Committee for Risk Assessment

RAC

Annex 1

Background document

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

isoeugenol; [1];
(E)-2-methoxy-4-(prop-1-enyl)phenol; [2];
(Z)-2-methoxy-4-(prop-1-enyl)phenol; [3];

EC Number: 202-590-7; [1]; 227-678-2; [2]; 227-633-7; [3];
CAS Number: 97-54-1; [1]; 5932-68-3; [2]; 5912-86-7; [3];

CLH-O-0000001412-86-98/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

10 March 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name:

2-methoxy-4-(prop-1-enyl)phenol

2-methoxy-4-((E)prop-1-enyl)phenol

2-methoxy-4-((Z)prop-1-enyl)phenol

EC Number: **202-590-7**

227-678-2

227-633-7

CAS Number: **97-54-1**

5932-68-3

5912-86-7

Index Number: **not applicable**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOEUGENOL; [1]; (E)-2-METHOXY-4-(PROP-1-ENYL)PHENOL; [2]; (Z)-2-METHOXY-4-(PROP-1-ENYL)PHENOL; [3];

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Version number: 1.0 **Date:** **May 2015**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	*Isoeugenol* *2-methoxy-4-(prop-1-enyl)phenol*
EC number:	*202-590-7*
CAS number:	*97-54-1*
Annex VI Index number:	-
Degree of purity:	*confidential*
Impurities:	*confidential*

Substance name:	*Isoeugenol* *2-methoxy-4-((E)prop-1-enyl)phenol (IUPAC-name)*
EC number:	*227-678-2*
CAS number:	*5932-68-3*
Annex VI Index number:	-
Degree of purity:	*unknown*
Impurities:	*unknown*

Substance name:	*Isoeugenol* *2-methoxy-4-((Z)prop-1-enyl)phenol (IUPAC-name)*

EC number:	*227-633-7*
CAS number:	*5912-86-7*
Annex VI Index number:	-
Degree of purity:	*unknown*
Impurities:	*unknown*

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Skin. Sens. 1A H317: May cause an allergic skin reaction
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin. Sens. 1A H317: May cause an allergic skin reaction

1.3 Proposed harmonised classification and labelling based on CLP Regulation

This is a CLH proposal for isoeugenol with CAS 97-54-1 and EC 202-590-7. Isoeugenol is a mixture of two diastereomers, this CLH-proposal covers the racemic mixture and both isomers (i.e. 2-methoxy-4-((E)prop-1-enyl)phenol and 2-methoxy-4-((Z)prop-1-enyl)phenol).

The scope of this proposal is limited to human health hazard assessment, and furthermore targeted to classification for skin sensitisation.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Not evaluated
2.2.	Flammable gases	None		None	Not evaluated
2.3.	Flammable aerosols	None		None	Not evaluated
2.4.	Oxidising gases	None		None	Not evaluated
2.5.	Gases under pressure	None		None	Not evaluated
2.6.	Flammable liquids	None		None	Not evaluated
2.7.	Flammable solids	None		None	Not evaluated
2.8.	Self-reactive substances and mixtures	None		None	Not evaluated
2.9.	Pyrophoric liquids	None		None	Not evaluated
2.10.	Pyrophoric solids	None		None	Not evaluated
2.11.	Self-heating substances and mixtures	None		None	Not evaluated
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not evaluated
2.13.	Oxidising liquids	None		None	Not evaluated
2.14.	Oxidising solids	None		None	Not evaluated
2.15.	Organic peroxides	None		None	Not evaluated
2.16.	Substance and mixtures corrosive to metals	None		None	Not evaluated
3.1.	Acute toxicity - oral	None		None	Not evaluated
	Acute toxicity - dermal	None		None	Not evaluated
	Acute toxicity - inhalation	None		None	Not evaluated
3.2.	Skin corrosion / irritation	None		None	Not evaluated
3.3.	Serious eye damage / eye irritation	None		None	Not evaluated
3.4.	Respiratory sensitisation	None		None	Not evaluated
3.4.	Skin sensitisation	Skin. Sens. 1A; H317	None	None	
3.5.	Germ cell mutagenicity	None		None	Not evaluated
3.6.	Carcinogenicity	None		None	Not evaluated
3.7.	Reproductive toxicity	None		None	Not evaluated
3.8.	Specific target organ toxicity –single exposure	None		None	Not evaluated
3.9.	Specific target organ toxicity – repeated exposure	None		None	Not evaluated
3.10.	Aspiration hazard	None		None	Not evaluated
4.1.	Hazardous to the aquatic	None		None	Not evaluated

	environment				
5.1.	Hazardous to the ozone layer	None		None	Not evaluated

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

Labelling: Signal word: Warning
Hazard statements: H317: May cause an allergic skin reaction..
Precautionary statements: Not harmonised

Proposed notes assigned to an entry:

: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Isoeugenol has not previously been assessed for harmonised classification by RAC or TC C&L.

2.2 Short summary of the scientific justification for the CLH proposal

This proposal is based on available animal studies and human data from patch testing and epidemiological studies. There is no registration of isoeugenol (updated in July 2014). Animal tests, local lymph node assay and guinea pig maximisation test, showed that isoeugenol is a substance with a high potency of sensitization. Information on skin sensitisation is described in many studies where diagnostic human patch test data showed a relative high incidence at low exposure levels. Observational epidemiological studies showed there is a high incidence of allergic contact dermatitis at relative low exposure. Therefore the dossier submitter argued that based on the available animal and human evidence for isoeugenol, a classification as *Skin Sens. 1A – H317: May cause an allergic skin reaction* is proposed for isoeugenol. Classification for the individual isomers is based on limited data supported by read-across.

2.3 Current harmonised classification and labelling

Isoeugenol has currently no harmonised classification (Annex VI, CLP Regulation).

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

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

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

The self-classification as available from the C&L Inventory Database on 16 June 2014 includes self-classification of a total of 1051 notifiers for acute toxicity, skin irritation, skin sensitisation, respiratory irritation and eye irritation.

4 out of 1051 notifiers (0.4%) did not consider self-classification for skin sensitisation.

Self-classification for skin sensitisation was done by 1047 notifiers. These notifications included 1031 (98%) self-classifications for Skin Sens 1, 16 (1.5%) self-classifications for Skin Sens 1A, none (0%) self-classification for Skin Sens 1B.

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

RAC general comment
Isoeugenol comprises two isomers, (E)-2-methoxy-4-(prop-1-enyl)phenol and (Z)-2-methoxy-4-(prop-1-enyl)phenol. Most studies were performed with isoeugenol without specifying the ratio between the cis- and trans-isomer. Apart from the HMT test by RIFM (1980d) conducted with the Z-isomer and the patch test by Tanaka *et al*. (2004) conducted with the E-isomer (although in this case the test outcome might be due to a cross-reaction), there is very limited information available on the skin sensitising potential of each of the isomers. It was noted by the DS that the double bond configuration that differs between the two isomers was not expected to be relevant for the activation before protein binding. Therefore, the results obtained with isoeugenol are considered relevant for the individual isomers and for the racemic mixture.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Isoeugenol is currently not classified according to Annex VI of CLP. However, based on patch testing, epidemiological data, animal tests, local lymph node assay and guinea pig maximisation test it is warrant to classify isoeugenol as Skin Sens. 1A. Therefore, the self-classification applied by the majority of the C&L notifiers is considered incorrect and resulting in a GCL for mixtures of 1.0% instead of 0.1%. This justifies a proposal for harmonised classification. Through the harmonised classification of isoeugenol as a skin sensitiser category 1A the information about the presence of the substance in mixtures is improved.

As isoeugenol is a strong sensitiser, classification of mixtures containing isoeugenol should already occur at a concentration as low as 0.1% while the substance should be indicated on the label starting at 0.01% as required according to Table 3.4.6 of Annex I of CLP. In this way mixtures containing isoeugenol would be easily recognized and preventing measures can be applied by informed consumers and other professional handlers of isoeugenol containing mixtures.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	202-590-7
EC name:	isoeugenol
CAS number (EC inventory):	97-54-1
CAS number:	
CAS name:	Phenol, 2-methoxy-4-(1-propen-1-yl)-
IUPAC name:	2-methoxy-4-(1-propenyl)-phenol
CLP Annex VI Index number:	not applicable
Molecular formula:	$C_{10}H_{12}O_2$
Molecular weight range:	164.21

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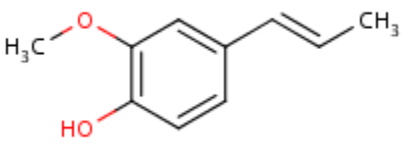
EC number:	227-678-2
EC name:	(E)-2-methoxy-4-(prop-1-enyl)phenol
CAS number (EC inventory):	5932-68-3
CAS number:	
CAS name:	Phenol, 2-methoxy-4-(1E)-1-propen-1-yl-
IUPAC name:	2-methoxy-4-((E)prop-1-enyl)phenol
CLP Annex VI Index number:	not applicable
Molecular formula:	$C_{10}H_{12}O_2$
Molecular weight range:	164.21

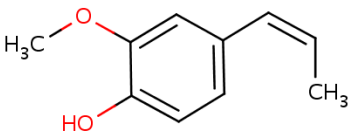
EC number:	227-633-7
EC name:	(Z)-2-methoxy-4-(prop-1-enyl)phenol
CAS number (EC inventory):	5912-86-7
CAS number:	
CAS name:	Phenol, 2-methoxy-4-(1Z)-1-propen-1-yl-
IUPAC name:	2-methoxy-4-((Z)prop-1-enyl)phenol
CLP Annex VI Index number:	not applicable
Molecular formula:	$C_{10}H_{12}O_2$
Molecular weight range:	164.21

Structural formula:

E-isomer:



Z-isomer:



1.2 Composition of the substance

Isoeugenol is a mixture of two diastereomers (i.e. 2-methoxy-4-((E)prop-1-enyl)phenol and 2-methoxy-4-((Z)prop-1-enyl)phenol)

Current Annex VI entry: no harmonized classification

Due to the absence of a registration dossier, information on impurities or additives is not available.

1.2.1 Composition of test material

The test material concerns isoeugenol with unknown purity and isomer ratio, unless otherwise specified in the individual studies.

1.3 Physico-chemical properties

Due to the absence of a registration the available physical-chemical information is limited. The available property data, including references, are from EPI Suite 4.10 or Syracuse Research Corporation.

Table 5: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Liquid	Merck Index	
Melting/freezing point	- 10 °C	Merck Index	
Boiling point	270.60 °C (Adapted Stein & Brown method) 266 °C (experimental database)		
Relative density	1.080 g/cm^3	Merck Index	
Vapour pressure	0.00381 mm Hg at 25 °C (Modified Grain Method) 0.012 mm Hg at 25 °C (experimental database)		
Surface tension	No information available		
Water solubility	356 mg/L at 25 °C	MEYLAN,WM ET AL. (1996)	
Partition coefficient n-octanol/water	3.04	GRIFFIN,S ET AL. (1999)	Experimental data
Flash point	No information available		
Flammability	No information available		
Explosive properties	No information available		
Self-ignition temperature	No information available		
Oxidising properties	No information available		
Granulometry	No information available		
Stability in organic solvents and identity of relevant degradation products	No information available		
Dissociation constant	9.88 at 25 °C	SERJEANT,EP & DEMPSEY,B (1979)	Experimental Data
Viscosity	No information available		

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this report.

2.2 Identified uses

Isoeugenol is used as fragrance and flavouring agent in numerous non-food and food products and as an anaesthetic for fishes.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this report.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

EPMAR from European Medicines Agency on isoeugenol (EPMAR 2011) has included a literature study (GLP status unstated) which used radiolabelled isoeugenol to investigate the metabolism of the compound in the male Fischer 344 rat. Following a single oral dose of 14[C] isoeugenol (156 mg/kg bw, 50 microCi/kg bw), greater than 85% of the administered dose was excreted in the urine predominantly as sulfate or glucuronide metabolites by 72 hours. Approximately 10% was recovered in the faeces, and less than 0.1% was recovered as CO or expired organics. The parent compound isoeugenol was not detected in the blood at any of the time points analysed (0.25 to 72 hours). Following intravenous administration (15.6 mg/kg bw, 100 microCi/kg bw), isoeugenol disappeared rapidly from the blood. The half-life was 12 minutes, the volume of distribution was 13.96 l/kg, mean residence time (MRT) was 11.6 minutes and the systemic clearance was 1.9 l/min/kg. Excretion characteristics were similar to those seen following oral administration. The total amount of radioactivity remaining in selected tissues (heart, kidneys, liver, muscle, subcutaneous adipose tissue and testicular adipose tissue) by 72 hours was less than 0.25% of the dose following both oral and intravenous administration. Based on the findings of this study, it can be concluded that isoeugenol is rapidly metabolised in the rat and is excreted predominantly in the urine as phase II conjugates of the parent compound.

The mechanism of action of isoeugenol was also discussed in a technical report from US National Toxicology Program (NTP 2010). It has been shown although isoeugenol is detoxified by phase II conjugation of its free phenolic group, that direct single-electron oxidation is a fifth pathway that results in formation of the quinone-methide metabolite (cited from NTP 2010: Thompson et al., 1993, 1998; Bertrand et al., 1997; Burkey et al., 2000; Badger et al., 2002). The formation of quinone or quinonemethide metabolites is thought to be responsible for skin sensitization caused by both isoeugenol and eugenol (cited from NTP 2010: Thompson et al., 1993, 1998; Bertrand et al., 1997; Burkey et al., 2000) and could be responsible for other toxic responses. The formation of a quinone-methide metabolite is further supported by other studies, which indicate that the biosynthesis of eugenol and isoeugenol proceeds by NADPH-dependent reduction of their quinone-methide, formed from coniferyl acetate (cited from NTP 2010: Louie et al., 2007; Koeduka et al., 2008). It should be noted that eugenol, isoeugenol, and coniferyl alcohol form the same quinone-methide and that presence of a phenolic hydroxyl group para to the propenyl group is essential for its formation. Studies in mice (cited from NTP 2010: Bertrand et al. 1997) suggested that the two chemicals form reactive quinone-methide haptens by different mechanistic pathways. Isoeugenol sensitization is consistent with direct oxidation to its p-quinone-methide without first undergoing demethylation. By analogy, isoeugenol, which also has a free phenolic hydroxyl group, can undergo a similar direct oxidation to form the identical quinone-methide. Another study (cited from NTP 2010: Rastogi and Johansen, 2008) indicated that substantial amounts of isoeugenyl acetate are now

present in some perfumed products, apparently to decrease the amount of isoeugenol needed to provide a desired fragrance; however, this substitution does not allay concern about isoeugenol exposure because skin may readily metabolize the acetate ester to isoeugenol, perhaps exerting concomitant contact allergy in sensitive individuals.

4.1.2 Human information

Isoeugenol is absorbed into the systemic circulation after dermal application or ingestion. Application of 10 mM of ^{14}C-isoeugenol to human cadaver skin using various vehicles (ethanol:water, propylene glycol, liquid paraffin, lotions, white petrolatum, or macrogol ointment) resulted in penetration values ranging from 0.29% to 4% (water- based vehicles) and 0.05% to 11% (lotions and ointments) (cited from NTP 2010: Jimbo et al., 1983)

4.1.3 Summary and discussion on toxicokinetics

Isoeugenol is rapidly metabolised and eliminated. Oral toxicokinetic studies show no signs of metabolic saturation. Skin penetration studies *in vitro* and *in vivo* show isoeugenol rapidly penetrates the skin. Moreover, it has been found that the formation of quinone or quinonemethide metabolites might be the mechanism by which isoeugenol and other isoeugenol derivate cause sensitisation.

4.2 Acute toxicity

Not evaluated in this report

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

Not evaluated in this report

4.4 Irritation

4.4.1 Skin irritation

Not evaluated in this report

4.4.2 Eye irritation

Not evaluated in this report

4.4.3 Respiratory tract irritation

Not evaluated in this report

4.5 Corrosivity

Not evaluated in this report

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

Isoeugenol has been chosen for a full risk assessment by HERA (Human and Environmental Risk Assessment on ingredients of household cleaning products) program because of its known skin sensitising properties (HERA, 2005). These assessments results are integrated in this section. Due to the public unavailability of a.o. the RIFM studies cited in HERA, 2005, these studies are not individually evaluated. No information is provided in Hera (2005) as to whether positive and negative controls were included in these studies and their results. In absence of detailed information, it is assumed that the results of the negative controls for all the studies are 0%. For the same reason, information on the dose-selection of most studies (mainly confidential studies) is not available. However, some studies (Kimber et al., 1991; Basketter and Scholes, 1992; Hilton et al., 1996; Takeyoshi et al., 2008) stated that preliminary irritation tests were carried out to determine the concentrations of the test substances suitable for induction of sensitization and for sensitization challenge.

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The skin sensitization potential of isoeugenol has been evaluated in different animal tests systems. Table 6 presents an overview of available guinea pig maximisation tests (GPMT) with isoeugenol. In the guinea pig maximization test according to the Magnusson-Kligman protocol (one of the reference methods in OECD TG 406), positive results were obtained (Table 6) showing that isoeugenol has a clear potential to induce cell-mediated contact allergy.

Table 6 Guinea pig maximization tests (GPMT) on isoeugenol (cited from HERA, 2005)*

Induction		Challenge	Results	Reference
Intra-dermal	**Topical**			
5% in saline	30% in Petrolatum	1% in Petrolatum	1/20 (5%)	RIFM (1985b)
		3% in Petrolatum	2/20 (10%)	
		10% in Petrolatum	10/20 (50%)	
5% in saline	25% in Petrolatum	"subirritant" concentratum (specific concentration is unknown)	Some sensitization	Klecak et al. (1977)
0.15% in saline	25% in Acetone PEG 400	5% in Acetone PEG 400	100% (total number of animals is unknown)	Basketter and Scholes (1992); Barratt and Basketter (1992)
0.15% in saline	25% in Acetone PEG 400	5% in Acetone PEG 400	100% (total number of animals is unknown)	Hilton et al. (1996)
0.15% in DOBS saline	25% in Acetone PEG 400	5% in Acetone PEG 400	10/10 (100%)	Kimber et al. (1991)
1.0% in Ethanol	100%	100%	10/10 (100%)	Tsuchiya et al. (1982); Tsuchiya et al. (1985)
Modified test No intra-dermal administration of Isoeugenol	3% in Petrolatum	0.5% in Petrolatum	10/10 (100%)	Maurer and Hess (1989)
5% in olive oil	5% in olive oil	5% in olive oil	100% sensitization (total number of animals is unknown)	Takeyoshi et al. (2008)

* *This overview table is cited from HERA 2005. Not all the references are public available. The information on positive and negative controls are given below when the individual studies are public available.*

In a study (Klecak et al. 1977) isoeugenol (and 32 other compounds) was tested by the Open Epicutaneous Test (OET) technique, and, for the purpose of comparison, by three intradermal techniques, namely the Draize Test (DT), the Maximization Test (MT) and the Freund's Complete Adjuvant Test (FCAT). For MT on day 0 the animals (number unknown) were injected intradermally with 0.1 ml of a 5% solution of isoeugenol, with 0.1 ml of a 5% emulsion of

isoeugenol in Freund's complete adjuvant (FCA) and with 0.1 ml of FCA alone, each injection being given twice. In addition, 250 mg of the compound dissolved in petrolatum at a concentration of 25% was applied on day 8 to a lipped skin area of the neck and was kept under occlusive bandage for 2 days (total dose 20 mg intra-dermally plus 250 mg epicutaneously). On day 21 an occlusive patch test with the compound at a sub-irritant concentration in petrolatum was applied to the flank for 24 h. The reactions were read 24 and 48 h after removing the patch. It has been found that isoeugenol induces sensitization in all used testing systems (Table 7).

Table 7 Skin irritating and sensitizing properties of isoeugenol in Guinea Pigs (Klecak et al., 1977)

Compound	OET				Allergenicity in Guinea Pigs			
	Minimum Irritating Conc. in %		Minimum Sensitizing Conc. in %	Minimum Eliciting Conc. in %	OET	DT	MT	FCAT
	After 1 Application	After 21 Applications						
Isoeugenol	30	10	10	1	+	+	+	+

In another study (Tsuchiya et al., 1982) on contact hypersensitivity in the guinea pig, several allergens including isoeugenol was tested using Freund's complete adjuvant test (FCAT) method, Open epicutaneous test (OET) method, Guinea pig maximization test (GPMT) method, and cumulative contact enhancement test (CCET) method. The results (Table 8) showed that the sensitization ratio of isoeugenol is 100% using GPMT method.

Table 8 Sensitization ratio of isoeugenol in different concentrations examined in 4 different sensitization test methods (positives/total) (Tsuchiya et al., 1982)

Animal strain	Induction method	Induction concentration (%)	Isoeugenol
Pirbright	FCAT	5	8/8
		0	0/8
	CCET	100	2/6
		30	6/6
		10	6/6
		0	0/6
	OET	100	6/6
		30	6/6
		10	5/6
		3	2/6
		0	0/8
Hartley	CCET	100	5/10
	GPMT	1 (Topical challenge conc (%): 100)	10/10

Maurer and Hess (1989) assessed the skin sensitization potential of several compounds including isoeugenol using GPMT method. When the concentrations of isoeugenol used for induction and challenge were 3% and 0.5%, respectively, the incidence of positive sensitization reactions was 100% (10/10).

The sensitization potential of isoeugenol was also tested in another study (Kimber et al. 1991). In GPMT test, isoeugenol (injection: 0.15% in DOSB (dodecyl benzene sulphonate)/saline; patch: 25% in aceton/PEG 400; challenge: 5% in aceton/PEG 400) induced 100% sensitization response of

the tested animals. Isoeugenol showed positive in LLNA performed in four different laboratories. The lowest concentration yielding a positive response is 2.5%.

Basketter and Scholes (1992) compared GPMT with LLNA for the detection of a range of contact allergens. GPMT results showed that 100% of tested animals had sensitization response. LLNA results showed that isoeugenol is a sensitizer (A chemical was regarded as a sensitizer in the LLNA if at least one concentration of the chemical resulted in a three-fold or greater increase in ^{3}HTdR incorporation compared with control values.). In another study (Barratt and Basketter 1992), the sensitization potential of isoeugenol has been examined using GPMT. The test concentrations of isoeugenol were 0.15% for induction injection, 25% for topical induction patch and 5.0% for topical challenge patch. The results showed that the response of sensitization to isoeugenol is 100%.

Another study reported the differences in skin sensitization potencies for isoeugenol and two types of dimer, ß-O-4-dilignol and dehydrodiisoeugenol (DIEG), as evaluated by the non-radioisotopic local lymph node assay (non-RI LLNA) and guinea pig maximization test (Takeyoshi et al., 2008). In the guinea pig maximization test, isoeugenol, ß-O-4-dilignol and DIEG were classified as extreme, weak and moderate sensitizers, respectively (Table 9). As for the results of non-RI LLNA, the EC3 for isoeugenol, ß-O-4-dilignol and DIEG were calculated as 12.7%, > 30% and 9.4%, respectively (Table 10).

Table 9 Results of the guinea pig maximization test for isoeugenol and isoeugenol dimers (Takeyoshi et al., 2008)

Chemical name	Sensitization rate (%)	Grade[a]	Classification[a]
isoeugenol	100	V	Extreme
ß-O-4-dilignol	0	I	Weak
DIEG	50	III	Moderate

[a] Classifications were made according to the criterion of Magnusson and Kligmann (1969)

Table 10 Results (stimulation index and EC3-values) of non-RI LLNA for isoeugenol and isoeugenol dimers (Takeyoshi et al., 2008)

	Isoeugenol		ß-O-4-dilignol		DIEG	
% tested	Mean	SE	Mean	SE	Mean	SE
1%	1.00	0.10	1.00	0.12	1.00	0.11
3%	1.52	0.49	1.02	0.27	1.95	0.42
10%	2.43	0.45	1.19	0.30	3.09	0.31*
30%	6.73	0.88*	1.05	0.20	5.37	0.50*
EC3 (%)	12.7		>30		9.4	

Results represent mean values and standard errors in four mice
The stimulation index (SI) was calculated by dividing the mean value obtained in each treatment group by that of the control group.
** Significantly different from the concurrent vehicle control (0%) at p < 0.05 (Dunnett's test)*

Other adjuvant tests (Freund's Complete Adjuvant Test and Optimization Test) also revealed the sensitization potential of isoeugenol (Table 11) while the Cumulative Contact Enhancement Test (Table 12) showed a dose-response relationship as well as vehicle effects (data not shown).

Table 11 Freund's complete adjuvant tests (FCAT, optimization test) on isoeugenol (cited from HERA 2005)*

Induction concentration	Challenge Concentration	Results	Comments	References
1% in Ethanol 3% in Ethanol 10% in Ethanol	1% in Ethanol 3% in Ethanol 10% in Ethanol	5/10 (FCAT) 9/10 10/10	Intra-dermal induction. Topical challenge (FCAT)	RIFM (1985b)
50% in Adjuvant	"subirritant concentration"	(FCAT) Sensitisation observed	FCAT. Results only reported in summary form	Klecak et al. (1977)
5% in Ethanol	5% in Ethanol	(FCAT) 8/8	FCAT. Results only reported in summary form	Tsuchiya et al. (1982); Tsuchiya et al. (1985)
3% in Acetone	0.3% in Acetone 1% in Acetone 3% in Acetone	(FCAT) Moderate sensitisation at all concentrations	Modified FCAT. Results only reported in summary form	Hausen et al. (1995)
0.1% in 30% Ethanol	Intra-dermal challenge : 0.1% in 30 % Ethanol Topical challenge: 0.5% in Petrolatum	Optimization test 17/20 20/20	Optimization test. Like FCAT except intra-dermal and topical challenges	Maurer et al. (1979)

* *This overview table is cited from HERA 2005. Not all the references are public available. The information on positive and negative controls are given below when the individual studies are public available.*

Table 12 Cumulative contact enhancement tests (CCET) on isoeugenol (cited from HERA 2005)*

Induction Conditions	Challenge Conditions	Results	Comments	References
100%	100%	5/10	Standard CCET	Tsuchiya et al. (1982); Tsuchiya et al. (1985)
100%	100% 30% in Ethanol 10% in Ethanol	2/6 6/6 6/6	Multi-dose CCET	Tsuchiya et al. (1982); Tsuchiya et al. (1985)
10% in Ethanol	10% in Ethanol	0/9	Standard CCET	Tsuchiya et al. (1985)
10% in Ethanol	10% in liquid paraffin (low viscosity)	2/9	Standard CCET	Tsuchiya et al. (1985)
10% in Ethanol	10% in liquid paraffin (high viscosity)	0/9	Standard CCET	Tsuchiya et al. (1985)
10% in liquid paraffin (low viscosity)	10% in liquid paraffin (low viscosity)	8/10	Standard CCET	Tsuchiya et al. (1985)
10% in liquid	10% in Ethanol	8/10	Standard CCET	Tsuchiya et al. (1985)

paraffin (low viscosity)				
10% in liquid paraffin (low viscosity)	10% in liquid paraffin (high viscosity)	7/10	Standard CCET	Tsuchiya et al. (1985)
10% in liquid paraffin (high viscosity)	10% in liquid paraffin (high viscosity)	1/10	Standard CCET	Tsuchiya et al. (1985)
10% in liquid paraffin (high viscosity)	10% in Ethanol	1/10	Standard CCET	Tsuchiya et al. (1985)
10% in liquid paraffin (high viscosity)	10% in liquid paraffin (low viscosity)	6/10	Standard CCET	Tsuchiya et al. (1985)

* *This overview table is cited from HERA 2005. Not all the references are public available. The information on positive and negative controls are given below when the individual studies are public available.*

The allergenic potential of isoeugenol is also evident from non-adjuvant tests. Early studies using the Modified Draize Test on Guinea Pigs had already indicated this (Table 13). In the Buehler Test (Table 14), a clear dose/response relationship was observed. However, because of the dose levels chosen, no test displayed a non-inducing dose although this would seem to be close to 1% when the skin at the site of induction was intact (Kaminsky and Szivos,1986; Kaminsky and Szivos, 1990).

Table 13 Modified Draize Tests (Guinea Pigs) on isoeugenol (cited from HERA 2005)*

Induction conditions (intra-dermal)	Challenge conditions (intra-dermal)	Results	Comments	References
1% in peanut oil	1% in peanut oil	2/2	Old study	Griepentrog (1961)
0.1% in saline	0.1% in saline	Sensitization reported	No details were reported	Klecak at al. (1977)

* *This overview table is cited from HERA 2005. Not all the references are public available. The information on positive and negative controls are given below when the individual studies are public available.*

Table 14 Buehler Tests on isoeugenol (cited from HERA 2005)*

Induction conditions (topical)	(Re-)challenge conditions (topical)	Results	Comments	References
10% in diethylphthalate	3% in diethylphthalate 10% in diethylphthalate 30% in diethylphthalate	2/20 1/20 5/20	Standard test	RIFM (1987a)
5% in ethanol/water 80/20)	3% in diethylphthalate 9% in diethylphthalate	0/20 0/20	Standard test	RIFM (1986)

	30% in diethylphthalate	1/20		
4% in petrolatum for first 5 inductions, then 1% in petrolatum for 6th induction	2% in petrolatum Re-challenge at 1% in petrolatum	5/10 (24 hours) 1/10 (48 hours)	Standard test with intact skin	Kaminsky and Szivos (1986); Kaminsky and Szivos (1990)
4% in petrolatum for first 5 inductions, then 1% in petrolatum for 6th induction	2% in petrolatum Re-challenge at 1% in petrolatum	2/10 (24 hours) 1/10 (48 hours) 7/10 (24 hours) 2/10 (48 hours)	Use of abraded skin in induction phase	Kaminsky and Szivos (1986); Kaminsky and Szivos (1990)
30% in petrolatum for first 5 inductions, then 20% for the 6th induction	2% in petrolatum Re-challenge at 1% in petrolatum	8/10 (24 hours) 4/10 (48 hours) 9/10 (24 hours) 2/10 at 48 hours	Standard test with intact skin	Kaminsky and Szivos (1986); Kaminsky and Szivos (1990)
3% in petrolatum	1% in petrolatum	5/8 (24 hours) 4/8 (48 hours)	Standard test with intact skin	Kaminsky and Szivos (1986); Kaminsky and Szivos (1990)
3% in petrolatum	1% in petrolatum	9/10 (24 hours) 5/10 (48 hours)	Abraded skin at sites of induction	Kaminsky and Szivos (1986); Kaminsky and Szivos (1990)
30% in petrolatum	1% in petrolatum	7/10 (24 hours) 6/10 (48 hours)	Abraded skin at sites of induction	Kaminsky and Szivos (1986); Kaminsky and Szivos (1990)
1% in petrolatum	1% in petrolatum Re-challenge at 1% in petrolatum	1/9 (24 hours) 0/9 (48 hours) 1/9 (24 hours) 0/9 (48 hours)	Standard test with intact skin	Kaminsky and Szivos (1986); Kaminsky and Szivos (1990)
1% in petrolatum	1% in petrolatum Re-challenge at 1% in petrolatum	3/9 (24 hours) 2/9 (48 hours) 3/9 (24 hours) 1/9 (48 hours)	Abraded skin at sites of induction	Kaminsky and Szivos (1986); Kaminsky and Szivos (1990)
30% in petrolatum	1% in petrolatum Re-challenge at 1% in petrolatum	7/9 (24 hours) 3/9 (48 hours) 8/9 (24 hours) 2/9 (48 hours)	Standard test with intact skin	Kaminsky and Szivos (1986); Kaminsky and Szivos (1990)
10% in petrolatum	0.1% in petrolatum 1% in petrolatum	8/20 16/20	Standard test with extra challenges with chemical analogues	Goh and Yuen (1994)
	0.1% acetyl isoeugenol 0.1% eugenol	2/6 1/6	Cross-challenges only on animals that had been sensitive to isoeugenol at 0.1%	
	1% acetyl isoeugenol 1% eugenol	3/6 1/6	Cross-challenges only on animals that had been sensitive to isoeugenol at 1%	

* *This overview table is cited from HERA 2005. Not all the references are public available. The information on positive and negative controls are given below when the individual studies are public available.*

Epicutaneous tests, involving open application and closed patch testing (Table 15), showed no clear dose/response relationship except in the challenge doses that were able to elicit reactions.

Table 15 Epicutaneous tests (open: OET & closed: CET) in guinea pigs on isoeugenol (cited from HERA 2005)*

Induction conditions (topical)	Challenge conditions (topical)	Results	Comments	References
10% (vehicle not specified)	1% (vehicle not specified)	Sensitization observed	Standard OET but only summary of results reported	Klecak et al. (1977)
100%, 30%, 10% and 3% in ethanol	30% in ethanol	No reactions	Standard OET	RIFM (1986)
100%, 30%, 10% and 3% in ethanol	100% in ethanol 30% in ethanol 10% in ethanol 3% in ethanol	6/6 6/6 5/6 2/6	Standard multi-dose OET	Tsuchiya et al (1982); Tsuchiya et al. (1985)
8% (vehicle not specified)	8% (vehicle not specified)	No reactions	Standard OET but only summary of results reported	Klecak (1979)
10% in petrolatum	1% in petrolatum 3% in petrolatum 10% in petrolatum	7/20 14/20 15/20	Standard CET (48 hours occlusion at induction and challenge)	RIFM (1985b)
10% (vehicle not reported)	1% (vehicle not reported)	16/20	CET with (48 hours occlusion)	Ishihara et al. (1986)

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In the murine tests (Table 16), the Mouse Ear Swelling Test (MEST) confirmed the allergenicity of isoeugenol. The Local Lymph Node Assay (LLNA) also gave positive reactions in numerous tests. These tests were performed according to OECD TG. Some insight into the mechanism has been provided by local lymph node assays conducted with and without an inhibitor of epidermal cytochrome P4501A which showed that the inhibition of this enzyme increased degree of allergenic reaction (Scholes et al., 1994).

Table 16 Murine tests (mouse ear swelling test: MEST, Local Lymph Node Assay: LLNA) on isoeugenol (cited from HERA 2005)*

Induction conditions (AOO = acetone:olive oil	Challenge conditions	Results	Comments	References

[4:1])				
5% (vehicle not specified)	5% (vehicle not specified)	Significant ear swelling after 24 hours	MEST	Yamazaki et al. (1998)
10%, 25%, 50% and 75% in AOO	10%, 25%, 50% and 75% in AOO	Sensitization at all dose level	MEST	Garrigue et al. (1994)
3% and 10% (vehicle not specified)	3% and 10% (vehicle not specified)	100% mice were sensitized at both levels	MEST	Thorne et al. (1991)
5%, 10% and 25% in AOO	-	Sensitization at all levels	LLNA	Hilton et al. (1996)
1.3 and 5% in AOO	-	Stimulation index (SI) was 4.16 at 1.3%	LLNA: only two doses	Dearman et al. (1999)
2.5%, 5% and 10% in AOO	-	Sensitization effects at all doses	LLNA	Basketter and Scholes (1992)
2.5%, 5% and 10% in AOO	-	Sensitization effects at all doses	LLNA	Kimber et al (1991)
0.25%, 0.5%, 1%, 2.5% and 5% in AOO	-	Number of labs with positive effects 0.25% (1/5) 0.5% (0/5) 1% (1/5) 2.5% (3/5) 5% (5/5)	LLNA: interlaboratory comparison (5 labs) sensitization effects (SI >3) recorded	Loveless et al. (1996)
2.5%, 5% and 10% in AOO	-	SI: 8.5 at 2.5%, 12.1 at 5% and 16.5 at 10%	LLNA: SI were recorded but EC3 not calculated	Bertrand et al. (1997)
2.5%, 5% and 10% in AOO	-	EC3: 3.3%, 3.5% or 3.8% depending on method of calculation	LLNA: comparison of different methods of calculating EC3	Basketter et al. (1999)
0.5%, 1%, 2.5%, 5% and 10% in following solvents: Acetone/olive oil (AOO) Dimethyl sulphoxide (DMSO) Methyl ethyl ketone (MEK)	-	EC3 values as indicated 1% (AOO) (250 µg/cm^2) 0.9% (DMSO) (225 µg/cm^2) 1% (MEK) (250 µg/cm^2)	LLNA To determine effect of using 7 different vehicles	Wright et al. (2001a); Wright et al. (2001b)

Dimethyl formamide (DMF)		1.4% (DMF) ($350\ \mu g/cm^2$)		
Propylene glycol (PG)		2.5% (PG) ($625\ \mu g/cm^2$)		
Ethanol/water [50/50] (E/W)		4.9% (E/W) ($1225\ \mu g/cm^2$)		
Ethanol/water [90/10] (E/W)		1.8% (E/W) ($450\ \mu g/cm^2$)		
0.25%, 0.5%, 1%, 2.5% and 5% in AOO	-	EC3: 1.54% ($390\ \mu g/cm^2$)	LLNA	RIFM (2001)
0.25%, 0.5%, 1%, 2.5% and 5% in AOO	-	EC3: 0.64% ($160\ \mu g/cm^2$)	LLNA	RIFM (2001)
Not given	-	EC3: 1.3% ($325\ \mu g/cm^2$)	LLNA: Report of unpublished study	Basketter et al. (2002); Basketter et al. (2003); Dearman et al. (1999)
0.5%, 1% and 5% in AOO	-	EC3 values between 0.5% and 2.6% (125 – 653 $\mu g/cm^2$). Mean of 300 $\mu g/cm^2$ with SD of 0.6%	29 separate LLNA studies where isoeugenol was used as a positive control	Basketter and Cadby (2004)
5% in olive oil	5% in olive oil	EC3: 12.7%	Non-radioisotopic LLNA	Takeyoshi et al. (2008)

* *This overview table is cited from HERA 2005. Not all the references are public available. The information on positive and negative controls are given below when the individual studies are public available.*

The sensitizing potential of isoeugenol was evaluated in mice and guinea pigs (Hilton J. et al., 1996). From the negative results from mouse IgE test it is concluded that isoeugenol has no significant potential to cause sensitization of the respiratory tract. The mouse IgE test seeks to identify chemical respiratory allergies by their ability to induce increases in serum concentration of IgE. The local lymph node assay response provoked by isoeugenol was substantially greater than that observed with the same concentrations of eugenol. Under the assay conditions employed, isoeugenol was also found to exhibit greater activity in the guinea pig maximization test. A 100% response rate was recorded with isoeugenol and a 30% response rate with eugenol (Table 17). No dermal responses were observed in guinea pigs that had received vehicle alone and were subsequently challenged with eugenol or isoeugenol.

Table 17 Assessment of the contact sensitization potential of eugenol and isoeugenol using the guinea pig maximization test (Hilton et al., 1996)

Test substance	Intradermal induction	Induction patch	Challenge patch	Response[a] rate
Eugenol	0.1% in dobs/saline[b]	100%	25% in acetone/PEG	30% (0.81)
Isoeugenol	0.15% in dobs/saline	25% in acetone/PEG[c]	5% in acetone/PEG	100% (1.5)

[a] *Response rate is expressed as a percentage of test animals judged sensitized . The mean erythema score from positive animals is shown in parentheses.*
[b] *0.01% Dodecyloxybenzene sulphate in 0 .9% sodium chloride.*
[c] *70:30 Acetone :polyethylene glycol 400*

In the study of Loveless and co-workers (1996), sensitizing potential of seven test materials including isoeugenol was evaluated in the LLNA-test performed by five independent laboratories. In each laboratory all skin sensitizing chemicals examined elicited positive responses of comparable magnitude as judged by the derived lowest concentration of test chemical required to elicit a 3-fold or greater increase in the proliferative activity of draining lymph node cells compared with vehicle-treated controls. The results of isoeugenol are summarized below (Table 18).

Table 18 Comparison of results on isoeugenol from five laboratories including statistical analysis of lymph nodes from individual mice

Exposure concentration (%)	Lab A		Lab B		Lab C		Lab D		Lab E	
	dpm	SI	dpm	SI	dpm	SI	dpm	SI	dpm	SI
AOO	501		441		251±22		313±57		43±12	
0.25	741	1.5	458	1.0	729±105	2.9	228±39	0.7	53±11	1.2
0.50	1111	2.2	588	1.3	435±112	1.7	230±37	0.7	74±77	1.7
1.00	1270	2.5	921	2.1	584±40	2.3	272±10	0.9	112±16	2.6
2.50	2437	4.9	1033	2.3	953±145	3.8	649±113	2.1	184±35	4.3
5.00	5050	10.0	1794	4.1	1718±259	6.8	2242±487	7.2	479±96	11.0

To have insights into the mode of action of isoeugenol, Bertrand et al. (1997) have synthesized a series of modified isoeugenol which were tested in the mouse LLNA for their skin sensitizing potential. All isoeugenol derivatives fulfil the criteria for a chemical to be classified as a sensitizer in the LLNA. The sensitization potential of isoeugenol in the mouse was not substantially affected (Table 19) when the methoxy group was replaced by the isopropoxy group (2-Isopropoxy-4-propenylphenol). Methyl substitution in the 6-position of isoeugenol (6-Methylisoeugenol) had no discernible effect on the sensitization potential, whereas methyl substitution in the 3- and 5-positions of isoeugenol (3-Methylisoeugenol and 5-Methylisoeugenol, respectively) led to a reduction in sensitization potential. Introduction of a tert-butyl substituent at the ϒ-position of the alkyl chain (9,9,9-Trimethylisoeugenol) resulted in a strong decrease of the sensitizing capacity. The results indicated that isoeugenol act via a mechanism not involving demethylation.

Table 19 Cell proliferation induced by isoeugenol and derivatives in the LLNA (Bertrand et al., 1997)

Chemical	Concentration (w/w %)	Stimulation Index (SI)
Isoeugenol	Control	-
	2.5 (0.15 M)	8.5
	5 (0.30 M)	12.1
	10 (0.61 M)	16.5
6-Methylisoeugenol	Control	-
	2.5 (0.14 M)	5.9
	5.5 (0.31 M)	11.1
	11 (0.62 M)	15.7
5-Methylisoeugenol	Control	-
	2.5 (0.14 M)	5.4
	5.5 (0.31 M)	5.2
	11 (0.62 M)	7.0
3-Methylisoeugenol	Control	-
	2.5 (0.14 M)	2.2
	5.5 (0.31 M)	4.3
	11 (0.62 M)	6.0
2-Isopropoxy-4-propenylphenol	Control	-
	0.6(31 mM)	3.0
	1.2 (62 mM)	5.7
	3 (0.16 M)	11.1/10.7
	6 (0.31 M)	11.6
	12 (0.62 M)	11.9
9,9,9-Trimethylisoeugenol	Control	-
	6.3 (0.30 M)	3.2
	12.6 (0.61 M)	4.7
	31.4 (1.52 M)	8.0

In another study (Dearman et al., 1999) groups of mice (n = 14) received 25 µl of two application concentrations (1.3% and 5%) of isoeugenol in AOO vehicle, or an equal volume of AOO, on the dorsum of both ears daily for three consecutive days. Five days following the initiation of treatment, mice (10 per group) were terminated, draining auricular lymph nodes were excised, a single-cell suspension of lymph node cells were prepared aseptically and viable cell yield was determined by trypan blue exclusion. Lymph node cellularity for each treatment group is expressed as total lymph node cell count per lymph node. The remainder of the animals (four per group) were injected intravenously with 250 µl of PBS containing 20 µCi of tritiated thymidine [H]TdR. Draining auricular lymph nodes were excised 5 h later, a single-cell suspension was prepared and [^{3}H]TdR incorporation was measured by β-scintillation counting. The results showed that isoeugenol gave positive response in LLNA (Table 20).

Table 20 Influence of exposure to isoeugenol upon lymph node cellularity and incorporation of tritiated thymidine (dpm node-1 and stimulation index, SI) (Dearman et al., 1999)

Exposure	Cellularity (x10^{7} cells node $^{-1}$)	dpm node^{-1}	SI
AOO	0.36	300	-
1.3% isoeugenol (EC3)	0.55	1249	4.16
5% isoeugenol	0.88	3137	10.46

To compare different statistical approaches to derive EC3 values from LLNA dose responses, ten chemicals including isoeugenol were examined for their sensitization potentials (Basketter et al., 1999). The activity of isoeugenol in LLNAs is displayed in Table 21. Included are details of the test concentrations, the vigour of LNC proliferative responses as judged by [^{3}H]TdR incorporation (dpm node)$^{-1}$, the derived stimulation indices, and the EC3 values derived by each of the three statistical

approaches. It was found that in most instances, the derived EC3 values obtained using each of the three statistical approaches were very similar.

Table 21 LLNA data for isoeugenol and EC3 values derived using different methods of statistical analysis (Basketter et al., 1999)

Chemical	Concentration (%, w/v)	dpm node^{-1}	SI	EC3 value		
				Linear EC3	Quadratic EC3	Richard's EC3
Isoeugenol	0	441	1			
	0.25	458	1.0			
	0.5	588	1.3	3.3	3.5	3.8
	1.0	921	2.1			
	2.5	1033	2.3			
	5.0	1794	4.1			

In a study of Wright and co-workers the effects of vehicle on skin sensitizing potency of four chemicals including isoeugenol were assessed using LLNA method (Wright et al. 2001b). The four chemicals were applied in each of seven different vehicles (acetone: olive oil [4:1]; dimethylsulphoxide; methylethylketone; dimethyl formamide; propylene glycol; and both 50:50 and 90:10 mixtures of ethanol and water). It was found that the vehicle in which a chemical is presented to the epidermis can have a marked effect on sensitizing activity. EC3 values ranged from 0.9 to 4.9% for isoeugenol (Table 22).

Table 22 LLNA data for isoeugenol (Wright et al., 2001b)

Venicle /conc. (%)	AOO		MEK		DMF		PG		DMSO		EthOH/ddw (90:10)		EthOH/ddw (50:50)	
	dpm node^{-1}	SI	dpm node^{-1}	SI	dpm node^{-1}	SI	dpm node^{-1}	SI	dpm node^{-1}	SI	dpm node^{-1}	SI	dpm node^{-1}	SI
0	307	1	360	1	281	1	260	1	279	1	324	1	295	1
0.5	552	1.8	322	0.9	736	2.6	216	0.8	518	1.9	594	1.8	293	1.0
1.0	898	2.9	1149	3.2	765	2.7	418	1.6	894	3.2	652	2.0	377	1.2
2.5	2364	7.7	1785	5.0	1046	3.7	784	3.0	2062	7.4	1235	3.8	586	2.0
5.0	3389	11.1	1768	4.9	2101	7.5	1369	5.3	5549	20.0	1890	5.8	896	3.0
10.0	3598	11.7	2926	8.1	3315	11.8	2201	8.5	4780	17.1	4065	12.6	1606	5.4

In the study of Basketter and Cadby (2004), a considerable body of data has been accumulated which demonstrates that the local lymph node assay (LLNA) can provide a valuable estimation of the contact allergenic potency of a substance. This estimate is obtained via interpolation of the LLNA dose-response curve and is expressed as the concentration of the chemical required to evince a 3-fold stimulation of proliferation in lymph nodes draining the site of application compared to the vehicle-treated controls (EC3). In the study isoeugenol gave EC3 values ranging from 0.5 to 2.6% (n = 29), with a mean and standard deviation of 1.2 ± 0.6%. Given that EC3 values for a variety of contact allergens range over several orders of magnitude, these results further endorse the utility of EC3 values as a reliable indicator of human contact allergenic potency.

4.6.1.2 Human information

In human volunteers, the Human Maximization Test (HMT) (Kligman, 1966) and Human Repeat Patch Test (HRIP Test) (Table 23) have been extensively used.

Table 23 HMT and HRIP tests on isoeugenol (cited from HERA 2005)

Test	Induction conditions	Challenge conditions	Results	Comments	Reference
Human Maximization Test)	10% in petrolatum	10% in petrolatum	19/25		RIFM (1979c)
	8% in petrolatum	8% in petrolatum	0/25		RIFM (1971)
	8% in petrolatum	8% in petrolatum	20/24 (in Japanese-Americans)		RIFM (1979c)
	8% in petrolatum	8% in petrolatum	8/29		RIFM (1979e)
	8% in petrolatum	8% in petrolatum	5/29		RIFM (1980d)
	8% in petrolatum	8% in petrolatum	10/32		RIFM (1980d)
	8% in petrolatum	8% in petrolatum	0/25		RIFM (1980d)
	8% in petrolatum	8% in petrolatum	21/33		RIFM (1980d)
	8% in petrolatum	8% in petrolatum	7/25		RIFM (1980d)
	8% in petrolatum	8% in petrolatum	5/29		RIFM (1980d)
	8% in petrolatum	8% in petrolatum	4/28		RIFM (1980d)
	8% in petrolatum	8% in petrolatum	Only irritant Reactions in 25		RIFM (1980d)
	8% in petrolatum	8% in petrolatum	4/27		RIFM (1980d)
	8% in petrolatum	8% in petrolatum	3/21*		RIFM (1980d)
	8% in petrolatum (with 8% eugenol)	8% in petrolatum (with 8% eugenol)	10/22		RIFM (1980d)
	8% in petrolatum with 8% dipropylene glycol	8% in petrolatum with 8% dipropylene glycol	8/35		RIFM (1980d)
	8% in petrolatum with 8% limonene	8% in petrolatum with 8% limonene	9/25		RIFM (1980d)
	1% in petrolatum with 20% fragrance compound	1% in petrolatum with 20% fragrance compound	0/25		RIFM (1980d)
	0.6% in petrolatum with 20% fragrance compound	0.6% in petrolatum with 20% fragrance compound	Only irritant Reactions in 30		RIFM (1980d)
	1.8% in petrolatum with 20% fragrance compound	1.8% in petrolatum with 20% fragrance compound	1/29		RIFM (1980d)
	0.6% in petrolatum with 20% fragrance compound	8% in petrolatum with 20% fragrance compound	4/35		RIFM (1980d)

	1.8% in petrolatum (contains 20% fragrance compound)	8% in petrolatum	4/34		RIFM (1980d)
	1% in petrolatum	1% in petrolatum	6/7		Kligman and Gollhausen (1986)
	8% in petrolatum (90% cis-isoeugenol)	8% in petrolatum (90% cis-isoeugenol)	21/31		RIFM (1980d)
	5% in hydrophilic ointment	1% in hydrophilic ointment	5/25		RIFM (1979e)
Human Repeat Patch Test (HRIP Test)	1.25% in 95% ethanol (970 µg/cm^2) Nine 24 hour semi-occluded patches	1.25% in 95% ethanol	2/40	11 male & 29 female volunteers Re-chllenge at 5 months gave 1/40	RIFM (1964)
	1.25% in 95% ethanol (970 µg/cm^2) Nine 24 hour semi-occluded patches	1.25% in 95% ethanol	0/41	7 male & 34 female volunteers	RIFM (1964)
	1% in SDA ethanol (800 µg/cm^2) Nine 24 hour semi-occluded patches	1% in SDA ethanol	2/38	10 male & 28 female volunteers	RIFM (1973)
	0.5% in SDA ethanol (260 µg/cm^2) Nine 24 hour semi-occluded patches	0.5% in SDA ethanol	2/53	Re-chllenge after 2 weeks gave no reactions	RIFM (1980b)
	10% in petrolatum (11,800 µg/cm^2) Nine 48 hour occluded patches	10% in petrolatum	16/25	7 male & 18 female volunteers	RIFM (1979d)
	5% in SDA ethanol (5,900 µg/cm^2) for first 3 weeks. Therefore after 2.5% (semi-occlusive) (2,950 µg/cm^2) Nine 24 hour occluded patches	2.5% in SDA ethanol	3/49	Irritation with 5% isoeugenol under occlusion gave irritant reactions. Induction changed to 2.5% semi-occlusion	RIFM (1987b)
	1.25% in unknown vehicle	1.25% in unknown vehicle	1/81	Details not provided	Thompson et al. (1983)
	1% in unknown vehicle	1% in unknown vehicle	1/38	Details not provided	Thompson et al. (1983)
	0.5% in unknown vehicle	0.5% in unknown vehicle	0/56	Details not provided	Thompson et al. (1983)
	8% in ethanol (2,500 µg/cm^2) Ten 48-72 hour occluded patches	8% in ethanol	9/73	Severe induction conditions	Marzulli and Maibach (1980)
	32 µg/cm^2 in petrolatum		ED50%	Estimated	Johansen et al. (1996)

	< 0.4 µg/cm^2 in petrolatum	No effect	Observed	Johansen et al. (1996)
	< 0.0005% (0.15 µg/cm^2)	No effect	Observed	Andersen et al. (2001)
Repeated Open Application Test (ROAT test)	5.6 µg/cm^2 in ethanol	63% positive	Observed	Johansen et al. (1996)
	2.2 µg/cm^2 in ethanol	42% positive	Observed	Andersen et al. (2001)
	9.0 µg/cm^2 in ethanol	67% positive	Observed	Andersen et al. (2001)
	0.167 µg/cm^2 in deodorant matrix	23% positive	Observed	Bruze et al. (2005)
	0.53 µg/cm^2 in deodorant matrix	69% positive	Observed	Bruze et al. (2005)
	1.67 µg/cm^2 in deodorant matrix	77% positive	Observed	Bruze et al. (2005)

* although in HERA (2005) the results are presented as 21/3, it is assumed this is a typographical error and that this should be 3/21. The original study report of RIFM (1980d) is not available.

Thompson and co-workers (1983) evaluated the potential of isoeugenol to induce delayed contact hyper-sensitivity or to elicit pre-existing sensitization reactions in humans by analysing patch-test data from dermatitis and non-dermatitis subjects. Results from a total of 6512 patch tests (involving approximately 5850 subjects) on isoeugenol alone and on various consumer products and fragrance blends containing isoeugenol, were collected from fragrance and formulator companies (Table 24). One induced reaction in 32 patch tests was attributable to isoeugenol at a concentration of 0.02% while another induced reaction in 23 patch tests was attributable to the same concentration of isoeugenol though being dissolved in an isoeugenol-eugenol mixture. One elicited reaction at an isoeugenol concentration of 0.04% occurred in the 6512 patch tests was reported in this survey. This single elicitation was related to an isoeugenol-eugenol mixture, but the specific causative agent was not identified.

Table 24 Human sensitization survey: isoeugenol in consumer products and in fragrance blends (Thompson et al., 1983)

Product type	**Isoeugenol concentration In patch test mixture**	**No. of tests**	**No of sensitization reactions**	
			Elicited	**Induced**
Personal care	0.02-0.05%	504	0	0
	0.02%	32	0	1
	0.000009-0.009%	2307	0	0
Household	0.02%	23	0	1#
	0.0000003-0.0001%	612	0	0
Fragrance	0.8%	56	0	1
	0.05-0.1%	360	0	0
	0.05%	20	0	0

0.04%	50	0	0
0.04%	83	1*	0
0.01-0.03%	840	0	0
0.01%	51	0	0
0.00006-0.008%	1399	0	0

Related to a 2:5 isoeugenol-eugenol mixture.

* *Related to a 4:9 isoeugenol-eugenol mixture.*

Kligman and Gollhausen (1986) collected a panel of 7 volunteers whom they had sensitized to isoeugenol by the maximization procedure. These persons reacted on the arms in varying intensity to 48 h exposures. First, isoeugenol was applied at 1% contractions in petrolatum on opposite arms for 48 h. Two days later the exposures were repeated on the same arm, separated by a distance of 2 cm. The results showed that the reactions were the same whether isoeugenol was in close proximity or on opposite arms.

The clinical implications of sensitization to isoeugenol were studied in 19 subjects using patch testing and a Repeated Open Application Test (ROAT) (Johansen et al. 1996). In patch test with isoeugenol in petrolatum 4/19 (20%) of the test subjects had a threshold response at 0.01% or lower. The ROAT was performed with a test solution of 0.2% isoeugenol in ethanol with maximum exposure period of 4 weeks. The upper arm was used as test site for the first 14 days and the upper arm as well as the neck for the next 14 days. The results showed that 12/19 (63%) of test subjects had a positive ROAT. Of the responders, 4 out of 12 (33%) reacted beyond day 7, but none after day 14. Use testing on the neck for 14 days did not add any further ROAT-positive cases, compared with testing on the upper arm.

In the study of Andersen *et al*. (2001) 27 isoeugenol-sensitive patients participated in serial dilution patch tests with isoeugenol and a double-blinded ROAT using two concentrations of isoeugenol, 0.2 and 0.05%. Seven controls without isoeugenol allergy were also included. The participants applied 3.72 ± 1.57 (mean ± SD) mg/cm^2 of coded isoeugenol solutions twice a day to a 3 x 3 cm^2 area on the volar aspect of the right and left arm, respectively. For each test site the applications continued until a reaction appeared or for a maximum of 28 days. The minimal criteria for a positive reaction regarded as allergic contact dermatitis was persistent erythema at the ROAT test site. All controls were negative and 16/24 (66.7%) of the included isoeugenol-sensitive subjects showed a positive ROAT to the 0.2% solution within the study period (Fisher's test, p=0.0024). Ten of the positive patients also reacted to the 0.2% solution after 7 days and after 15 days for the 0.05% solution. There was a highly significant correlation between the patients' patch test threshold and the number of days until a positive ROAT. In conclusion, the time until an isoeugenol allergic individual reacts in a ROAT depends on the individual sensitivity as well as the exposure concentrations; for low concentrations of the allergen or low degree of sensitivity, the allergic contact dermatitis may develop after several weeks of exposure. Therefore, a negative ROAT after 7 days may be a false negative.

In order to investigate the significance of isoeugenol in deodorants for the development of axillary dermatitis when used by people with and without contact allergy to isoeugenol, patch tests with deodorants and ethanol solutions with isoeugenol, as well as repeated open application tests (ROAT) with roll-on deodorants with and without isoeugenol at various concentrations, were performed in 35 dermatitis patients, 10 without and 25 with contact allergy to isoeugenol (Bruze *et*

al. 2005). A positive ROAT was observed only in patients hypersensitive to isoeugenol (P<0.001) and only in the axilla to which the deodorants containing isoeugenol had been applied (P<0.001) (Table 25). Deodorants containing isoeugenol in the concentration range of 0.0063–0.2% used 2 times daily on healthy skin can thus elicit axillary dermatitis within a few weeks in people with contact allergy to isoeugenol.

Table 25 Data on sex and ages of the 13 test (patients' no. 1-13) and 10 control patients (patients' no. 14-23) and average dose of deodorant used for each application, as well as the results of the patch tests and repeated open application tests (ROAT) (Bruze et al., 2005)

		Patch test				ROAT						
patient no.	**Sex**	**Iso in ethanol**	**perfumed deodorant**	**unperfumed deodorant**	**ethanol**	**Low#**		**Medium#**		**High#**		**Deodorant used in mg/applications**
						perfumed	**unperfumed**	**perfumed**	**unperfumed**	**perfumed**	**unperfumed**	
1	F	0.125*	0.2*	-	-	-	-	-	-	1+	-	228
2	F	0.002	0.02	-	-	1	-	Not tested	Not tested	Not tested	Not tested	167
3	F	0.0005	0.063	-	-	1.5	-	Not tested	Not tested	Not tested	Not tested	219
4	F	0.002	0.2	-	-	-	-	1	-	Not tested	Not tested	188
5	F	0.008	0.2	-	-	-	-	1	-	Not tested	Not tested	212
6	F	2.0	-	-	-	-	-	-	-	-	-	476
7	F	1.0	-	-	-	-	-	-	-	-	-	364
8	F	0.125	-	-	-	1	-	Not tested	Not tested	Not tested	Not tested	348
9	F	1.0	-	-	-	-	-	1.5	-	Not tested	Not tested	189
10	M	0.004	0.063	-	-	-	-	2	-	Not tested	Not tested	293
11	F	0.008	0.2	-	-	-	-	1	-	Not tested	Not tested	217
12	F	0.25	0.2	-	-	-	-	1	-	Not tested	Not tested	203
13	F	0.063	0.2	-	-	-	-	-	-	-	-	353
14-23	M F	-	-	-	-	-	-	-	-	-	-	117-586

Iso: isoeugenol
- : negative test reaction
** Lowest concentration (w/v) of test solution eliciting a positive test reaction.*
 # Set of perfumed and unperfumed deodorants with isoeugenol at the concentrations 0.0063% w/v (low), 0.02% (medium) and 0.063% (high).
+ Time in week when ROAT became positive e.g. patient no. 1 did not react to any deodorant during the first 4 weeks, but after application of the deodorant with isoeugenol at 0.063% for 1 week, a positive ROAT was observed.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOEUGENOL; [1]; (E)-2-METHOXY-4-(PROP-1-ENYL)PHENOL; [2]; (Z)-2-METHOXY-4-(PROP-1-ENYL)PHENOL; [3];

There are many published reports of studies in which isoeugenol produces positive reactions in "Fragrance Mix-sensitive", "perfume-sensitive" and "cosmetic-sensitive" patients in routine diagnostic patch testing (Table 26, 27 and 28, respectively).

Table 26 Clinical patch testing of isoeugenol in "Fragrance Mix-sensitive" patients

Patch test conditions	Number tested	Number reacting	Scores	Comments	References
No dose reported 24 hrs occlusion Finn Chambers®	160	24	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Temesvari et al. (2002)
No dose reported	32	9	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Sieben et al. (2001)
1% in petrolatum 48 hrs occlusion	226	45	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Brites et al. (2000)
1% in petrolatum 48 hrs occlusion over 15 years	1112	231	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Buckley et al. (2000b)
1% in petrolatum Finn Chambers® or Scanport®, 48 hrs occlusion	40	8	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Katsarma and Gawkrodger (1999)
1% in petrolatum Finn Chambers® or Scanport®, 48 hrs occlusion	38	9	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Katsarou et al. (1999)
Different concentrations (serial dilution study on isoeugenol - sensitive patients who had	19	18	Different scores recorded for different patients	Patients probably reacted to other test materials in the same study.	Johansen et al. (1996d)

previously reacted to Fragrance-Mix)					
1% or 2% in petrolatum 48 hrs occlusion in Finn Chambers® or Scanport®, tape	367	68	+ to +++ reactions	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Johansen and Menne (1995)
No conditions given	50	3	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Becker et al. (1994)
1%, 3% and 5% in petrolatum (serial dilutions)	6	1	Not given	Patients probably reacted to other test materials in the same study.	De Groot et al. (1993)
2% in petrolatum 48 hrs occlusion in Finn Chambers®	20	4	Not given	Patients probably reacted to other test materials in the same study.	Safford et al. (1990)
1% in petrolatum	162	27	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Enders et al. (1989)
1% in petrolatum 48 hrs occlusion in Finn Chambers® or Scanpore®	54	12	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Santucci et al. (1987)
Not given	42	19	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Rudzki and Grzywa (1986)
1% in petrolatum	144	6	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Angelini et al. (1985)
Not reported	80	7	Not given	Not a primary study. Review of several studies	Romaguera et al. (1983)

				or multicentre study. Patients probably reacted to other test materials in the same study.	
2% in Petrolatum	172	48	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Calanan et al. (1980)
Not given	50	8	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Bordalo et al. (2000)
1% in petrolatum 48 hrs patch tests	4900 consecutive patients	173	51 gave + reactions to 1% isoeugenol and to 8% Fragrance-Mix. 60 gave + reactions to 1% isoeugenol but ++ or +++ reactions to 8% Fragrance -Mix. 56 gave ++ or +++ reactions to both the Fragrance-Mix and Isoeugenol 6 gave ++ or +++ reactions to isoeugenol but only + reactions to Fragrance-Mix.	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Schnuch et al. (2002)
5% isoeugenol in petrolatum	520	15	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Ohela and Saramies (1983)

Table 27 Clinical patch testing of isoeugenol in “perfume-sensitive” patients as well as patients reacting to other fragrance ingredients

Patch test conditions	**Number tested**	**Number reacting to isoeugenol**	**Scores**	**Comments**	**Refences**

1% in Petrolatum 48 hours occlusion	747 "Perfume-sensitive" patients	40	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Wohrl et al. (2001)
4% in petrolatum 48 hrs occlustion using Finn Chambers or Scanpore	167 "Perfume-sensitive" patients	23	Irritant reactions in 6 allergic reactions in 23	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Larsen et al. (1996)
2% in petrolatum	8 "Perfume-sensitive" patients	0	-	-	Safford et al. (1990)
2.5% in petrolatum	21 "Perfume-sensitive" patients	7	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Meynadier et al. (1986)
2% and 5% in petrolatum	21 "Perfume-sensitive" patients	5	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Larsen (1997)
1% in petrolatum 48 hrs occlusion in Finn Chambers	1072 "Perfume-sensitive" patients	30	20++ to +++ 10+ or?	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Frosch et al. (1995a)
Not reported	97 "Perfumery plant workers with occupational eczema"	0	-	-	Gutman and Somov (1968)
1% in petrolatum	367 "Perfume sensitive"	15	9++ to +++ 4+ and 2 doubtful	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Ruhnek et al. (1989)
2% in petrolatum 24 hrs occusion using Finn	102 "Peru-balsam sensitive" patients	28	7+, 11++ and 10+++	Not a primary study. Review of several studies or multicentre study.	Hausen (2001)

Chambers or Scanpore				Patients probably reacted to other test materials in the same study.	
5% in petrolatum	1 “Peru-balsam sensitive” patients	1	Not given	Patients probably reacted to other test materials in the same study.	Bruynzeel et al. (1984)
5% in petrolatum 48 hrs occlusion in Lysaplast patches	74 “Peru-balsam sensitive” patients	45	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Hjorth (1961c)
2% in petrolatum 48 hrs occlusion in Lysaplast patches	55 “Peru-balsam sensitive” patients	33	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Hjorth (1961c)
0.5% in petrolatum 48 hrs occlusion in Lysaplast patches	22 “Peru-balsam sensitive” patients	20	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Hjorth (1961c)
2% in petrolatum 48 hrs occlusion in Lysaplast patches	17 “Peru-balsam sensitive” patients	6	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Hjorth (1961b)
5% in petrolatum 48 hrs occlusion in Lysaplast patches	28 “Peru-balsam and vanillin-sensitive” patients	25	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Hjorth (1961a)
5% in petrolatum 48 hrs occlusion in Lysaplast patches	32 “Peru-balsam and vanillin-sensitive” patients	15	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Hjorth (1961a)
8% in petrolatum	242 patients sensitive to Peru-balsam, wood tar, eugenol and coumarin	36	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the	Van Joost et al. (1984)

				same study.	
Not reported	31 “Oak moss-sensitive”patients	9	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Goncalo et al. (1988)
Not reported	6 “Lichen-sensitive”patients	2	Not given	Patients probably reacted to other test materials in the same study.	Stinchi et al. (1997)
Not reported	16 “Musk ambrette phto-sensitive” patients	3	Not given	Patients probably reacted to other test materials in the same study.	Wojnarowska and Calnan (1986)
Not reported	3 “Musk ambrette phto-sensitive” patients	1	Not given	Patients probably reacted to other test materials in the same study.	Ducombs et al. (1986)
2% in petrolatum	5 “Wood tar sensitive” patients in 667 patients	5	Not given	Patients probably reacted to other test materials in the same study.	Van Joost et al. (1984)
1% in petrolatum 48 hrs occlusion in Finn Chambers or Scanpore	2261 consecutive dermatitis patients	40	Not given		Tanaka et al. (2004)
	Concomittent reactions in 40 patients sensitive to trans-isoeugenol	36			
	19 patients sensitive to isoeugenyl acetate	13			
	4 patients sensitive to isoeugenyl benzoate	3			
	16 patients sensitive to isoeugenyl phenyl acetate	15			
	4 patients sensitive to isoeugenyl methyl ether	0			
	2 patients sensitive to isoeugenyl	0			

Table 28 Clinical patch testing of isoeugenol in "cosmetic-sensitive" and other dermatitis patients

Patch test conditions	Number tested	Number reacting to isoeugenol	Scores	Comments	References
2% in petrolatum with + 1% sorbitan sesquioleate	757 "cosmetic sensitive" patients	16	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Hendriks and van Ginkel (1999)
5% in petrolatum	64 "cosmetic sensitive" patients	4	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Haba et al. (1993)
No dose reported 48 hrs occlusion	462 "Cosmetic-sensitive" patients	33	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Dooms-Goossens et al. (1992)
2% in petrolatum 48 hrs occlusion in Finn Chambers or Scanpore	115 "Cosmetic-sensitive" patients	5	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Remaut (1992)
5% (vehicle and patches not reported)	310 "Cosmetic-sensitive" patients	13	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Itoh et al. (1986) Itoh et al. (1988)
Not reported	258 "Cosmetic-sensitive" patients	22	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the	Asoh and Sugai (1986) Asoh and Sugai (1987)

				same study. Abstract only in English.	
Not reported	156 “Cosmetic-sensitive” patients	16	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Broeckx et al. (1987)
1% in petrolatum 48 hrs occlusion in closed patch tests	117 “Cosmetic-sensitive” patients	7	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Hayakawa and Japan Patch Test Research Group (1986)
3% in petrolatum 48hrs occlusion in van der Bend Chmbers	119 “Cosmetic-sensitive” patients	2	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	De Groot et al. (1988)
Dose not reported Finn Chambers or Scanpore	122 “Cosmetic-sensitive” patients	4	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Asoh and Sugai (1985)
Dose not reported Finn Chambers or A1-test patches 48 hrs occlusion	399 “Cosmetic-sensitive” patients	10	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Adams and Maibach (1985)
4% in petrolatum 48 hrs occlusion in Finn Chambers or Scanpore	16 “Cosmetic-sensitive” patients	0	-	Not a primary study. Review of several studies or multicentre study.	Emmons and Marks Jr. (1985)
8% in petrolatum 48 hrs occlusion under Sliver patches	179 “Cosmetic-sensitive” patients	36	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	De Groot et al. (1985)

5% (vehicle and conditions not reported)	155 "Cosmetic-sensitive" patients	8	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Ishihara et al. (1981)
1 – 5 % in petrolatum	133 "Cosmetic-sensitive" patients	3	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Ishihara et al. (1979)
Dose not reported 48 hrs occusion	70 "Cosmetic-sensitive" patients	2	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Schorr (1974)
5% (vehicle and conditions not reported)	212 "Cosmetic-sensitive" patients	9	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Nishimura et al. (1984)
Dose vehicle not reported A-1 test strips or Finn Chambers for 48 hrs	149 Dermatitis patients	10	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Eiermann et al. (1982)
5%	159 Dermatitis patients	11	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Ishihara et al. (1981)
5% in petrolatum 48 hrs occlusion	155 Dermatitis patients	8	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the	Itoh (1982)

				same study. Abstract only in English.	
1% in petrolatum in Finn Chambers or Scanpore	22 Dermatitis patients	3	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Nagareda et al. (1992)
1% in petrolatum 48 hrs occlusion	117 Dermatitis patients	7	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Hayakawa and Japan Patch test Research Group (1986)
1% in petrolatum	155 consecutive dermatitis patients	8	3 questionable reactions also observed		White et al. (1999)
Not reported	19546 consecutive dermatitis patients	39	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Angelini et al. (1997)
Dose not reported 48 hrs occlusion	83 children	Some reactions	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Shah et al. (1997)
Dose not reported 48 hrs occlusion	95 children	2	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Stables et al. (1996)
Dose not reported 48 hrs occlusion	63 consecutive dermatitis patients	1	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Shah et al. (1996)

1% in petrolatum 48 hrs occlusion in Finn Chambers or Scanpore	702 consecutive dermatitis patients	17	6 irritant reactions also observed 6 additional reactions observed when 1% sorbitan sesquioleate added to patch test vehicle	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Frosch et al. (1995b)
5% in petrolatum	677 consecutive dermatitis patients	15	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	De Groot et al. (1993)
1% in petrolatum 48 hrs occlusion using Finn Chambers or Scanpore	106 consecutive dermatitis patients	2	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Hashimoto et al. (1990)
Not reported	50 consequtive dermatitis patients	15	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Miranda et al. (1990)
5% in petrolatum 24 or 48 hrs occlusion in Finn Chambers	1967 consecutive dermatitis patients	90	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Malanin and Ohela (1989)
4% in petrolatum 48 hrs or 72 hrs occlusion in Finn Chambers or Scanpore	1012 consecutive dermatitis patients	24	5 additional questionable reactions	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Storrs et al. (1989)
Not reported ICDRG recommendations	403 consecutive dermatitis patients	1	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to	Macfarlane et al. (1989)

followed				other test materials in the same study.	
Not reported ICDRG recommendations followed	125 children with dermatitis	4	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Rademaker and Forsyth (1989)
5% in petrolatum 48- hrs or 72- hrs occlusion A1-test strips or Finn Chambers or Scanpore	89 consecutive dermatitis patients including 19 with eyelid dermatitis	4	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Nethercott et al. (1989)
5% (vehicle and conditions not reported) in Finn Chambers®	520 Dermatitis patients	15	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Ohela and Saramies (1983)
1% in Petrolatum 48 hrs occlusion in Finn Chambers® or Scanpore®	884 Dermatitis patients	78	+ to +++ reactions	Not a primary study. Review of several studies or multi-centre study. Patients probably reacted to other test materials in the same study.	(Johansen et al., 1997)
1% in petrolatum 48 hrs occlusion in Finn Chambers or Scanpore	335 Dermatitis patients	27	+ to +++ reactions		Johansen et al., (1996b)
1% in petrolatum 48 hrs occlusion in Finn Chambers or Scanpore	1072 Dermatitis patients	20	+ to +++ reactions with an additional 10 questionable reactions	Not a primary study. Review of several studies or multi-centre study. Patients probably reacted to other test materials in the same study.	Frosch et al. (1995a)
Conditions not specified	5315 Dermatitis patients	299	Not given	Not a primary study. Review of several studies or multi-centre study. Patients probably reacted to other test materials in the same study.	Rudzki and Grzywa (1986)
5% in petrolatum 24 hrs occlusion	82 Dermatitis patients	2	Not given	Not a primary study. Review of several studies or multi-centre study.	Ishihara (1977) Ishihara (1978)

				Patients probably reacted to other test materials in the same study. Abstract only in English.	
2% (vehicle not reported) A1-test and Dermicel 48 hrs occlusion	273 consecutive dermatitis patients	14	Not given	Not a primary study. Review of several studies or multi-centre study. Patients probably reacted to other test materials in the same study.	Rudner (1977) Rudner (1978)
2% in petrolatum	1836 2461	31 48	Not given	Not a primary study. Review of several studies or multi-centre study. Patients probably reacted to other test materials in the same study.	Cronin (1985)
2% in paraffin in Finn Chambers	241 consecutive dermatitis patients	13	Not given	Not a primary study. Review of several studies or multi-centre study. Patients probably reacted to other test materials in the same study.	Ferguson and Sharma (1984)
2% (vehicle not reported) 48 hrs occlusion	25 dermatitis patients	2	Not given	Not a primary study. Review of several studies or multi-centre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Asoh et al. (1985)
5% in petrolatum 2% in petrolatum 1% in petrolatum 48 hrs occlusion in A1-patches or Torii-ban patches or Finn Chambers	357 357 357 Patients with facial dermatitis	13 11 11	Not given Not given Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Mid-Japan Contact Dermatitis Research Groups (1984)
5% vehicle and conditions not reported	275 non-cosmetic dermatitis patients	17	Not given	Not a primary study. Review of several studies or multi-centre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Nishimura et al. (1984)
Dose not reported Finn Chambers or	152 Dermatitis	9	Not given	Not a primary study. Review of several studies or multi-	Sugai T. et al.

Scanpore	patients			centre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	(1983)
4% in petrolatum 48 hrs occlusion in Finn Chambers or Scan pore	15 Dermatitis patients	0	-	Patients probably reacted to other test materials in the same study.	Emmons and Marks, Jr. (1985)
4% in petrolatum Open application under Scanpore tape	15 Dermatitis patients	0	-	Patients probably reacted to other test materials in the same study.	Emmons and Marks, Jr. (1985)
Dose not reported Finn Chambers or Scanpore	408 consecutive dermatitis patients	24	Not given	Not a primary study. Review of several studies or multi-centre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Itoh et al. (1986) Itoh et al. (1988)
Not reported	120 consecutive dermatitis patients	4	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Goodfield and Saihan (1988)
5% in petrolatum 48 hrs occlusion in Finn Chambers or Scanpore	1200 consecutive dermatitis patients	14	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study.	Santucci et al. (1987)
0.05 – 0.5% in a base cream or in 99% ethanol	54 Dermatitis patients	1	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Takenaka et al. (1986)
2% in paraffin 48 hrs occlusion in A1-test patches or Scanpore	457 consecutive dermatitis patients	8	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the	Addo et al. (1982)

				same study.	
5% (vehicle and patch test conditions not reported	159 consecutive dermatitis patients	11	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Ishihara et al. (1981)
1 – 5% in petrolatum	86 dermatitis patients	4	Not given	Not a primary study. Review of several studies or multicentre study. Patients probably reacted to other test materials in the same study. Abstract only in English.	Ishihara et al. (1979)

4.6.1.3 Summary and discussion of skin sensitisation

Isoeugenol has been chosen for a full risk assessment by HERA (Human and Environmental Risk Assessment on ingredients of household cleaning products) program because of its known skin sensitising properties (HERA, 2005). The assessments results are integrated in this CLH report.

Isoeugenol shows a definite skin sensitization potential in a wide variety of predictive test systems and is classified as a moderate skin sensitizer according to ECETOC standards. The evidences include the positive results obtained in GPMTs (Tsuchiya et al., 1982; Tsuchiya et al., 1985; Maurer and Hess, 1989; Kimber et al., 1991; Basketter and Scholes, 1992; Barratt and Basketter, 1992; Hilton et al., 1996; Takeyoshi et al., 2008), in FCATs (Klecak et al., 1977; Maurer et al., 1979; Tsuchiya et al., 1982; Tsuchiya et al., 1985; RIFM, 1985b), in CCETs (Tsuchiya et al., 1982 and 1985), in Buehler Tests (Kaminsky and Szivos, 1986 and 1990; RIFM, 1986 and 1987a; Goh and Yuen, 1994), in OETs and CETs with guinea pigs (Klecak et al., 1977; Tsuchiya et al, 1982; Tsuchiya et al., 1985; RIFM, 1985b; Ishihara et al., 1986), in MESTs (Thorrne et al., 1991; Garrigue et al., 1994; Yamazaki et al., 1998), as well as in LLNAs (Kimiber et al, 1991; Basketter and Scholes, 1992; Hilton et al., 1996, Bertrand et al., 1997; Dearman et al., 1999; Basketter et al., 1999; Takeyoshi et al., 2008).

Non-adjuvant tests in animals and maximized tests carried out on human subjects offer a sound basis for a "weight of evidence" judgment on what exposure levels are unlikely to induce allergy in naïve individuals during use of household products. The LLNA places this level at around 500 µg/cm^2 (with a some degree of variability) while the HRIP Test places this at around 260 µg/cm^2 on the basis of two tests carried out on a total of 97 subjects. SSCS in its opinion on fragrance allergens in cosmetic products has pointed out that the EC3 value of isoeugenol is 0.54% (M = 0.033), based on a report submitted by RIFM (2009). Studies on animals and humans demonstrate that isoeugenol is a skin sensitiser of moderate allergenic potency. This is substantiated by clinical

data that show possible allergy to isoeugenol. However, very few cases of allergy are clearly attributable to the presence of isoeugenol in any specific consumer products.

A number of experimental *in vitro* techniques provided indications of the positive allergenicity of iseugenol (Dearman et al., 1994; Dearman et al., 1999; Guironnet et al., 2000; Sieben et al., 2001; Verrier et al., 1999a; Verrier et al., 1999b; Verrier et al., 2001). The methods used in these studies have not been validated or related in any quantitative way to studies in animals or humans.

There are many published reports of studies in which isoeugenol produces positive reactions in patients in routine diagnostic patch testing. Although there have been numerous reports of patients giving frank allergic responses to isoeugenol in clinical patch testing on dermatological patients, many of these studies do not establish a clear causal relationship according to currently accepted criteria (Lachapelle, 1997; Lachapelle and Maibach, 2003; Maibach and Hostynek, 2003).
A publication by Hostynek and Maibach (2004) has pointed out that reactions seen in dermatological clinics, while genuinely allergic in nature, may only occur under the severe conditions use in clinical diagnosis and may not relate to adverse effects from the use of consumer products. In a separate publication, the same authors (Hostynek and Maibach, 2003c) have also defined criteria by which possible causality can be assessed. These criteria have been applied by these authors to a number of other proposed allergens (Hostynek and Maibach, 2003b; Hostynek and Maibach, 2003a). The same criteria have been used here to assess the strength of a causal link between the observed clinical reaction and everyday exposure to an isoeugenol-containing product.

Isoeugenol is one of the eight components of the "Fragrance Mix" used by dermatologists to detect possible sensitivity to fragrances. This mix was first proposed (Larsen, 1975; Calnan et al., 1980), on the basis of the components of a fragrance used in a popular Tri-Adcortyl cream (Mycolog®, Squibb Corp.) (Larsen, 1979). It was concluded that the use of this ointment in treating eczematous and ulcerous skin may have contributed significantly to the cases of clinical dermatitis that had been ascribed to this substance (Larsen, 1979). Clinical patch testing of patients who have already shown positive reactions to the "Fragrance Mix" frequently gives positive reactions to isoeugenol although in such cases, it is rare that isoeugenol is the only component of this "Fragrance Mix" to produce positive reactions. In the cases reported in Table 26, no clear causal link could be established with the use of consumer products using the criteria of Hostynek and Maibach (2003c). In a large multi-centre study covering nearly 60,000 patients tested in German clinics from 1996 to 2002 (Schnuch et al., 2004), the frequency of reactions to isoeugenol in patients reacting to the fragrance mix was reported to be about 13%. These patients have frequently reacted to other constituents of the fragrance mix (for instance 47.6% and 56.7% of patients reacting to chemically-dissimilar geraniol and amylcinnamic aldehyde respectively, also reacted to isoeugenol).

It has been reported that while the proportion of patients reacting to the "Fragrance Mix" has been relatively constant over 17 years, there is a 5% yearly increase in the proportion of patients reacting to isoeugenol (Buckley et al., 2000a) having reached an average 16.7% and 15.4% of "Fragrance Mix-sensitive" males and females respectively. However, the full significance of these findings has been questioned (Wesley NO and Maibach, 2003).

A European multicentre study a total of 1072 patients were patch tested in 9 different centres of which 20 out of 1072 patients (1.86%) had a positive reaction to isoeugenol at a concentration of 1% (Frosch P.J. et al., 1995). In another study, 20 perfume allergic patients were tested with several screening series of fragrances. Isoeugenol at a concentration at 2% gave a positive reaction in 5/20 (25%) of the patients (Larsen W.G. 1977). Adams and Maibach (1985) identified causal link

between cutaneous reactions in 713 patients and cosmetic products. In 578 out of 713 cases sensitisation were observed. In 10 out of 713 subjects isoeugenol was found to be one of the causative ingredients as judged by patch testing. In another study in which 156 patients with contact allergy to cosmetic products were identified, isoeugenol was one of the causative ingredients in 16 cases (10.3%), as determined by patch testing (Broneck W. et al., 1987). In a European multicentre study involving 6 countries, 78 patients positive to one of two different fragrance mixes (both containing isoeugenol), were tested with the individual constituents of the mixes. Results showed that 16/78 (20.5%) were positive to 2% isoeugenol (Wilkinson J.D. et al., 1989). Furthermore, the frequency of contact allergy to isoeugenol in patients positive to the fragrance mix, is reported in a range of studies from different countries: 22% of the contact allergy reactions were due to Isoeugenol present in fragrance mix in Italy (Santussi B. et al, 1987), 18.5 % in Denmark (Johansen J.D. and Menné T. 1995), 6% in Hungary (Becker K. et al., 1994), 16.6% in Germany (Enders F. et al., 1989) and 17% in France (Artigou C. et al., 1989). In addition, isoeugenol has been found to cause sensitisation in 12-36% of healthy volunteers (Thompson G.R. et al., 1983; Marzulli F.N. and Maibach H.I., 1980). Isoeugenol was restricted in the IFRA (International Fragrance Association) guideline[1] to 0.2% until May 1998, where the concentration was lowered to 0.02%.

Most studies were performed with isoeugenol without specification of the ratio between the cis and the trans isomer. Also very limited information is available on the skin sensitising potential of the specific isomers. However, the HMT with 8% isoeugenol in petrolatum of which 90% was specified as cis-isoeugenol shows (positive response in 21/31 patients) that the cis-isomer has skin sensitising potential (RIFM, 1980d). The clinical patch test with 1% isoeugenol in petrolatum shows that the trans-isomer has the potential to induce an allergic reaction in sensitised people although a cross-reaction cannot be excluded (Tanaka et al, 2004). In addition there is a clear structural similarity between both isomers as can be expected for isomers. In addition the double bond that differs between the two isomers is not expected to be relevant for the activation before protein binding. Therefore, the results obtained with isoeugenol are considered relevant for the individual isomers and for the racemic mixture.

There is some information available that indicates that the skin sensitisation response might be dependent on the type of vehicle used. In the LLNA study of Wright et al. (2001a/b) isoeugenol was tested using various vehicles, i.e. acetone/olive oil, dimethyl sulphoxide, methyl ethyl ketone, dimethyl formamide, propylene glycol, ethanol/water (50/50) and ethanol/water (90/10). These data show that the vehicle might affect the skin sensitisation response, though this is considered limited (up to a factor of 5). EC3 values ranged from 0.9% to 4.9% for isoeugenol. Further, the CLP-regulation does not provide options to include vehicle-dependency in the classification itself or the setting of SCLs for skin sensitisation. Based on this, no full evaluation of the dependency of the skin sensitisation response on the type of vehicle is included in the discussion and conclusion of this endpoint.

4.6.1.4 Comparison with criteria

In the CLP Regulation, it is stated that substances shall be classified as sensitisers in accordance with the criteria:

[1] http://www.ifraorg.org/en-us/guidelines#.VNDrcmd0xjo

Category	***Criteria***
Category 1	*Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:* *(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or* *(b) if there are positive results from an appropriate animal test*
Sub-category 1A	*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.*
Sub-category 1B	*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.*

Animal test results for sub-category 1A and 1B can include data with values of:

Category	***Assay***	***Criteria***
1A	*Local Lymph Node Assay (LLNA)*	*EC3 value ≤ 2 %*
	Guinea Pig Maximisation Test *(GPMT)*	*≥ 30 % responding at ≤ 0.1 % intradermal induction dose or ≥ 60% responding at > 0.1 % to ≤ 1% intradermal induction dose*
	Buehler Assay	*≥ 15 % responding at ≤ 0.2 % topical induction dose or ≥ 60% responding at > 0.2 % to ≤ 20 % topical induction dose*
1B	*Local Lymph Node Assay (LLNA)*	*EC3 value > 2 %*
	Guinea Pig Maximisation Test *(GPMT)*	*≥ 30 % to < 60% responding at > 0.1 % to ≤ 1 % intradermal induction dose or ≥ 30% responding at > 1 % intradermal induction dose*
	Buehler Assay	*≥ 15 % to < 60% responding at > 0.2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose*

Human evidence for sub-category 1A can include:

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 in relation to relatively low exposure.*

Human evidence for sub-category 1B can include:

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 in relation to relatively high exposure.*

The results of animal tests have showed that in LLNAs EC3 values of isoeugenol is between 0.5 and 3.8 at applied concentrations, and a SI of three or more has been observed in LLNA of isoeugenol from the test concentration of 1.3% (Kimber et al, 1991; Basketter and Scholes, 1992; Hilton et al., 1996, Bertrand et al., 1997; Dearman et al., 1999; Basketter et al., 1999; Takeyoshi et al., 2008). 100 % responding from 0.15 % intradermal induction dose of isoeugenol have been detected in most of the GPMT studies (Tsuchiya et al., 1982; Tsuchiya et al., 1985; Maurer and Hess, 1989; Kimber et al., 1991; Basketter and Scholes, 1992; Barratt and Basketter, 1992; Hilton et al., 1996; Takeyoshi et al., 2008). Above evidence supports that isoeugenol is sub-category 1A skin sensitiser. The outcomes from the most of the Buehler assay however indicate that isoeugenol falls into sub-category 1B.

In human tests, a number of HRIPT (RIFM, 1964, 1973, 1979d, 1980b, 1987b; Marzulli and Maibach, 1980; Johansen et al., 1996) give the evidence that isoeugenol is sub-category 1A skin sensitiser (positive responses at $\leq$ 500 $\mu g/cm^2$). Besides this, relatively high and substantial incidence of allergic contact dermatitis caused by isoeugenol and mixtures containing isoeugenol are observed in diagnostic patch test and in epidemiological studies.

Overall there is clear evidence for classification in category 1A from animal tests (LLNA and GPMT) and human tests and human data. Only the results from the Buehler tests indicate category 1B. As human data is considered more relevant than animal data and the Buehler assay is considered less sensitive compared to the LLNA and the GPMT, classification in category 1A is warranted.

The GCL for Skin Sens. 1A substance is 0.1%. According to the 'Guidance on the Application of the CLP Criteria' (paragraph 3.4.2.2.5), specific concentration limits can be set based on potency. Tables 3.4.2-f/g/h of this CLP Guidance present the potency classes for the mouse LLNA-test, Guinea Pig Maximisation test and the Buehler assay, respectively. The results of the LLNA-studies and the GPMT-tests are sufficient for classification into category 1A. Based on the results of the LLNA-studies (EC3 0.5-3.8%), no EC3-value $\leq$0.2% (w/v) was observed. Thus according to the criteria in table 3.4.2-f of the CLP-guidance, this would correspond to a strong potency class. Further, the results of the GPMT tests (100% positive response following a 0.15% intradermal induction dose) also indicate a strong potency class following the criteria in table 3.4.2-g of the CLP-Guidance. For this potency class, the GCL of 0.1% applies (Table 3.4.2-I of the CLP-Guidance). However, when a 100% response in the GPMT is observed at 0.15% intradermal induction it can be expected that a response above 60% will occur at 0.1% induction. This would indicate an extreme sensitising potency and justify a SCL of 0.001%. Based on all available information consisting of the LLNA data showing strong but no extreme potency and the GPMT indicating extreme potency, a strong potency for isoeugenol is considered justified. Hence, setting of a SCL for isoeugenol is not needed.

4.6.1.5 Conclusions on classification and labelling

Based on the available animal and human evidence for isoeugenol, a classification as Skin Sens. 1A – H317: May cause an allergic skin reaction is required for isoeugenol. Based on the available animal studies, setting of a SCL for isoeugenol is not necessary.

RAC 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. The DS proposed to classify isoeugenol as a skin sensitiser in category 1A (Skin Sens. 1A; H317) based on test data from several LLNA (Local Lymph Node Assay) and GPMT (Guinea Pig Maximisation Tests) as well as from some human studies. The DS also pointed out that isoeugenol had been chosen for a full risk assessment by HERA (Human and Environmental Risk Assessment on ingredients of household cleaning products), a voluntary industry programme, because of its known skin sensitising properties (HERA, 2005).

The GCL for Skin Sens. 1A substances is 0.1% w/v. As the EC3-values of 0.2 - 2.0% (w/v) were observed in the LLNA studies, indicating a strong (but not "extreme") potency class, which was also supported by the results of several GPMT tests (100% positive response following a 0.15% intradermal induction dose) indicating a strong potency class (resulting in a generic concentration limit of 0.1% w/v), no SCL was proposed by the DS.

Comments received during public consultation

Three Member State Competent Authorities (MSCA) commented during the public consultation. Each supported the proposed classification (Skin Sens. 1A; H317) but one suggested some revisions regarding the argumentation.

Industry did not provide any comments.

Assessment and comparison with the classification criteria

Isoeugenol quickly penetrates the human skin according to skin penetration studies *in vitro* and *in vivo*. The free phenolic group of the substance is detoxified by phase II conjugation. Isoeugenol is rapidly metabolised and eliminated without achieving metabolic saturation. The formation of quinone or quinonemethide metabolites might be the mechanism by which isoeugenol and its derivatives cause sensitisation.

According to the CLP criteria, effects seen in either humans or animals will normally justify classification in a weight of evidence approach for skin sensitisers. As isoeugenol showed clear sensitising effects in a range of experimental animal studies and in human patch tests, there is evidence that isoeugenol is a skin sensitiser. However, according to the CLP Regulation, sub-categorisation is only possible if data are sufficient. RAC considers that the data available for isoeugenol are sufficient for sub-categorisation as Skin Sens. 1A.

Human data

In an HRIPT (Human Repeat Insult Patch Test) conducted by RIFM (1980b) assessing induction using 0.5% isoeugenol in SDA (specially denatured alcohol) ethanol (corresponding to 260 $\mu g/cm^2$ isoeugenol) and challenge with 0.5% in SDA ethanol, positive results were seen in 2 of 53 volunteers. Johansen *et al*. (1996) achieved positive results in an HRIPT with induction using 32 $\mu g/cm^2$ isoeugenol. However, as described in the study report, this HRIPT test was performed on isoeugenol-sensitive patients, so the results are considered to relat to elicitation rather than induction. In the HMT (Human Maximisation Test) by Kligman and Gollhausen (1986), 6/7 volunteers showed positive results after an induction dose of 1% isoeugenol in petrolatum applied for 48 h and a challenge dose of 1% isoeugenol in petrolatum two days later also applied for 48 h. Also, several other positive HMT and HRIPT studies (and a few negative HMT studies) with

isoeugenol at higher induction doses, from 1.25% to 10%, as well as a few negative HRIPT studies at very low induction doses were included in the CLH report. According to the CLP criteria, human evidence for sub-category 1A can include positive responses at ≤ 500 $\mu g/cm^2$ (HRIPT, HMT – induction threshold) corresponding to ≤ 1% induction concentration (CLP Guidance, Table 3.4.2-c) and human evidence for sub-category 1B can include positive responses at > 500 $\mu g/cm^2$ (HRIPT, HMT – induction threshold) corresponding to > 1% induction concentration. Therefore, RAC concluded that based on the results from the human HRIPT study (RIFM, 1980b) and one HMT (Kligman and Gollhausen, 1986) with relatively low exposure to isoeugenol (≤ 1.0% or ≤ 500 $\mu g/cm^2$) a sub-categorisation in Skin Sens. 1A is justified. In the other positive HMT and HRIPT studies in which induction concentrations > 1% were tested, it could not be concluded whether these concentrations were the induction thresholds since no lower concentrations were tested and therefore sub-categorisation was not possible based on the results of these tests.

Patch testing with serial dilutions and Repeated Open Application Test (ROAT) are performed on sensitised individuals in order to indicate the degree of sensitivity and safe limits of exposure (CLP Guidance Table 3.4.2-a). In Johansen *et al*. (1996), patch testing with serial dilutions of isoeugenol and a ROAT were performed in 19 subjects to study the clinical implications of sensitisation to isoeugenol. 4/19 (20%) of the test subjects had a threshold response at concentrations 0.01% or lower in the patch test and 12/19 (63%) of the test subjects had a positive ROAT with a test solution of 0.2% isoeugenol in ethanol with a maximum exposure period of 4 weeks. In the ROAT study by Andersen *et al*. (2001), 66.7% of the isoeugenol-sensitive subjects showed a positive result with 0.2% isoeugenol in ethanol and a 42% positive response was observed with 0.05% isoeugenol in ethanol following application for up to 28 days. In Bruze *et al*. (2005) the patch test was used to identify the minimal eliciting concentration of isoeugenol in ethanol and in perfumed deodorant in patients who previously had been shown to be hypersensitive to isoeugenol. The controls had previously been shown to produce negative patch test results to the fragrance mix. The results of the patch tests showed that relatively low concentrations of isoeugenol in ethanol and in deodorants (range from 0.0005% to 2% in ethanol and from 0.063% to 0.2% in perfumed deodorant) applied for 48 h, with the result read on days 3 and 7, led to positive results in hypersensitive dermatitis patients whereas the controls were negative. A positive ROAT was also observed only in patients hypersensitive to isoeugenol and only in the axilla to which the deodorants containing isoeugenol had been applied (3/13 sensitised individuals at 0.0063% isoeugenol). It was concluded in Bruze *et al*. (2005) that deodorants containing isoeugenol in the concentration range of 0.0063–0.2% used 2 times daily on healthy skin can elicit axillary dermatitis within a few weeks in people with contact allergy to isoeugenol. A survey of approximately 6500 consumer patch tests on isoeugenol alone or in various consumer products and fragrance blends containing isoeugenol was performed by Thompson *et al*. (1983) at concentrations ranging from $3x10^{-7}$ to 0.8% isoeugenol in consumer products and at conentrations of 1.0% and 1.25% of neat isoeugenol. Induction reactions following exposure to consumer products of isoeugenol was reported in one out of 32 patch tests at 0.02% isoeugenol, one out of 23 patch tests at 0.02% isoeugenol-eugenol mixture, and one out of 56 patch tests at 0.8% isoeugenol. For neat isoeugenol 1/81 patch tests and 1/38 patch tests showed an induction reaction at 1.25% and 1.0% isoeugenol, respectively. Due to these studies isoeugenol is considered potent in elicitating allergic responses in sensitised individuals. However, as patch testing with serial dilutions and ROAT are performed solely on sensitised individuals in order to estimate the elicitation threshold of an allergen, the CLP Guidance (Tables 3.4.2-b-d) on sub-categorisation is not applicable to the data obtained via these tests, since this table refers to induction doses.

There is also information on the number of reacting patients vs. number of tested patients in numerous clinical patch tests on "fragrance mix-sensitive", "perfume-sensitive" and "cosmetic-sensitive" patients showing a high frequency of positive responses to "isoeugenol". However, since it was reported in relation to each study that "patients

probably reacted to other test materials in the same study" these studies were not considered reliable for determining the isoeugenol-induced frequency of occurrence of skin sensitisation. In addition, there was no information on the (presumed) use estimates of products containing isoeugenol for these patients, and therefore the exposure index could not be calculated (and this is needed for sub-categorisation according to the CLP Guidance).

Animal data

In the CLH report a large volume of animal data was provided by the DS. These data included results of LLNA, GPMT and the Buehler assays as well as of Open Epicutaneous Tests (OET), Draize Tests (DT), Freunds Complete Adjuvant Tests (FCAT), Cumulative Contact Enhancement Tests (CCET), optimization test, Modified Draize Tests and Mouse Ear Swelling Tests (MEST). Since according to the CLP Guidance the LLNA, GPMT and Buehler assays are the currently recognised and officially accepted animal test methods for skin sensitisation and the results from these studies can be used directly for classification and potency evaluation (see the table above), RAC assessed only the results of these animal studies. According to CLP Guidance (section 3.4.2.2.3.4) there is often a degree of uncertainty associated with the derivation of allergenic potencies from the Buehler and GPMT assays. This is because Guinea pig tests should be conducted at the highest induction dose causing mild (Buehler Assay) or mild-to-moderate (GPMT) skin irritation. As a consequence, it is unlikely that substances (other than strong irritants) would be tested at the low concentration given in the CLP Regulation, Annex 1, table 3.4.3, triggering classification as a skin sensitiser in sub category 1A. RAC notes that the information on the dose-selection for most studies is not available (apart from Kimber *et al*. (1991), Basketter and Scholes (1992), Hilton *et al*. (1996) and Takeyoshi *et al*. (2008)).

The outcomes from most of the Buehler Tests fit with the CLP criteria for a sub-category 1B (≥ 15% to 60% responding at > 0.2% to ≤ 20% topical induction dose or ≥ 15% responding at > 20% topical induction dose) for isoeugenol, although a sub-category 1A (≥ 15% responding at ≤ 0.2% topical induction dose or ≥ 60% responding at > 0.2% to ≤ 20% topical induction dose) cannot be excluded in the absence of dose-response and dose-selection information. The Buehler studies by Kaminsky and Szivos (1986; 1990) meet the CLP criteria for a sub-category 1A (≥ 60% responding at > 0.2% to ≤ 20% topical induction dose). All in all, the reliability of the available Buehler tests in estimating the potency of isoeugenol is questionable as there is no information available on the dose-selection for these studies.

Some of the GPMT results (RIFM (1985b) and Takeyoshi *et al*. (2008)) indicate that isoeugenol has at least moderate potency and meets the CLP criteria for sub-category 1B, although classification in sub-category 1A cannot be excluded in Takeyoshi *et al*. (2008) as lower intradermal induction concentrations were not tested. However, several of the GPMT tests indicate a high potency, warranting classification in sub-category 1A. In these studies, the response rate was 100% with an intradermal induction dose of 0.15% isoeugenol (Kimber *et al*. (1991), Basketter and Scholes (1992), Hilton *et al*. (1996)) or 100% with an intradermal induction of 1.0% isoeugenol (Tsuchiya *et al*. (1982), Tsuchiya *et al*. (1985)). Since according to the DS, Kimber *et al*. (1991), Basketter and Scholes (1992) and Hilton *et al*. (1996) have tested concentrations of the test substances suitable for induction of sensitisation and for sensitisation challenge in the GPMT studies, RAC considers these studies as reliable for sub-categorisation, and that they fulfil the criteria for the 1A sub-category.

All the reported LLNA studies showed sensitising effects with a Stimulation Index ≥ 3. In nine studies an EC3 value ≤ 2% was obtained and the different EC3 values were attributable to different solvents used. In the Wright *et al*. (2001a and 2001b) studies the EC3 values were 0.9%, 1%, 1.4%, 1.8% and 2.0%. In the RIFM studies, the EC3 values were 1.54% and 0.63%. In Basketter *et al*. (2002) the EC3 value was 1.3% and in Basketter and Cadby (2004) the EC3 values were 0.5% and 2.6%. Hence, the criteria for

the 1A classification of isoeugenol are also fulfilled in a number of LLNA tests.

Conclusion of RAC

Isoeugenol is a strong skin sensitiser. This was clearly shown in various sets of data from experimental animals and in studies on human volunteers designed to determine the induction threshold (the Human Maximisation Test (HMT) and Human Repeat Insult Patch Test (HRIPT)), justifying classification as Skin Sens. 1A; H317 according to the CLP regulation. In addition, patch tests with serial dilutions and ROATs on isoeugenol in ethanol and in deodorant showed that isoeugenol is potent in elicitating allergic responses in sensitised individuals.

In addition, the proposal to classify isoeugenol as a skin sensitisiser in Cat. 1A is consistent with the findings in the SCCS opinion on Fragrance allergens in cosmetic products from 2012 (http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_102.pdf). This SCCS opinion is an update of the Opinion of the Scientific Committee on Cosmetic products and Non-Food Products (SCCNFP) from 1999 (SCCNFP/0017/98), with a systematic and critical review of the scientific literature to identify fragrance allergens relevant to consumers, including isoeugenol. Clinical, epidemiological and experimental studies were evaluated. The studies conducted and assessed since the SCCNFP opinion on fragrance allergy in consumers confirmed that the fragrance allergens including isoeugenol identified by SCCNFP in 1999 are still relevant fragrance allergens for consumers from their exposure to cosmetic products.

RAC agrees with the reasoning of the DS that due to the structural similarity between the isoeugenol isomers, the results obtained with isoeugenol and with any ratio between the cis- and trans-isomer can be assumed to be comparable. Thus, RAC concludes that the same classification (Skin Sens. 1A) should also apply to both isoeugenol isomers and for any isomeric ratio of these.

Setting of Specific Concentration limit (SCL)

According to the SCCNFP (2001) opinion (SCCNFP/0392/00, final), isoeugenol should not be used such that the level in finished cosmetic products exceeds 0.02% (based on test results showing sensitising potential (IFRA guidelines)).

RAC acknowledges that this concentration limit is below the generic concentration limit (0.1%) for substances classified as Skin Sens. 1A (skin sensitisation induction), but the data used for this SCCNFP (2001) opinion was not available to RAC.

According to CLP Guidance, a substance can be considered an extreme potency sensitiser (warranting an SCL of 0.001%) based on a GPMT study if there is ≥ 60% positive response with an intradermal induction concentration of ≤ 0.1%. Most GPMT results referred to in the CLH report gave a 100% positive response following an intradermal induction concentration $\underline{\geq}$ 0.15%. Considering that at the lowest induction concentration used, these results fit the criteria for an extreme potency sensitiser, then if the SCL was based on these data alone 0.001% would be appropriate. However, extreme potency was not indicated in any of the LLNA data (no EC3 value ≤ 0.2%) or in any of the Buehler assays and all the evidence needs to be carefully weighed.

Limited support for an SCL was available from the human induction data. In an HRIPT conducted by RIFM (1980b) using isoeugenol at 0.5% in SDA ethanol (corresponding to 260 $\mu g/cm^2$ isoeugenol – well below the threshold of ≤ 500 $\mu g/cm^2$ for classification as Skin Sens. 1A) and a challenge with 0.5% in SDA ethanol, positive results were seen in 2 of 53 volunteers. Furthermore, in the survey by Thompson *et al*. (1983) of around 6500 patch tests with concentrations ranging from $3x10^{-7}$ to 0.8%, isoeugenol induction reactions following exposure was reported at 0.02% isoeugenol in one out of 32 patch tests and another 1/23 patch tests where in addition to the isoeugenol, eugenol was also

present.

Further support for a lower SCL comes from data assessing elicitation-reactions following human exposure to isoeugenol. In the study by Johansen *et al*. (1996), 20% of the test subjects had a threshold response at 0.01% or lower in the patch test, and 63% of the test subjects had a positive ROAT with a test solution of 0.2% isoeugenol in ethanol. The study by Bruze *et al*. (2005) showed that 3/13 sensitised individuals had a positive ROAT at 0.0063% isoeugenol in perfumed deodorants. In addition, in the survey by Thompson *et al*. (1983), one positive elicitation reaction out of 83 patch tests was reported following exposure to a 0.04% isoeugenol-eugenol mixture.

Taken together, data from the GPMT studies indicate that isoeugenol could be an extreme potency sensitiser (which would warrant an SCL of 0.001%), but extreme potency is not indicated in the findings from the LLNA or Buehler assays. Data from humans indicate that induction and elicitation can occur at concentrations lower than the GCL (0.1%) and there is evidence for elicitation (not induction) occurring at concentrations $\leq$ 0.01%.

Overall, RAC considers that there are both animal and human data to support a concentration limit lower than the GCL (0.1%). Greatest weight was given to the evidence for extreme potency (and an SCL of 0.001%) from the GPMT in comparison with the evidence for strong potency (and the GCL of 0.1%) from the LLNA and Buehler assays, and therefore an SCL of 0.01% was considered appropriate (being intermediate between 0.1% and 0.001% in terms of order of magnitude). Some evidence is also provided by the human studies (mainly involving elicitation) for a lower SCL than the GCL to be applied. RAC therefore concludes that an SCL of 0.01% is warranted[2].

4.6.2 Respiratory sensitisation

Not evaluated in this report

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

Not evaluated in this report

4.8 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this report

4.9 Carcinogenicity

Not evaluated in this report

4.10 Toxicity for reproduction

Not evaluated in this report

[2] Note: because isoeugenol is 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 isoeugenol at a concentration ≥ 0.001% (CLP Annex II, section 2.8), to protect already sensitised individuals.

4.11 Other effects

Not evaluated in this report

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this report

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7 ANNEXES