Committee for Risk Assessment

RAC

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

Adopted

10 March 2016

10 March 2016

CLH-O-0000001412-86-98/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **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];**

The proposal was submitted by the **Netherlands** and received by RAC on **10 June 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **30 June 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **14 August 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Bjørge**

Co-Rapporteur, appointed by RAC: **Sonja Kapelari**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **10 March 2016** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	xxx-xxx-xx-x	isoeugenol; [1]; (E)-2-methoxy-4-(prop-1-enyl)phenol; [2]; (Z)-2-methoxy-4-(prop-1-enyl)phenol; [3];	202-590-7; [1]; 227-678-2; [2]; 227-633-7; [3];	97-54-1; [1]; 5932-68-3; [2];...	Skin Sens. 1A	H317	GHS07 Wng	H317		-	
RAC opinion	xxx-xxx-xx-x	isoeugenol; [1]; (E)-2-methoxy-4-(prop-1-enyl)phenol; [2]; (Z)-2-methoxy-4-(prop-1-enyl)phenol; [3];	202-590-7; [1]; 227-678-2; [2]; 227-633-7; [3];	97-54-1; [1]; 5932-68-3; [2];...	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C $\geq$ 0,01 %	
Resulting Annex VI entry if agreed by COM	xxx-xxx-xx-x	isoeugenol; [1]; (E)-2-methoxy-4-(prop-1-enyl)phenol; [2]; (Z)-2-methoxy-4-(prop-1-enyl)phenol; [3];	202-590-7; [1]; 227-678-2; [2]; 227-633-7; [3];	97-54-1; [1]; 5932-68-3; [2];...	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C $\geq$ 0,01 %	

GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUATION

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 $\geq$ 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%). Greater 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**[1].

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and by RAC (excluding confidential information).

[1] 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.