 and December 1991 a total of 677 patients were patch tested with FM. Out of the 677 tested patients a total 61 patients were positive to FM. Cinnamaldehyde (2%) as a single constituent was tested in the FM positive patients.

The method of patch testing was not described by de Groot et al., 1993.

The results of the study showed that 34% of the selected FM positive patients (21/61) were tested positive for cinnamaldehyde at 2% in petrolatum.

3.1.2.20 STUDY 21 (Patch test, selected)

Study reference:

Enders, F., Przybilla, B., Ring, J.: Patch testing with fragrance mix at 16% and 8%, and its individual constituents. Contact Dermatitis 20, 237-238, 1989

Detailed study summary and results:

Test type

Enders et al., reports a study of 1845 patients patch tested with a fragrance mix in 1987 at the Dermatologische Klinik und Poliklinik, Germany. A total of 162 of the tested patients with a positive reaction to the fragrance mix were retested with the individual constituents including cinnamaldehyde at 1% (vehicle not reported).

The method of patch testing was not described by Enders et al., 1989.

The results of the study showed that 21% of the 162 selected fragrance mix positive patients were tested positive for cinnamaldehyde at 1%.

3.1.2.21 STUDY 22 (Patch test, selected)

Study reference:

Wilkinson, J.D., Andersen, K., Camarasa, J., Ducombs, G., Frosch, P., Lahti, A., Menné, T., Rycroft, R.J.G., White, I.: Preliminary results of the effectiveness of two forms of fragrance mix as screening agents for fragrance sensitivity. In Frosch, P.J. et al. (eds.): Current Topics in contact dermatitis. Heidelberg: Springer-Verlag: 127-131, 1989.

As *cited in* Opinion concerning Fragrance Allergy in Consumers. A Review of the Problem. The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. Adopted 8. December 1999 (SCCNFP 1999).

Detailed study summary and results:

Test type

2455 consecutive patients attending patch test clinics in England, Denmark, Spain, France, Germany and Finland were tested to 8% Hermal/Larsen fragrance mix and 9.5% Hausen fragrance mix. When one or the other of the mixes was positive all the individual fragrance compounds contained in the mixes were tested. Patch test technique and readings were as recommended by the ICDRG and, for positive results; an assessment of clinical relevance was also made.

The overall incidence of fragrance sensitivity was 7.8% using Hermal/Larsen mix and 6.7% with the Hausen mix. In 146 patients a direct comparison of the two fragrance mixes could be made: in 32 of these the

reactions were marginal or weak and in 114 the reactions were 1+ or greater. Among the 114 patients with solid reactions to one or other fragrance mix, 78 were tested to individual fragrance materials.

1% Cinnamaldehyde gave positive reactions in 12.8% (10/78).

3.1.2.22 STUDY 23-24 (Patch test, selected)

Study reference:

Santucci, B., Cristaudo, A., Cannistraci, C., Picardo, M.: Contact dermatitis to fragrances. Contact Dermatitis 16, 93-95, 1987

Detailed study summary and results:

Test type

The aim of the study by Santucci et al., 1987 was to evaluate the incidence of contact dermatitis to fragrances in Roma, Italy, and the influence of limited variations in fragrance and perfume mix concentrations on patch test responses. Two large groups of patients with contact dermatitis were patch tested with a range of mixed fragrances including cinnamaldehyde between 1983 and 1984 (n=1200) and 1984 and 1985 (n=1500). A total of 63 and 54 patients were tested positive in the first and second group, respectively. Patients reacting positive to any of the mixed fragrances were tested after 3 months with the individual components of the mix. In the 1983-1984 group the 2% cinnamaldehyde in petrolatum were used and in the 1984-1985 1% cinnamaldehyde in petrolatum were used.

Description of patch test as cited from Santucci et al., 1987: "*Using Finn Chambers on Scanpor'''. The tests were read at 48, 72 and 96 h, according to the ICDRG scale; the last reading was taken as definitive*."

The results of the study are showed in the table:

Number of tested patients	Number of positives	Percent positive	Test concentration of cinnamaldehyde
63	9	14.3%	2%
54	3	5.6%	1%

3.1.2.23 STUDY 25 (Patch test, selected)

Study reference:

Adams, R.M., Maibach, H.I.: A five-year study of cosmetic reactions. Journal of the American Academy of Dermatology 13, 1062-1069, 1985.

Detailed study summary and results:

Test type

A total of 713 cosmetic related cases were identified among 13216 patch tested contact dermatitis patients from various sections of the United States between 1977 and 1983. To identify the exact cause of their reactions the patients were patch tested with a range of cosmetic ingredients including the cosmetic products

used by the patient. A sub-group of 403 selected patients were patch tested with single ingredients including cinnamaldehyde.

Description of patch test as cited from Adams et al., 1985: "*Patch tests were applied to the upper back for 48 hours according to the methods outlined in the North American Contact Dermatitis Group (4) and the International Contact Dermatitis Group. Readings were made at 48 hours and 72 hours. In most centres, additional readings at 96 hours or 120 hours were also made. The patch test was either the Al test or the Finn Chamber (Hermal Pharmaceutical Labs., Inc., Oak Hill, NY; Allerderm Laboratories, Mill Valley, CA).*"

The results of the study showed that 1.5% of the selected patients (6/403) were tested positive for cinnamaldehyde (vehicle and concentration not specified).

3.1.2.24 STUDY 26 (Patch test, selected)

Study reference:

Malten, K.E., van Ketel, W.G., Nater, J.P., Liem, D.H.: Reactions in selected patients to 22 fragrance materials. Contact Dermatitis 11, 1-10, 1984.

Detailed study summary and results:

Test type

A total of 182 patients with suspected contact sensitisation to cosmetics were patch tested with a series of 22 fragrance and flavour raw materials including cinnamaldehyde at 0.5% in petrolatum.

Description of patch test as cited from Malten et al., 1984: "*The patch test reactions were read at 48 and 72 h; the last reading was recorded as definitive*."

The results of the study showed that 3.7% of the 182 selected patients were tested positive for 0.5% cinnamaldehyde in petrolatum.

3.1.2.25 STUDY 27 (Patch test, selected)

Study reference:

Larsen W. G.: Perfume dermatitis. a study of 20 patients. Archives of Dermatology 113, 623-626, 1977

Detailed study summary and results:

Test type

A total of 20 perfume-sensitive patients were patch tested with several screening sets of fragrance materials including cinnamaldehyde at 1% in petrolatum.

Description of patch test as cited from Larsen 1977: "*The standard patch-testing technique with use of an aluminium-backed strip was employed. Patch tests were applied to the patient's back and were left for 48 hours. Readings were made at the time of removal or 24 hours after removal. Patients were instructed to*

return if an additional delayed reaction occurred. All the fragrance allergens were tested on 50 control patients with negative results. To avoid the "angry back" phenomenon, patients were tested during a period of several months."

The results of the study showed that 30% of the selected patients (6/20) were tested positive for cinnamaldehyde at 1% in petrolatum.

3.1.2.26 STUDY 28 (Patch test, unselected/consecutive)

Study reference:

Mann J, McFadden JP, White JML, White IR, Banerjee P: Baseline series fragrance markers fail to predict contact allergy. Contact Dermatitis, 70, 276–281, 2014.

Detailed study summary and results:

Test type

The St Johns' Institute of Dermatology at St Thomas' Hospital, UK has performed a retrospective study of patch test data by reviewing the records of 1951 eczema patients, routinely tested with the 26 fragrance substances requiring labelling and with an extended European baseline series (FMI and FMII) in 2011 and 2012. The objective was to determine the frequencies of positive test reactions to the 26 fragrance substances for which labelling is mandatory in the EU, and how effectively reactions to fragrance markers in the baseline series (FMI and FMII) predict positive reactions to the fragrance substances that are labelled. The study thus explored whether routine patch testing with all individual fragrance substances that are labelled above a threshold identified cases of fragrance contact allergy that would have remained undetected when using the baseline series.

Description of test method as cited from Mann et al.: *The patch test records of all eczema patients who underwent routine testing with the fragrance series and the European baseline series during 2011 and 2012 were retrieved from the database at St John's Institute of Dermatology at St Thomas' Hospital, London. The data recorded at the time of consultation included the age, sex and occupation of patients, the primary site affected by eczema, and the duration of eczema. Positive reactions, on or after day 4 of testing, to fragrance markers in the European baseline series (FM I, FMII, Myroxylon pereirae, and HICC) or allergens from the fragrance series (the26 labelled fragrances and trimethylbenzenepropanol, but excluding HICC) were tabulated with spss™ version 12. Data were also collected for patients who reacted to colophonium and epoxy resin. The concentrations and constituents of the fragrance markers are shown in Table 1, and those of the allergens used in the fragrance series are shown in Table 2. Limonene and linalool were used in their un-oxidized forms throughout the study. Patch testing was performed with aluminium Finn Chambers® provided by Bio-Diagnostics® (Upton-Upon-Severn, United Kingdom) and allergens provided by Bio-Diagnostics®, Trolab® (Hermal Almirall, Reinbeck, Germany) and Chemotechnique® (Vellinge, Sweden). Allergens were in petrolatum. Reactions were read on days 2 and 4, according to the*

recommendations of the International Contact Dermatitis Research Group. Reactions documented as questionable or irritant were considered to be negative.

The results showed that 1.38% (27/1951) (95% CI: 0.9-1.9%) of the selective patients had positive reactions for cinnamaldehyde when tested at 1% in petrolatum.

Overall the study showed that >40% of those patients reacting to a substance in the fragrance series would have been missed if evidence of fragrance allergy had been investigated exclusively with the European baseline series, and that a similar proportion of those reacting to FM I or FM II constituents did not react to the mixes themselves. In general the study indicates a very high rate of fragrance allergy as >14% of the patients reacted to either a fragrance marker or a substance in the fragrance series.

3.1.2.27 STUDY 29-31 (Patch test, unselected/consecutive)

Study reference:

Zug K. A., Pham A. K., Belsito D. V., DeKoven J. G., DeLeo V. A., Fowler, J. F. Jr., Fransway A. F., Maibach H. I., Marks J. G. Jr., Mathias C. G. T., Pratt M. D., Denis Sasseville D., Storrs F. J., Taylor J. S., Warshaw E. M., Zirwas M. J.: Patch Testing in Children From 2005 to 2012: Results From the North American Contact Dermatitis Group. DERMATITIS, Vol 25, No 6, 2014

Detailed study summary and results:

Test type

The North American Contact Dermatitis Group (NACDG) has performed a retrospective study of 41 unselected children age 0-5 years, 838 unselected children age 6-18 years and 17 213 unselected adults (> 18 years) patch tested with a total of 87 different allergens, including 1% cinnamaldehyde in petrolatum, between 2005 and 2012.

Description of test method as cited from Zug et al. 2014: *"Deidentified patch test results from patients aged 18 years or younger who were referred on suspicion of having ACD and underwent patch testing between January 1, 2005 and December 31, 2012, by members of the NACDG were retrieved from a central database. This study qualified for an exempt from review from the Committee for the Protection of Human Subjects at Dartmouth-Hitchcock (CPHS no. 24202). During this test period, the NACDG underwent four 2-year cycles of patch testing (2005-2006, 2007-2008, 2009-2010, and 2011-2012) and 4 slightly modified allergen screening series were used for testing. A total of 87 different allergens, of varying chemical composition, delivery vehicles, or concentrations, were tested from 2005 to 2012. The patch testing was performed using a standard series of 65 (2005-2008; Chemotechnique Diagnostics AB, Malmö, Sweden) or 70 (2009-2012; allergEAZE by SmartPractice, Calgary, Alberta, Canada) allergens individually housed in Finn Chambers (SmartPractice, Phoenix, AZ) and applied to the patients' upper back in the standard fashion. At the clinician's discretion and depending on the clinical situation, a patient may have been patch-*

tested with additional supplemental allergens. Details on the number of supplemental allergens tested, if any, are not part of the data set. One or more allergens may have been omitted from testing in an individual patient if the patient had a known history of strongly reacting to that allergen. The patch tests were removed and then evaluated at 48 hours, and second, delayed final reading and interpretation were performed between days 3 and 7 after placement.".

The results for cinnamaldehyde showed that during the period 2005-2012 4.9% of the 41 unselected children age 0-5 years, 1.2% of the 838 unselected children age 6-18 years and 3.0% of the 17 213 unselected adults were tested positive.

3.1.2.28 STUDY 32 (Patch test, unselected/consecutive)

Study reference:

Heisterberg MV, Menné T, Johansen JD: Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. Contact Dermatitis, 65 (2011), 266–275 and corrigendum in: Contact Dermatitis, 67 (2012), 58.

Detailed study summary and results:

Test type

The Department of Dermato-Allergology, Copenhagen University Hospital, Gentofte has performed a retrospective study on consecutive eczema patients patch tested with cinnamaldehyde. The objective of the study was to investigate frequencies of sensitization to the 26 individual fragrances and evaluate the sensitivity of the standard fragrance screening markers (FM I and FM II), i.e. would testing with the individual substances reveal fragrance allergy that is not detected when using the standard fragrance markers. Patients (n = 1508) were patch tested with at least one of the 26 fragrance ingredients in the period from January 2008 to July 2010 were included in the study. 1503 patients were patch tested with cinnamaldehyde.

Description of patch test as cited from Heisterberg et al., 2011: "*The patch tests were performed according to international guidelines, with Finn Chambers applied on the back with Scanpor tape for a period of 2 days. Readings were performed on days 2, 3 or 4, and 7, according to the recommendations of the International Contact Dermatitis Research Group. Not all subjects were patch tested with limonene and linalool, as the patch test material during the study period changed from being the pure compounds to oxidized materials, because several studies have shown that it is the oxidized products that cause allergy. In this study, we report the results of patch testing with the pure compounds. Methyl 2-octyonate 1% was not patch tested in all of the subjects routinely patch tested, because active sensitization was observed in two patients, and we then stopped patch testing with it; thus only 211 patients were tested. Data management and statistical analysis were performed using SPSS™ version 15. Percentages of positive patch test reactions and*

confidence intervals were calculated with www.openepi.com. Chi-square tests and Fisher's exact tests for characteristic differences were performed, and p < 0.05 was considered to be significant."

The results showed that 1.3% of the consecutive patients (20/1503) were tested positive for cinnamaldehyde at 1% in petrolatum. It was furthermore concluded that 11.7% of fragrance allergy subjects would be undetected with a fragrance allergy if they had not been patch tested with the fragrance series, which underlines the value of patch testing all subjects with a fragrance series.

3.1.2.29 STUDY 33 (Patch test, unselected/consecutive)

Study reference:

Van Oosten EJ, Schuttelaar M-L A, Coenraads PJ: Clinical relevance of positive patch test reactions to the 26 EU-labelled fragrances. Contact Dermatitis, 61, 217–223, 2009.

Detailed study summary and results:

Test type

The Department of Dermatology, University of Groningen, the Netherlands performed a prospective study of patients with eczema suspected of being contact allergy to fragrances or cosmetics. In the study 320 patients were patch tested with the 26 EU-declared fragrance chemicals, FM I and FM II. The objective of the study was to describe frequencies of contact allergy to these 26 fragrance substances, and to evaluate clinical relevance of these positive reactions.

Description of test method as cited from Van Oosten et al., 2009: "*All 320 patients were tested with the series of 26 EU fragrance ingredients that are labelled. Additionally, the European baseline series (TRUE® test, Mekos laboratories, Denmark), which includes FM I, was tested in 295 patients, and the FM II (Hermal/Trolab, Reinbek, Germany) was tested in 227 patients. The fragrance compounds were obtained from Hermal/Trolab and from other international suppliers (International Flavors & Fragrances, USA; Robertet, France; Givaudan, Switzerland, Milennium Speciality Chemicals Inc., USA; Bedoukian Research Inc., USA; Rhodia, France; Symrise, Germany and Firmenich, Switzerland). All fragrances were dissolved in petrolatum, except for Evernia furfuracea which was dissolved in di-ethyl phthalate (Table 1). Patch tests were performed and read according to the guidelines of the International Contact Dermatitis Research Group (ICDRG) (12). The patches were applied for 2D. Final reading was done on D3. (7, 13). Reading of doubtful reactions was done up to D7 after the application of the patch test material. The relevance of the positive reactions (1+ through 3+) was determined and categorized as certain, probable, possible or not relevant. Contact allergy was defined as clinically relevant according to the following criteria: (i) certain exposure to the sensitizer and (ii) the patients dermatitis can be explained by the exposure (8, 11, 14, 15)".*

The results of the study showed that 1.6% of the unselected eczema patients (5/320) had positive reactions to cinnamaldehyde when tested at 1% in petrolatum.

3.1.2.30 STUDY 34 (Patch test, unselected/consecutive)

Study reference:

Zug K. A., Warshaw E. M., Fowler Jr J. F., Maibach H. I., Belsito D. L., Pratt M. D., Sasseville D., Storrs F. J., Taylor J. S., Mathias C. G. T., DeLeo V.A., Rietschel R. L.: Patch-Test Results of the North American Contact Dermatitis Group 2005–2006. Dermatitis, Vol 20, No 3, pp 149–160, 2009.

Detailed study summary and results:

Test type

The North American Contact Dermatitis Group (NACDG) performed patch test op 4 454 unselected patients with 26 different allergens, including 1% cinnamaldehyde in petrolatum, between January 1. 2005, and December 31. 2006. Results were compared to previous test cycles (including 2003-2004).

Description of test method as cited from Zug et al. 2009: "*Sixty-five allergens (Chemotechnique Diagnostics AB, Malmo¨, Sweden) were tested by the 13 members of the NACDG in 2005 and 2006. Patch testing was performed with a standardized technique using Finn Chambers (Epitest Ltd Oy, Tuusula, Finland) on Scanpor tape (Norgesplaster Alpharma A/S, Vennesla, Norway). Patches were left in place for 48 hours. First and second patch-test readings were performed at 48 to 72 hours and 72 to 168 hours, respectively, after initial patch-test placement. Allergic patch-test reactions were graded as +, ++, or +++, based on the intensity of positive reactions manifested by erythematous papules, vesicles, or a spreading reaction (sometimes with crusting and ulceration). Doubtful reactions (macular erythema) were generally coded as nonallergic reactions. If the clinical history suggested relevance, or if other positive reactions to the same allergen but in a different vehicle were found, or if a cross-reacting substance was identified, the investigator had the discretion to make the final determination that the macular erythema represented an allergic reaction. Irritant and allergic reactions were differentiated by each investigator, who considered the morphology and timing of the reaction at each reading.".*

The results for cinnamaldehyde showed that during the period 2005-2006 3.1% of the 4 435 unselected patients were tested positive. These 4435 patients are also included in the retrospective 2005-2012 study, Zug et al. (2014), and does therefore not have a separate entrance in the CLH report under Zug et al., 2009. Zug et al., 2009 does, however, also give results for the 2003-2004 test cycle were 2.4% of 5 138 unselected patients had positive patch test reactions for cinnamaldehyde. These results are included in the CLH report.

3.1.2.31 STUDY 35-40 (Patch test, unselected/consecutive)

Study reference:

Nguyen S. H., Dang T. P., MacPherson C., Maibach H., Maibach H. I.: Prevalence of patch test results from 1970 to 2002 in a multi-center population in North America (NACDG). Contact Dermatitis, 58, 101–106, 2008

Detailed study summary and results:

Test type

The North American Contact Dermatitis Group (NACDG) conducted a retrospective study on more than 34000 unselected allergic contact dermatitis (ACD) patients patch tested between 1970 and 2002. The number of patients tested with 1% cinnamaldehyde (in petrolatum according to Zug et al., 2009) was: year 1984-1985: 1199 patients; year 1985-1989: 3964 patients; year 1992-1994: 3528 patients; year 1994-1996: 3112 patients; year 1996-1998: 3443 patients and year 1998-2000: 4735 patients.

Description of patch test as cited from Nguyen et al., 2007: "*The patients were patch tested in a standardized manner as outlined previously (1–8), using Finn Chambers (Epitest Ltd Oy, Tuusula, Finland) on Scanpor tape (Norgesplaster Aksjeselskap, Venessia, Norway) applied to the back. Allergens were purchased from Hermal Pharmaceutical Laboratories, Inc. (Delmar, NY, USA) or Chemotechnique Diagnostics AB (Malmo, Sweden). The patches remained in place for 2–3 D and read at 3–7 D after placement. Patch test reactions were interpreted to be a 1+, 2+, or 3+ reaction manifested by erythematous papules, vesicles, or a spreading reaction with crust and ulceration (1–8).*".

The number and percentage of unselected ACD patients tested positive with 1% cinnamaldehyde in petrolatum can be seen in the table:

	1984-1985	1985-1989	1992-1994	1994-1996	1996-1998	1998-2000
Positive	1199	3964	3528	3112	3443	4735
%	5.9	3.1	2.7	2.4	2.8	3.6

3.1.2.32 STUDY 41 (Patch test, unselected/consecutive)

Study reference:

Schnuch A, Uter W, Geier J, Lessmann H, Frosch, PJ: Sensitization to 26 fragrances to be labelled according to current European regulation. Contact Dermatitis, 57, 1–10, 2007.

Detailed study summary and results:

Test type

The IVDK (a network of departments of Dermatology in Germany, Austria and Switzerland) has performed a retrospective study of patch test data from a multicentre project. During 2003-2004, 26 fragrances were

patch tested additionally to the standard series in a total of 21325 patients; the number of (consecutive, unselected) patients tested with each of the fragrances ranged from 1658 to 4238.

Description of patch test as cited from Schuch et al., 2007: "*Patch tests are performed in accordance with the recommendations of the International Contact Dermatitis Research Group and the German Contact Dermatitis Research Group (DKG). Patch test material is obtained from Hermal/Trolab, Reinbek, Germany. Patch test preparations are applied for 24 or 48 hr. Readings are done until at least 72 hr using the following grading based on international standards, further refined by the German Contact Dermatitis Group: neg, ?, +, ++, +++, irritant, follicular. The patch test results of every reading, a standardized history (including age, sex, atopic diseases, current and former occupation(s), presumptive causal exposures), along with final diagnoses and site(s) of dermatitis are assessed and documented.*
All data are transferred to the data centre in Göttingen in an anonymized format every 6 months. During 4 periods of 6 months each, from 1 January 2003 to 31 December 2004, 25 fragrances were successively patch tested additionally to the standard series, i.e. in unselected patients, by departments of the IVDK. In the first period 8, in the second 6, in the third 3, and in the last period 8 compounds were added to the standard series, the number of patients tested with each preparation ranging from 1658 (tree moss) to 4238 (farnesol; tested during 2 periods)."

The results showed that 1.0% (95% CI: 0.5-1.5%) of the consecutive patients (21/2063) were tested positive for cinnamaldehyde at 1% in petrolatum.

3.1.2.33 STUDY 42 (Patch test, unselected/consecutive)

Study reference:

Belsito D. V., Fowler Jr J. F., Sasseville D., Marks Jr J. G., De Leo V. A., Storrs F. J: Delayed-Type Hypersensitivity to Fragrance Materials in a Select North American Population. Dermatitis, Vol 17, No 1: pp 23–28, 2006

Detailed study summary and results:

Test type

Belsito et al. conducted a prospective study of 1603 selected patients with eczematous dermatitis patch tested with the North American Contact Dermatitis Groups (NACDG), screening tray (including cinnamaldehyde) addition to HMPCC.

Description of patch test as cited from Belsito et al., 2006: "*Patients were patch-tested in a uniform manner as previously described.(13) They returned for patch-test evaluation at 48 hours after the initial application and for a second evaluation 4 to 7 days after the initial application. Results were assigned scores of 1 to 6, based on reaction morphology as previously described.(13) Patients were considered allergic to a fragrance material if they had a +, ++, or +++ reaction."*

The results of the study showed that 1.7% (27/1603) of the selected patients was tested positive for cinnamaldehyde.

3.1.2.34 STUDY 43 (Patch test, unselected/consecutive)

Study reference:

Schnuch, A., Geier, J., Uter, W., Frosch, P.J.: Another look at allergies to fragrances: Frequencies of sensitisation to the fragrance mix and its constituents. Results from the Information Network on Departments of Dermatology (IVDK). Exogenous Dermatology 1, 231-237, 2002

Detailed study summary and results:

Test type

The IVDK (InformationsVerbund Dermatologischer Kliniken) a network of departments of Dermatology in Germany, Austria and Switzerland) has performed a retrospective study of patch test data from a multicentre project. During 1996-1999, fragrance mix (FM) (including cinnamaldehyde) was tested in a total of 35599 unselected patients and its single constituents were tested at 1% in petrolatum in a subgroup of 4900 patients.

Description of patch test as cited from Schnuch et al., 2002: "*The multicentre project 'Information Network of Departments of Dermatology' ('Informationsverbund dermatologischer Kliniken', IVDK) is an instrument of epidemiological surveillance of contact allergy and has been described in detail elsewhere [2, 8, 9]. Basically, patch tests are performed in accordance with the recommendations of the ICDRG, the International Contact Dermatitis Research Group [10] and the DKG, the German Contact Dermatitis Research Group [11]. Patch test material is obtained from Hermal/Reinbek, Germany, and applied for 24 or 48 h. Readings are performed until at least 72 h. All patch test results and a standardised history of all patients tested in the participating centres (see footnote) are recorded and transferred to the data centre in Göttingen.*"

The results showed that 1.9% of the 4900 unselected patients were tested positive for cinnamaldehyde (1% in pet.).

3.1.2.35 STUDY 44-45 (Patch test, unselected/consecutive)

Study reference:

Frosch, P.J., Pilz, B., Burrows, D., Camarasa, J.G., Lachapelle, J.M., Lahti, A., Menné, T., Wilkinson, J.D.: Testing with fragrance mix. Is the addition of sorbitan sesquioleate to the constituents useful? Results of a multicentre trial of the European Environmental and Contact Dermatitis Research Group (EECDRG). Contact Dermatitis 32, 266-272,1995a.

Detailed study summary and results:

Test type

A prospective multicentre study involving a total of 709 patients tested in 7 centres located in Europe was performed. The study involved testing of two types of fragrance mix (FM), its 8 constituents with 1% sorbitan sesquioleate (SSO) and its 8 constituents without SSO and 20% SSO. The concentration of cinnamaldehyde was 1% when tested as individual constituent.

Description of patch test as cited from Frosch et al., 1995a: *"The series was applied for 2 days to the back with Finn Chambers on Scanpor tape. Readings were made at 2 and 3 days (4 days in some centres), according to published guidelines (3). 7 centres participated in the study: Amersham in England (100 patients), Barcelona in Spain (100 patients), Belfast in Northern Ireland (100 patients), Brussels in Belgium (100 patients), Hellerup in Denmark (124 patients), Oulu in Finland (85 patients) and Dortmund in Germany (100 patients). The patients were unselected consecutive patients patch tested because of suspected contact dermatitis.*"

The results showed that 0.98% (7/702) reacted to the emulsifier 20 % SSO itself. Furthermore, 0.85% (6/702) and 0.14% (1/702) of the unselected patients were tested positive for cinnamaldehyde (1%) with and without SSO, respectively.

3.1.2.36 STUDY 46 (Patch test, unselected/consecutive)

Study reference:

Frosch, P.J., Pilz, B., Andersen, K.E., Burrows, D., Camarasa, J.G., Dooms-Goossens, A., Ducombs, G., Fuchs, T., Hannuksela, M., Lachapelle, J.M., Lahti, A., Maibach, H.I., Menné, T., Rycroft, R.J.G., Shaw, S., Wahlberg, J.E., White, I.R., Wilkinson, J.D.: Patch testing with fragrances: results of a multicentre study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. Contact Dermatitis 33, 333-342, 1995b.

Detailed study summary and results:

Test type

A prospective multicentre study involving a total of 1323 patients tested in 11 centres located in Europe was performed. The study involved testing of 48 frequently used constituents of perfumes, as well as patch testing with a standard series fragrance mix (FM) containing cinnamaldehyde. In 9 centres 1072 patients were patch tested with 1% cinnamaldehyde in pet. with 1% sorbitan sesquioleate (SSO)).

Description of patch test as cited from Frosch et al., 1995b: *"In each centre, a minimum of 100 consecutive patients were tested with the allocated FF* (Fenn fragrance) *materials and the 8% FM with its constituents. For each patient positive to any 1 of the FF materials, a questionnaire was filled out regarding clinical*

relevance and other sensitizations. Patch testing was performed with Finn Chambers on Scanpor tape applied for 2 days to the back. Readings were made following the guidelines of the ICDRG (16) on days 2 and 3, or in some centres on days 2 and 4".

The results showed that 0.93% (10/1072) of the unselected patients from a total of 9 European centres were patch tested positive for cinnamaldehyde at 1% in petrolatum with SSO.

3.1.2.37 STUDY 47 (ROAT)

Study reference:

Bruze, M., Johansen J. D., Andersen K. E., Frosch P., Lepoittevin J-P., Rastogi S., Wakelin S., White I., Menne T.: Deodorants: An experimental provocation study with cinnamic aldehyde. Journal of the American Academy of Dermatology 48, Number 2, 2003

Detailed study summary and results:

Test type

A clinical study were conducted involving 17 cinnamaldehyde-allergic patients and 20 healthy controls who were tested with a dilution series of cinnamaldehyde in a patch test and a use test - a repeated open application test (ROAT). The aim of the study was to investigate the significance of cinnamaldehyde in deodorants for the development of axillary dermatitis. For the patch test 2.0%, 1.0%, 0.5%, 0.25%, 0.125%, 0.063%, 0.031%, 0.016%, 0.008%, 0.004%, 0.002%, 0.001%, 0.0005%, 0.00025%, 0.00012%, and 0.00006% cinnamaldehyde in ethanol were used. In the first part of the ROAT 8 patients were exposed to unscented and scented deodorants at 3 concentrations (0.32%, 0.1%, and 0.032% wt/vol) in the axilla.

On the basis of the results of the first part of the study, deodorants with cinnamaldehyde at 0.1%, 0.032%, and 0.01% wt/vol were chosen for the second part were 9 patients were exposed in the axilla. Except for the content of cinnamaldehyde, the scented and unscented deodorants were identical with water, aluminium chlorohydrate, polypropyleneglycol-15, stearyl ether, steareth-2, steareth-21, dichlorobenzyl alcohol, and phenoxyethanol as the ingredients.

Description of patch test as cited from Bruze et al., 2003: "*The Finn Chamber technique was used. On each patch unit mounted on Scanpor tape, 15 µL of the respective test solution was applied. The patches were removed from the back after 48 hours and readings took place on day 3 and day 7 according to International Contact Dermatitis Research Group guidelines. Each test patient was tested with 15 ethanol solutions of cinnamic aldehyde, ethanol, and the unscented and scented deodorants. For those having reacted previously with a +++ reaction to cinnamic aldehyde the testing started at 1.0% and for all other test patients the testing started at 2.0%. Besides testing with the unscented deodorant and ethanol, the control patients were only tested with cinnamic aldehyde at 1.0%. The threshold of sensitivity (the minimal eliciting concentration [MEC]) was defined as the lowest concentration eliciting at least a + reaction (16) The positive test reactions were not always continuous. When the number of negative reactions, doubtful reactions, or both*

were followed by the same number or more of positive reactions, the lowest positive concentration was registered as the MEC. In all other situations, the concentration above the first negative or doubtful reaction was registered as the MEC (16)."

Description of use test as cited from Bruze et al., 2003: *"The use test was done as an ROAT (12) using the axillae as test sites. A pair of deodorants were always used with 1 unscented and 1 scented, which were applied twice daily to the respecive axilla that were randomly chosen for each participant. The deodorant to be used in the left axilla always had a red label and the deodorant for the right axilla, a blue label. Evaluation of the ROAT was made once a week, or at the request of the patient, with inspection including assessment of the following morphologic features: erythema, infiltration, papules, vesicles, and scaling. The involved area with dermatitis and the overall impression of the use-test reaction were also assessed (17)."*

Results of the patch test were that all 17 patients had at least 1 positive reaction to cinnamaldehyde. The lowest concentration that gave positive reactions was 0.002% and the highest were 2.0% in ethanol.

Results of the ROAT test were that 8/8 patients in the first part of the study and 8/9 patients in the second part of the study gave positive reactions in the axilla when tested with cinnamaldehyde in deodorants. Positive reactions were seen in 1/9 patients at the lowest concentration teste 0.01%.

3.1.2.38 STUDY 48 (ROAT)

Study reference:

Johansen J. D., Andersen K.E., Rastogi S.C., Menne T.: Threshold responses in cinnamic-aldehyde-sensitive subjects: results and methodological aspects. Contact Dermatitis, 34, 165-171, 1996

Detailed study summary and results:

Test type

A clinical study conducted at Gentofte Hospital and Odense University Hospital, Denmark involved 22 cinnamaldehyde-allergic patients and 20 healthy controls who were tested with a dilution series of cinnamaldehyde in a patch test and a repeated open application test (ROAT). The aim of the study was to provide quantitative information on the eliciting capacity of cinnamaldehyde to be considered in assessment of clinical relevance and health hazard. For the patch test 2%, 1%, 0.5%, 0.1%, 0.05%, 0.02% and 0.01% cinnamaldehyde in petrolatum were used along with 0.02%, 0.1% and 0.8% in ethanol. For the 6 week graded ROAT use test 0.8%, 0.1% and 0.02% cinnamaldehyde in ethanol were used. Ethanol and petrolatum were included as vehicle controls.

Description of patch test as cited from Johansen et al., 1996: "*The eczema patients were patch tested with coded concentrations of cinnamaldehyde applied to the upper back in a random order, changing for each patient. The control persons were tested with fragrance mix 8% pet. only. Scanpor® tape and Finn Chambers® were used. The patches were left in place for 2 days. Blind readings were done at D2, D3 and*

D7 in Gentofte and at D3 and D7 in Odense. The reactions were scored according to ICDRG scale (11). The threshold response was definded as the weakest concentration giving a visible skin reaction in a continuous line of patch test reactions starting with 2% pet."

Description of use test as cited from Johansen et al., 1996: *"The use test was done as a repeated open application test (ROAT) (14), using the outer aspect of the upper arm as test site. The test area was 5X5 cm. The cinnamic aldehyde solution was applied on one arm and the vehicle ethanol as control on the other. The solutions were coded and the solution of cinnamic aldehyde was, in a random, blinded manner, used in half the patients on the right and the other half on the left arm. An atomizer pump giving 0.05 ml per stroke was used for applications (15). The volunteers were instructed to use 0.1 ml 2X a day. The number of applications made by all persons were recorded, and the containers were weighted every 2 weeks.*

The use test was done with graded concentrations of cinnamic aldehyde. For the 1st 2 weeks, 0.02% cinnamic aldehyde was applied, for the next 2 weeks 0.1% and for the last 2 weeks 0.8%. The patients were asked to report if visible skin symptoms occurred at the test sites. The applications were continued until at least erythema was present or a week had passed form the first symptoms. Subjects with persistent skin reactions at the site of cinnamic aldehyde application and a negative control site were classified at positive no matter what the degree of reaction."

Results of the patch test were that 18/22 had at least 1 positive reaction to cinnamaldehyde and 4/22 had doubtful reactions. The lowest threshold concentration (minimum effect level) was 0.02%. Results of the ROAT use test were that 8 patients reacted to 0.1% and 5 to 0.8% cinnamaldehyde in ethanol. None reacted to 0.02% cinnamaldehyde in ethanol.

A total of 13/18 of the patients with a clearly positive patch test reaction to cinnamaldehyde (2% in pet.) also developed a positive reaction in the ROAT test. The 4 patients with doubtful response on patch test to cinnamaldehyde (2% in pet.) were all negative in the ROAT test.

3.1.2.39 STUDY 49 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 2004. Repeated insult patch test of cinnamaldehyde. RIFM report number 47158, November 22a. (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 3 in the publicly available part of the REACH registration (

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 94 volunteers (25 male and 69 female) using 0.5% cinnamaldehyde in 3:1DEP:EtOH.

Description of HRIPT as cited in Cocchiara et al., 2005: "*A 0.3 ml aliquot of 0.5% cinnamaldehyde in 3:1DEP:EtOH was applied to 25 mm Hilltop Chambers® and volatilized for a 15–40 min period. Patches were applied to the subjects skin between the left scapula and spinal mid-line for 24 h under occlusion. Induction applications were made to the same skin site (unless reactions became so strong that an adjacent site had to be employed) on a Monday–Wednesday–Friday schedule for three consecutive weeks. All patches were applied and removed by the laboratory staff except on Saturday when the individual subjects were instructed to remove them approximately 24 h after application. Reactions were read 24 or 48 h after patch removal. Following a two-week rest period, a 24-h challenge patch using 25 mm Hilltop Chambers was applied to a previously unpatched (virgin) site. Reactions to challenge were read at 24, 48, 72 and 96 h after patch removal*."

The results showed that no sensitization reactions were observed in the 94 volunteers when tested with 0.5% cinnamaldehyde in 3:1DEP:EtOH.

3.1.2.40 STUDY 50 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 2003a. Topical photoallergy screening test of cinnamaldehyde and cinnamic acid in male albino hairless guinea pigs (Crl: IAF(HA)-hrBR (Outbred), including primary irritation, phototoxicity and contact hypersensitivity evaluations. RIFM Report Number 41273, January 15 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 4 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 28 volunteers using 3% cinnamaldehyde in 3:1DEP:EtOH with 0.5% α-tocopherol.

Description of HRIPT as cited in Cocchiara et al., 2005: "*A 0.3 ml aliquot of 3% cinnamaldehyde in 3:1DEP:EtOH (with 0.5% α-tocopherol added to prevent peroxide formation) was applied to 25 mm Hilltop Chambers® and volatilized for a 15–40 min period. Patches were applied to the subjects skin between the left scapula and spinal mid-line for 24 h under occlusion. Induction applications were made to the same skin site (unless reactions became so strong that an adjacent site had to be employed) on a Monday–Wednesday–Friday schedule for three consecutive weeks. All patches were applied and removed by the laboratory staff*

except on Saturday when the individual subjects were instructed to remove them approximately 24 h after application. Reactions were read 24 or 48 h after patch removal. Following a two-week rest period, a 24-h challenge patch using 25 mm Hilltop Chambers® was applied to a previously unpatched (virgin) site. Reactions to challenge were read at 24, 48, 72 and 96 h after patch removal. The same procedure was repeated using 3% cinnamaldehyde dissolved in a 3:1EtOH:DEP vehicle (with 0.5% α-tocopherol added)."

Sensitization reactions were observed in 14% (4/28) of the volunteers exposed to 3% cinnamaldehyde in 3:1DEP:EtOH with 0.5% α-tocopherol. Two irritation reactions were observed in the 28 volunteers.

The 3% cinnamaldehyde dissolved in a 3:1EtOH:DEP vehicle (with 0.5% α-tocopherol added) study was aborted during the induction phase due to the number of irritant reactions (8/30) observed with this vehicle.

3.1.2.41 STUDY 51-52 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 2002. Repeated insult patch test of cinnamaldehyde. RIFM report number 41692, August 27 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 7 and 8 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 22 volunteers using 0.5% cinnamaldehyde in 3:1DEP:EtOH (with 0.5% α-tocopherol) and on 19 volunteers using 0.5% cinnamaldehyde dissolved in a 3:1EtOH:DEP vehicle (with 0.5% α-tocopherol).

Description of HRIPT as cited in Cocchiara et al., 2005: "*A 0.3 ml aliquot of 0.5% cinnamaldehyde in 3:1DEP:EtOH (with 0.5% α-tocopherol added to prevent peroxide formation) was applied to 25 mm Hilltop Chambers® and volatilized for a 15–40 min period. Patches were applied to the subjects skin between the left scapula and spinal mid-line for 24 h under occlusion. Induction applications were made to the same skin site (unless reactions became so strong that an adjacent site had to be employed) on a Monday–Wednesday–Friday schedule for three consecutive weeks. All patches were applied and removed by the laboratory staff except on Saturday when the individual subjects were instructed to remove them approximately 24 h after application. Reactions were read 24 or 48 h after patch removal. Following a two-week rest period, a 24-h challenge patch using 25 mm Hilltop Chambers® was applied to a previously unpatched (virgin) site. Reactions to challenge were read at 24, 48, 72 and 96 h after patch removal. The same procedure was*

repeated using 0.5% cinnamaldehyde dissolved in a 3:1EtOH:DEP vehicle (with 0.5% a-tocopherol added)."

Sensitization reactions were observed in 0% (0/22) of the volunteers exposed to 0.5% cinnamaldehyde in 3:1DEP:EtOH with 0.5% α-tocopherol. No irritation reactions were observed in the 22 volunteers.

Sensitization reactions were observed in 0% (0/19) of the volunteers exposed to 0.5% cinnamaldehyde in 3:1EtOH:DEP with 0.5% α-tocopherol. No irritation reactions were observed in the 19 volunteers.

3.1.2.42 STUDY 53-56 (HRIPT)

Study reference:

Danneman, P.J., Booman, K.A., Dorsky, J., Kohrman, K.A., Rothenstein, A.S., Sedlak, R.I., Steltenkamp, R.J., Thompson, G.R., 1983: Cinnamic aldehyde: a survey of consumer patch-test sensitization. Food and Chemical Toxicology 21, 721–725.

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Detailed study summary and results:

Human Repeat Insult Patch Tests (HRIPT) was conducted with cinnamaldehyde in ethanol on a total of 130 volunteers. 41 volunteers were tested with 0.1% cinnamaldehyde in ethanol, 38 volunteers were tested with 0.5% cinnamaldehyde in ethanol, 41 volunteers were tested with 1% cinnamaldehyde in ethanol and 10 volunteers were tested with 1.25% cinnamaldehyde in ethanol.

No reactions were observed when 0.1% cinnamaldehyde was tested in 41 volunteers or when 0.5% cinnamaldehyde was tested in 38 volunteers. 1.0% cinnamaldehyde produced 5/41 questionable reactions (subjects reacted at the induction site and not at the primary challenge site) and 5/10 reactions were observed with 1.25% cinnamaldehyde.

3.1.2.43 STUDY 57-58 (HRIPT)

Study reference:

Marzulli, F.N., Maibach, H.I., 1980. Contact allergy: Predictive testing of fragrance ingredients in humans by Draize and maximization methods. Journal of Environmental Pathology and Toxicology 3, 235–245.

and

Marzulli, F.N., Maibach, H.I., 1976. Effects of vehicles and elicitation concentration in contact dermatitis testing. I. Experimental contact sensitization in humans. Contact Dermatitis 2, 325–329.

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Detailed study summary and results:

Test type

Study summary as cited in Cocchiara et al., 2005: "*Using a modified Draize procedure, Marzulli and Maibach (1976) reported the effects of using two different vehicles to test cinnamaldehyde. A total of 108 volunteers were tested with cinnamaldehyde, 55 were tested with 1% cinnamaldehyde in 95% ethanol and 53 were tested with 1% cinnamaldehyde in petrolatum.*"

Description of test procedure as cited in Cocchiara et al., 2005: "*Each subject received ten 48-h (72 h on weekends) occluded applications, which were made 3 times a week to the same site. Two weeks after induction, a 72-h occluded challenge application was made to a new site.*"

1/55 sensitization reactions were observed in volunteers tested with 1% cinnamaldehyde in 95% ethanol.

No sensitization reactions were observed in 53 volunteers tested with 1% cinnamaldehyde in petrolatum.

3.1.2.44 STUDY 59 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 1973b. Repeated insult patch test. Unpublished report from IFF Incorporated, 23 January. Report number 12509 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 9 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 41 volunteers using 1% cinnamaldehyde in alcohol SDA 39C.

Description of HRIPT as cited in Cocchiara et al., 2005: "*A 0.5 ml aliquot was applied to semiocclusive patches, which were then applied to the upper arm of each subject for 24 h. After a 24–48 h rest period, subjects were again patched at the same site. A total of nine induction applications were made over a three week period. Approximately two weeks after the last induction patch, a 24-h semi-occluded challenge patch was applied to the same site and to a site not previously exposed. Reactions to challenge were read at 24 and 72 h after patch removal.* "

Sensitization reactions were observed in 12% (5/41) of the volunteers exposed to 1% cinnamaldehyde in alcohol SDA 39C. No irritation reactions were observed in the 41 volunteers.

3.1.2.45 STUDY 60 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 1965. Repeated insult patch test. Unpublished report from IFF Incorporated, 1October. Report number 12508 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 6 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 38 volunteers using 0.5% cinnamaldehyde in ethanol.

Description of HRIPT as cited in Cocchiara et al., 2005: *"A 0.5 ml aliquot was applied to semiocclusive patches, which were then applied to the upper arm of each subject. These patches were removed 24 h after application. After a 24–48 h rest period, subjects were again patched at the same site. Reactions were read 24–48 h after patch removal just prior to application of the next patch. A total of nine applications were made over a three-week period on a Monday–Wednesday–Friday schedule. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 24 h. Reactions to challenge were read at 24 and 72 h after patch removal."*

Sensitization reactions were observed in 0% (0/38) of the volunteers exposed to 0.5% cinnamaldehyde in ethanol. No irritation reactions were observed in the 38 volunteers.

3.1.2.46 STUDY 61 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 1964a. Repeated insult patch test. Unpublished report from IFF Incorporated, 3 April. Report number 12511 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 10 in the publicly available part of the REACH registration.

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 10 volunteers using 1.25% cinnamaldehyde in ethanol.

Description of HRIPT as cited in Cocchiara et al., 2005: *"A 0.5 ml aliquot of 1.25% cinnamaldehyde in ethanol was applied to semi-occlusive patches, which were then applied to the upper arm of each subject. These patches were removed 24 h after application. After a 24–48 h rest period, subjects were again patched at the same site. Reactions were read 24–48 h after patch removal just prior to application of the next patch. A total of nine applications were made over a three-week period on a Monday–Wednesday–Friday schedule. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 24 h. Reactions to challenge were read at 24 and 72 h after patch removal."*

Sensitization reactions were observed in 50% (5/10) of the volunteers exposed to 0.5% cinnamaldehyde in ethanol. No irritation reactions were observed in the 10 volunteers.

3.1.2.47 STUDY 62 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 1964b. Repeated insult patch test. Unpublished report from IFF Incorporated, 29 July and 25 November. Report number 12510 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 5 in the publicly available part of the REACH registration.

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on in total 41 volunteers using 0.125% cinnamaldehyde in ethanol. The study was conducted in two phases. In the first phase 31 male and female volunteers were tested and in the second phase 10 female volunteers were tested

Description of the first phase HRIPT as cited in Cocchiara et al., 2005: *"In the first phase on 31 male and female volunteers, a 0.5 ml aliquot of 0.125% cinnamaldehyde in ethanol was applied to semi-occlusive patches which were then applied to the upper arm of each subject. These patches were removed 24 h after*

application. After a 24–48 h rest period, subjects were again patched at the same site. Reactions were read 24–48 h after patch removal just prior to application of the next patch. A total of nine applications were made over a three-week period on a Monday–Wednesday–Friday schedule. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 24 h. Reactions to challenge were read at 24 and 72 h after patch removal."

Description of the second phase HRIPT as cited in Cocchiara et al., 2005: *"In the second phase, 0.125% cinnamaldehyde in ethanol produced no sensitization reactions in 10 female volunteers after nine 24-h semi-occluded induction applications followed approximately two weeks later by a 24-h semi-occluded challenge patch."*

Sensitization reactions were observed in 0% (0/41) of the volunteers exposed to 0.125% cinnamaldehyde in ethanol. No irritation reactions were observed in the 41 volunteers.

3.1.2.48 STUDY 63 (HMT)

Study reference:

Unpublished reports by the Research Institute for Fragrance Materials (RIFM), 1974a. Report on human maximization studies. RIFM report number 1779, August 22 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 2 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

A Human Maximization Test (HMT) was conducted with 3% cinnamaldehyde in butylene glycol on 25 healthy, male and female volunteers.

Description of the HMT as cited in Cocchiara et al., 2005: "*Application was under occlusion to the same site on the volar forearms of all subjects for five alternate-day 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate under occlusion. Reactions were read at patch removal and again 24 h after patch removal.*"

Sensitization reactions were observed in 12% (3/25) of the volunteers exposed to 3% cinnamaldehyde in butylene glycol.

3.1.2.49 STUDY 64 (HMT)

Study reference:

Unpublished reports by the Research Institute for Fragrance Materials (RIFM), 1973c. Report on human maximization studies. RIFM report number 1802, October 10 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 1 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

A Human Maximization Test (HMT) was conducted with 2% cinnamaldehyde in petrolatum on 25 healthy male volunteers.

Description of the HMT as cited in Cocchiara et al., 2005: *"Application was under occlusion to the same site on the volar forearms of all subjects for five alternate-day 48-h periods. Following a ten-day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Reactions were read at patch removal and again 24 h after patch removal."*

Strong to severe sensitization reactions were observed in 44% (11/25) of the volunteers exposed to 2% cinnamaldehyde in petrolatum.

3.1.2.50 STUDY 65 (Case study)

Study reference:

Guarneri F.: Occupational allergy to cinnamal in a baker. Contact Dermatitis 63, 294–294, 2010.

Detailed study summary and results:

Test type

After having changed his workplace and work habits, switching from production of bread to the preparation of sweet bakery goods, itching eczematous hand lesions were reported for a 33-year old baker. His work required to knead many ingredients, including cinnamon.

Patch tests were performed with the Italian Society of Allergological, Occupational and Environmental Dermatology baseline series, the bakers series, latex and dust mites, in Hayes' chambers. Readings at D2 and D4 according to International Contact Dermatitis Research Group guidelines showed sensitization to fragrance mix I and cinnamaldehyde.

With correct use of individual protection devices (latex, nitrile or polyvinylchloride gloves), resolution of the lesions occurred in about 4 weeks, with no relapses over 6 months.

3.1.2.51 STUDY 66 (Case study)

Study reference:

Decapite T.J., Anderson B. E.: Allergic contact dermatitis from cinnamic aldehyde found in an industrial odour-masking agent. Contact Dermatitis 51, 311–322, 2004

Detailed study summary and results:

Test type

A 47-year-old man suffered from dermatitis of his hands, feet, face and body. He routinely handled a powder used to mask the vinyl odour from vinyl covers used for car seat upholstery. The powder contained cinnamaldehyde. Patch testing with the North American Contact Dermatitis Group standard series was performed. The day 2 readings showed positive reactions to cinnamaldehyde and North American Contact Dermatitis Group standard series.

3.1.2.52 STUDY 67 (Case study)

Study reference:

Diba V. C., Statham B. N.: Contact urticaria from cinnamal leading to anaphylaxis. Contact Dermatitis 46, 115–119, 2003

Detailed study summary and results:

Test type

A 42-year old woman nurse had rash on her arms. She continued to experience irritation developing on her arms at work. A natural rubber latex prick test was negative. She was patch tested to the European standard series, medicaments series, latex gloves and glutaraldehyde. At D4, however, a + reaction to fragrance mix was seen. She was therefore patch tested to the constituents of fragrance mix, which were applied for just 20 min. A strong urticarial reaction was seen to cinnamaldehyde and after 40 min. she developed widespread pruritus and erythema, and 5 min later, started to feel faint. A blood pressure reading was unrecordable. She was treated with 10mg chlorphenamine maleate and 1mg adrenaline intramuscularly and made a good recovery. Review of the 20-min test sites at D4 identified a ++ reaction to cinnamaldehyde. All other tests were negative. It was concluded that she had immediate, as well as delayed, hypersensitivity to cinnamaldehyde and that this constituent of the fragrance mix was the most likely cause of the anaphylaxis.