Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

methyl oct-2-ynoate

EC Number: 203-836-6
CAS Number: 111-12-6

CLH-O-0000007360-81-01/F

Adopted

14 September 2023

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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 on **14 September 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **methyl oct-2-ynoate**

EC Number: **203-836-6**

CAS Number: **111-12-6**

Rapporteur, appointed by RAC: **Karine Angeli**

Administrative information on the opinion

Denmark has submitted on **28 September 2022** 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 **14 November 2022**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 January 2023**.

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 following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry, if agreed by the Commission.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	TBD	methyl oct-2-ynoate	203-836-6	111-12-6	Skin Sens. 1A	H317	GHS07 Wng	H317			
RAC opinion	TBD	methyl oct-2-ynoate	203-836-6	111-12-6	Skin Sens. 1A	H317	GHS07 Wng	H317			
Resulting Annex VI entry if agreed by COM	TBD	methyl oct-2-ynoate	203-836-6	111-12-6	Skin Sens. 1A	H317	GHS07 Wng	H317			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Methyl oct-2-ynoate (methyl heptine carbonate, folione) is a mono-constituent substance used in air care products, biocides (e.g. disinfectants, pest control products), perfumes and fragrances, polishes and waxes, washing & cleaning products and cosmetics and personal care products.

Methyl oct-2-ynoate is one of the 26 fragrance allergens stated by the Cosmetic Regulation (EC) No 1223/2009 to be listed on the ingredient label of a cosmetic product sold on the European market if the concentration is ≥ 10 ppm (0.001%) in leave-on products or ≥ 100 ppm (0.01%) in rinse-off products.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed to classify methyl oct-2-ynoate as a strong skin sensitiser; Skin Sens. 1A; H317: May cause an allergic skin reaction. The classification is based on the low EC3 values from the available LLNA studies, showing the strong potency of the substance to cause skin sensitisation, and are supported by human data.

Since no scientific information has been identified to support a specific concentration limit (SCL), the DS proposed to use the generic concentration limits of the sub-category 1A (0.1% w/v).

The DS proposed the additional labelling EUH208 – 'Contains methyl oct-2-ynoate. May produce an allergic reaction' for mixtures containing ≥ 0.01% methyl oct-2-ynoate.

Comments received during consultation

Two member states supported the DS's proposal. One of them mentioned that the OECD Expert Group on defined approaches for skin sensitisation selected methyl oct-2-ynoate as a reference substance and classified the substance as Skin Sens. 1 A based on both HMT/HRIPT and LLNA data.

Assessment and comparison with the classification criteria

In silico/in chemico/in vitro data

In the evaluation of the Research Institute for Fragrance Materials (Api *et al.* 2019), methyl oct-2-ynoate is reported to be positive in 3 *in chemico*/*in vitro* assays investigating the key events of the skin sensitisation Adverse Outcome Pathway (AOP) a direct peptide reactivity assay (DPRA) addressing key event 1 "covalent interaction with skin proteins"; a KeratinoSens™ assay addressing key event 2 "Keratinocyte

responses"; and a human cell line activation test (h-CLAT) addressing key event 3 ''Dendritic cell responses''. Based on those tests, as well as on the predictions from both Derek Nexus and OECD QSAR Toolbox, methyl oct-2-ynoate was one of the reference substances used to evaluate the performance assessment of the Defined Approaches (DAs) on skin sensitisation against sets of *in vivo* reference data (supporting document to the OECD TG 497 on Defined Approaches on Skin Sensitisation No 336, 2021; Urbisch *et al.*, 2015 supplementary data). While only the results are reported, hampering a full assessment of these *in chemico*/*in vitro* data, RAC takes note that the OECD Expert Group on DA on skin sensitisation predicted methyl oct-2-ynoate to be a skin sensitiser applying the "2 out of 3" DA for hazard identification and **as strong sensitizer (sub-category 1A)** applying the DA for skin sensitisation potency categorisation (integrated testing strategies ITSv1 and ITSv2) (Annex 2 of OECD No 336, 2021).

Animal data

Two local lymph node assays (LLNAs), one guinea pig maximisation test (GPMT), one guinea pig open epicutaneous test (OET) and one Buehler test with methyl oct-2-ynoate have been identified by the DS (Table 7 of the CLH report).

In a GLP-compliant LLNA, performed according to OECD TG 429 (reliability score of 1), female CBA mice (4/group) received topical applications of methyl oct-2-ynoate at 0.05, 0.1, 0.25, 0.5 or 1.0% in 1:3 ethanol/diethyl phthalate, daily, for 3 days. The proliferative activity of draining lymph node cells was determined by incorporation of radiolabeled thymidine. The reported concentration, producing a 3-fold increase in lymphocyte proliferation (EC3) was 0.45% (112.5 µg/cm^2) (ECHA dissemination site 2023; Kern *et al.* 2010; RIFM, 2006).

In a previous LLNA (GLP status not stated), performed according to OECD TG 429, CBA mice (4/group) received topical applications of methyl oct-2-ynoate at 0.5, 1.0, 2.0, 5.0 or 10.0% in 1:3 ethanol/diethyl phthalate, daily, for 3 days. The reported concentration producing a 3-fold increase in EC3 was lower than 0.5% (SCCS, 2012; RIFM, 2005).

The quality of the GPMT and the Buehler test reported in Hostynek and Maibach (2006) cannot be directly assessed (GLP status and guideline not stated). However, the authors rated them with a score of 5 (i.e. high degree of confidence, all test qualification criteria met). Therefore, RAC considers them as reliable with restriction. In both tests, methyl oct-2-ynoate was positive. In the GPMT, 90% of the animals responded after an intradermal induction dose of 0.625% while in the Buehler test, up to 70% of the animals responded after an induction dose of 2.5%.

In the OET, 1, 3, 10, 30 and 100% skin reactions are reported from 10% onwards. No validated guideline is available and the information in the REACH registration dossier is very limited, hampering the quality check of the study. The reliability is therefore not assignable (ECHA dissemination site 2023).

RAC notes that skin sensitisation is consistently induced by methyl oct-2-ynoate exposure in different *in vivo* test systems. Among the identified animal data, the reliable LLNA studies allow **sub-categorisation as 1A since the EC3 (0.45%) is below 2%** (CLP Annex I: 3.4.2.2.3.2.). While of lower reliability, the other animal studies further support that methyl oct-2-ynoate is a skin sensitiser with a strong potency.

Human data

Human Repeated Insult Patch Test (HRIPT) & Human Maximization Test (HMT)

In the review of Hostynek and Maibach (2006) results of several unpublished HMTs and HRIPTs are reported. This includes an HRIPT study involving 41 human volunteers, where methyl oct-2-ynoate 0.25% in ethanol gave two positive reactions, and two other studies, with 40 and 42 subjects, where methyl oct-2-ynoate 0.1% in ethanol induced no reaction. In 3 HRIPTs with methyl oct-2-ynoate 0.1% in ethanol and diethyl phthalate (3:1), there was 1, 0 and 0 reaction out of 36, 33 and 74 human volunteers respectively. Based on those data, the Research Institute for Fragrance Materials (RIFM) established a NOEL-HRIPT (induction) of 118 µg/cm^2, a LOEL-HRIPT/HMT (induction) of 194 µg/cm^2 and concluded that methyl oct-2-ynoate is a strong skin sensitizer, based on a weight of evidence analysis (Api *et al.*, 2019).

Diagnostic patch tests

As reported in Table 8 of the CLH report, eleven diagnostic patch test studies have been identified by the DS, in which methyl oct-2-ynoate has been tested at concentrations of 0.5 to 2% in petrolatum.

In seven retrospective studies on **unselected patients**, the frequency of skin sensitisation ranged from 0 to 1.67%: 2/120 eczema patients (1.67%) in France, date not indicated (Heisterberg *et al.*, 2010), 12/1951 eczema patients (0.62%) in United Kingdom tested between 2011-2012 (Mann *et al.*, 2014), 1/278 patients (0.4%) in North America, date not indicated (Mitchell *et al.*, 1982 as cited in SCCNFP, 1999), 6/2401 patients (0.2%) in Europe (IVDK data), tested between 2003-2004 (Schnuch *et al.*, 2007), 3/1870 patients (0.16%) in Europe (IVDK data), tested between 2007-2009 (Schnuch *et al.*, 2015), 1/988 patients suspected of allergic dermatitis (0.1%) in Germany, tested between 2005-2008 (Uter *et al.*, 2010) and 0/230 eczema patients in Denmark (0%), tested between 2007-2008 (Heisterberg *et al.*, 2010).

In three studies on **selected patients** (contact allergy related to cosmetics), the frequency of skin sensitisation ranged from 0.3 to 3%: 1/34 patients (3%) and 2/182 patients (1.1%), region and date not available Malten *et al.*, 1984 as cited in SCCNFP, 1999), 1/320 patients (0.3%) in Netherland tested between 2005-2007 (Van Oosten *et al.*, 2009).

In the single occupational study on **selected workers** involving four bakers with hand eczema, the frequency of occurrence of skin sensitisation was 25% (Malten, 1979 as cited in SCCNFP, 1999).

Regarding diagnostic patch test data, the Guidance on the Application of the CLP Criteria (CLP guidance, Section 3.4.2.2.3.1, Table 3.2) outlines how high or low frequency of occurrence of skin sensitisation shall be assessed as reported in the Table below.

Table: *Relatively high or low frequency of occurrence of skin sensitisation criteria (CLP guidance Table 3.2) applied to methyl oct-2-ynoate*

Human diagnostic patch test data	**High frequency**	**Low/ moderate frequency**	**Information on methyl oct-2-ynoate**
General population studies	≥ 0.2%	< 0.2%	No data
Dermatitis patients (unselected, consecutive)	≥ 1.0%	< 1.0%	Seven studies, frequency: 0 - 1.67% • 1 study : **High** • 6 studies: **Low/moderate**
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0%	< 2.0%	3 studies, frequency: 0.3 - 3% • 1 study: **High** • 2 studies: **Low/moderate**
Workplace studies: **1: all or randomly selected workers** **2: selected workers with known exposure or dermatitis**	≥ 0.4% ≥ 1.0%	< 0.4% < 1.0%	No data 1 study (n=4): **High** frequency (25%)
Number of published cases	≥ 100 cases	< 100 cases	< 100 cases **Low/moderate**

In the majority of the available patch test datasets including the largest and most relevant studies, **low to moderate frequency** of occurrence of skin sensitisation is observed. However, one out of seven patch tests in consecutive patients and one out of three patch tests in selected patients show a high frequency of occurrence (1.67% and 3% respectively). In the single patch test available in selected workers, the frequency of occurrence was also high (25%) but the headcount was limited to 4 bakers.

Case reports

From the five case studies reported (Table 8 of the CLH report), RAC considers that three of them show **active sensitisation from patch testing** with methyl oct-2-ynoate (Heisterberg *et al.*, 2010), of critical importance for classification purposes. The definition of active sensitisation is "a negative patch test with a flare up after 10–20 days, and a positive reaction within a few days after retesting with the suspected substance" (Wahlberg *et al.*, 2006). In the three reported cases, late reactions occurred two to four weeks after patch testing with concentration of 1% or 2% methyl oct-2-ynoate. Two patients who did the patch test with 1% methyl oct-2-ynoate were positive after retesting, which clearly indicate that sensitisation was induced by the patch testing procedure. While not retested, the case history of the third patient (patch tested with 2%) also supports active sensitisation. Based on these results, RAC notes that the **induction threshold is below 1%** methyl oct-2-ynoate in petrolatum corresponding to a dose below 400 µg/cm^2 (patch tests performed according to international guidelines

using Finn Chambers® of diameter 8 mm; area 0.5 cm^2; recommended 20 mg of petrolatum preparation).

Exposure consideration

Methyl oct-2-ynoate is registered under REACH to be manufactured in and/or imported in a relatively low tonnage (10 to 100 tonnes per annum).

In the SCCS opinion (2012), it is mentioned that, while the exposure to all 26 allergenic fragrances was considered foreseeable in daily life, it was highlighted that the exposure to methyl oct-2-ynoate appeared to be low. This statement was based on several market surveys performed on cosmetics and other consumer products (e.g. less than 1% of the products contained methyl oct-2-ynoate in a German dataset (n=3000), SCCS, 2012).

In the study by Schnuch *et al.* (2015), methyl oct-2-ynoate was also listed as a fragrance compound very rarely used based on four datasets of consumer products from Germany (n=5451), Netherland (n = 516), UK (n=300) and Denmark (n=88). The occurrence of methyl oct-2-ynoate in products ranged from 0% to 1%.

The low frequency of occurrence in consumer products could be explained by the standard limits introduced by the International Fragrance Association (IFRA) more than 20 years ago. The IFRA Standard is a global risk management system for the safe use of fragrance ingredients. For many years, the IFRA standard limit for methyl oct-2-ynoate in most categories of products was very low (0.01%). In the 2020 update, the new standard limits for the finished products are generally increased compared to the previous ones but still below 0.1% for leave-on products (Table 10 of the CLH report; IFRA, 2020B).

The CLP guidance (Section 3.4.2.2.3.1., Table 3.2) outlines how high or low exposure shall be assessed, as reported in the Table below.

***Table**: Relative high or low exposure criteria (CLP guidance Table 3.3) applied to methyl oct-2-ynoate*

Exposure data	**Indicator of relatively low exposure**	**Indicator of relatively high exposure**	**Assessment for methyl oct-2-ynoate**
Concentration / dose at induction	< 1.0% < 500 µg/cm^2 *(score 0)*	≥ 1.0% ≥ 500 µg/cm^2 *(score 2)*	Based on expected concentration of methyl oct-2-ynoate in consumer products (IFRA, 2020B) → **score 0**
Repeated exposure	< once/daily *(score 1)*	≥ once/daily *(score 2)*	Based on SCCS (2012) and Schnuch *et al.* (2015), daily exposure of **consumers** is not expected → **score 1** For certain **workers**, the exposure might be daily (beauty therapists, healthcare workers, cleaners, hairdressers, cooks and bakers…). → **score 2**
Number of exposures	<100 exposures *(score 0)*	≥100 exposures *(score 2)*	Given the type of consumer and professional uses, the exposure is likely more than 100 times. → **score 2**

The calculated additive exposure index for methyl oct-2-ynoate is 3 (0 + 1 +2) for consumers and 4 (0 + 2 +2) for workers, indicating a **relatively low exposure** according to CLP guidance since it is lower or equal to 4.

According to the classification criteria of Regulation (EC) 1272/2008 (Annex I section 3.4.2.2.2) human evidence for Sub-categories 1A and 1B, respectively, can include the following type of data:

Sub-category 1A

(a) positive responses at ⩽ 500 µg/cm^2 (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;

(c other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

Sub-category 1B

(a) positive responses at > 500 µg/ cm^2 *(HRIPT, HMT – induction threshold);*

(b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure;

(c) other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

While the reports of the HRIPTs and HMTs performed with methyl oct-2-ynoate are not available, RAC notes that the LOEL-HRIPT/HMT (induction) of 194 µg/ cm^2 set by RIFM suggests that the criteria for classification in sub-category 1A are fulfilled since the induction threshold is below 500 µg/cm^2. This is further supported by the cases of active sensitisation observed after patch testing with 1% methyl oct-2-ynoate in petrolatum (Heisterberg *et al.*, 2010), corresponding to an induction dose below 400 µg/cm^2.

Combining the frequency of occurrence of skin sensitisation with the exposure index, according to Table 3.4 of the CLP guidance, the relatively low frequency of occurrence and the relatively low exposure index would categorise methyl oct-2-ynoate in "category 1 or case by case evaluation". However, RAC notes that despite the existing IFRA standard limits, sensitisation is still observed in patch tests which seems to support a strong potency suggesting that sub-category 1A is more justified than category 1 despite the relatively low frequency of occurrence observed in the majority of the patch tests.

Conclusion

Human, animal and *in vitro* data provide consistent evidence that methyl oct-2-ynoate is a skin sensitizer.

The identified animal studies provide clear evidence of strong sensitising effects of methyl oct-2-ynoate. The results obtained in the two reliable LLNAs (EC3 values < 2%) allow the categorisation in sub-category 1A. While of lower reliability, the GPMT and Buehler test also support the strong sensitising potency.

Furthermore, human data corroborates classification in sub-category 1A based on the reported LOEL (induction) of 194 µg/cm^2 for the HRIPT as well as the three cases of active sensitisation observed after patch testing with 1% methyl oct-2-ynoate in petrolatum corresponding to an induction dose lower than 400 µg/cm^2.

The OECD Expert Group also considered that human data HMT/HRIPT and animal data LLNA support Cat. 1A as mentioned in the comment during the consultation.

Therefore, based on a weight of evidence approach, **RAC considers that classification Skin Sens. 1A; H317: May cause an allergic skin reaction, is warranted** (in alignment with DS conclusion).

Based on the EC3 obtained in the LLNA tests comprised between 0.2% and 2% indicating a strong potency (Table 3.6 of the CLP guidance), **the generic concentration limit of the sub-category 1A (0.1% w/v)** should apply.

Additional labelling

According to CLP Regulation (Table 3.4.6. and section 2.8 of Annex II), the label on the packaging of a mixture containing ≥ 0.01% methyl oct-2-ynoate **should bear the following statement**:

EUH208 – "Contains methyl oct-2-ynoate. May produce an allergic reaction"

Additional references

OECD (2021). Series on Testing and Assessment No. 336: Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Skin Sensitisation. Organisation for Economic Cooperation and Development, Paris. Available at: https://www.oecd.org/chemicalsafety/testing/series-testing-assessmentpublications-number.htm

Urbisch D, Mehling A, Guth K, Ramirez T, Honarvar N, Kolle S, Landsiedel R, Jaworska J, Kern PS, Gerberick F, Natsch A, Emter R, Ashikaga T, Miyazawa M, Sakaguchi H (2015). Assessing skin sensitization hazard in mice and men using non-animal test methods. Regul Toxicol Pharmacol. 71, 337-351.

Wahlberg J E, Lindberg M. Patch testing. In Contact Dermatitis. 4th edition, Frosch P J, Menne T, Lepoittevin J-P (eds): Berliner Heidelberg, Springer-Verlag, 2006, 382.

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 and additional information (if applicable).

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