Committee for Risk Assessment

RAC

Annex 1

Background document

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

(3*E*)-dec-3-en-2-one

EC Number: -
CAS Number: 18402-84-1

CLH-O-0000007098-68-01/F

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

Adopted

18 March 2022

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

(3*E*)-dec-3-en-2-one

EC Number:

CAS Number: **18402-84-1**

Index Number: **-**

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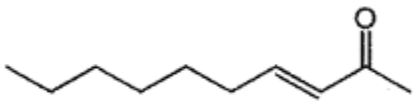
Version number: 2.0 **Date: April 2021**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	(3*E*)-dec-3-en-2-one
Other names (usual name, trade name, abbreviation)	(3*E*)-3-decen-2-one
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	-
EC name (if available and appropriate)	
CAS number (if available)	18402-84-1
Other identity code (if available)	-
Molecular formula	$C_{10}H_{18}O$
Structural formula	
SMILES notation (if available)	Not available
Molecular weight or molecular weight range	154.2
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The active substance may consist of the trans- and cis-isomer. The trans-isomer is the biological active substance and is available in abundance in the technical material and the cis-isomer is considered an impurity (<1%).
Description of the manufacturing process and identity of the source (for UVCB substances only)	Confidential information
Degree of purity (%) (if relevant for the entry in Annex VI)	980 g/kg (98.0% w/w)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
(3E)-dec-3-en-2-one	**98.0 % w/w**	Not applicable	H411: Toxic to aquatic life with long lasting effects.

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
			H315: Causes skin irritation. H332: Harmful if inhaled.

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Confidential	-			The impurities do not affect the classification

Current Annex VI entry: Not applicable

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Confidential	-				

Current Annex VI entry: N/A

Information on the purity of the tested material is included with every test when available. In most studies the purity was 98.57% or above and therefore considered relevant for (3E)-dec-3-en-2-one. However, no information regarding the cis/trans ratio of the laboratory scale batch used for the toxicological studies is available.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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		(3*E*)-dec-3-en-2-one		18402-84-1	Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1 Asp. Tox. 1 Aquatic Chronic 2	H332 H315 H317 H304 H411	GHS07 GHS08 GHS09 Dgr	H332 H315 H317 H304 H411	EUH071	inhalation: ATE = 1.5 mg/L (dusts or mists)	
Resulting Annex VI entry if agreed by RAC and COM		(3*E*)-dec-3-en-2-one		18402-84-1	Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1 Asp. Tox. 1 Aquatic Chronic 2	H332 H315 H317 H304 H411	GHS07 GHS08 GHS09 Dgr	H332 H315 H317 H304 H411	EUH071	inhalation: ATE = 1.5 mg/L (dusts or mists)	

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

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

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	Data lacking	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

(3E)-dec-3-en-2-one has not been previously classified by RAC or TC C&L. (3E)-3-Decen-2-one has been registered under REACH. The studies in the REACH Registration Dossier appear to be the same as those that were submitted for the active substance approval under Regulation (EC) No 1107/2009.

A Draft Assessment Report (DAR) and Proposed Decision of the Netherlands has been prepared in the context of the possible approval of (3E)-3-decen-2-one under Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market. All summaries are based on the DAR.

During the drafting of the DAR and the CLH proposal it became clear that the active substance actually placed on the market is the E-enantiomer with only a minor fraction of the Z-enantiomer. Therefore, the identity of the substance in this proposal is limited to this enantiomer.

RAC general comment

(3*E*)-3-decen-2-one is intended to be used as plant growth regulator in potatoes during storage. The product is applied by hot fogging.

(3*E*)-3-decen-2-one has not been previously classified by RAC or TC C&L. (3*E*)-3-decen-2-one has been registered under REACH. The studies in the REACH registration dossier appear to be the same as those that were submitted for the active substance approval under Regulation (EC) No 1107/2009 (PPP Regulation).

A Draft Assessment Report (DAR) and Proposed Decision of the Netherlands has been prepared in the context of the possible approval of (3*E*)-3-decen-2-one under the PPP Regulation.

During the drafting of the DAR and the CLH proposal it became clear that the active substance actually placed on the market is the E-enantiomer with only a minor fraction of the Z-enantiomer. Therefore, the identity of the substance in this CLH proposal is limited to this enantiomer.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

(3E)-dec-3-en-2-one is an active substance in the meaning of Regulation (EC) No 1107/2009 and therefore no justification is required.

5 IDENTIFIED USES

(3E)-3-Decen-2-one is intended to be used as plant growth regulator in potatoes during storage. The product is applied by hot fogging.

6 DATA SOURCES

This report has been prepared based on the data on (3E)-dec-3-en-2-one that was submitted for the evaluation under Regulation (EC) No 1107/2009 and the evaluation of these data in the DAR (https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-3e-3-decen-2-one.)

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	The active substance (3E)-dec-3-en-2-one is a colourless to pale yellow clear liquid with a ketone-like odour	Benton (2011)[a], Wo (2009)[a]	Visual and organoleptic assessment, respectively.
Melting/freezing point	< -40°C	Benton (2011)[a]	Measured
Boiling point	224°C (99.11%) 226.3°C (98.6%)	Wo (2009)[a], Benton (2011)[a]	Measured
Decomposition/sublimation point	No decomposition or breakdown was observed under the conditions of the test (up to 400°C).	Benton (2011)[a]	Measured
Relative density	0.847 (99.11%)	Wo (2009)[a]	Measured
Vapour pressure	430 Pa at 25°C (98.57%)	Pointer (2009)[a]	Measured
Volatility, Henry's law constant	473.8 $Pa.m^3.mol^{-1}$	Benton (2011)[a]	Calculation: ratio of vapour pressure and water solubility at 25°C
Surface tension	In Neat a.i. At 20°C: 29.4 mN/m At 40°C: 27.2 mN/m In saturated water solution At 20°C: 42.2 mN/m At 40°C: 44.1 mN/m (98.6%)	Benton (2011)[a]	Measured
Water solubility	pH 7: 0.14 g/L (at 24°C) (99.11%) (not determined at pH 5 and pH 9, does not dissociate)	Wo (2009)[a]	Measured
Solubility in organic solvents	n-Heptane >250g/L p-Xylene >250g/L 1,2-Dichloroethane >250g/L Methanol >250g/L Acetone >250g/L Ethyl Acetate >250g/L	Benton (2011)[a] Benton (2014)[a]	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
Partition coefficient n-octanol/water	Log Pow = 3.45 ± 0.02 at 24°C (98.1%) pH 4: 3.45 at 22°C pH 7: 3.47 at 22°C pH 9: 3.43 at 22°C	Wo (2009)[a] Benton (2011)[a]	Measured
Flash point	99°C (98.6%)	Benton (2011)[a]	Measured
Flammability	Not applicable.	-	-
Explosive properties	Not explosive	Neumans (2011)[a]	Based on its chemical structure and DSC profile
Self-ignition temperature	275°C (98.6%)	Benton (2011)[a]	Measured
Oxidising properties	Not oxidizing	Neumans (2011)[a]	Based on its chemical structure and DSC profile
Granulometry	Not applicable		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	No dissociation constant could be determined.	Pointer (2014)[a]	The data showed no difference in spectra between 200-800 nm and as the spectra were consistent across the pH range (pH 1.3-13.2) it is concluded that there was no dissociation of functional groups of 3-decen-2-one. 3-decen-2-one presents no chemical function that enables it to release or fix a free H+ proton in water solution. It has therefore no acidic or alkaline property. Since dissociation in water does not occur, the determination of pKa is not relevant.
Viscosity	Kinematic viscosity: 2.21 mm^2/s (25°C) 1.76 mm^2/s (45°C) Dynamic viscosity: 1.858 mPa.s (25°C) 1.475 mPa.s (45°C)	Bradbury (2010)[a]	Measured
pH	1% (w/w) in water: 4.33	Wo (2009)[a]	Measured

[a] As summarised in the DAR (Volume 3, annex B.2)

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
n.a.	Not explosive	Expert statement	Neumans (2011)

8.1.1 Short summary and overall relevance of the information provided on explosive properties

According to UN RTDG, (3E)-dec-3-en-2-one exhibits no explosive properties at screening based on its chemical structure and DSC profile (peak at 226.28°C with 271.106 J/g).

8.1.2 Comparison with the CLP criteria

No chemical groups associated with explosive properties and decomposition energy is < 500 J/g (DSC). The substance is concluded to be not explosive.

8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed.

8.2 Flammable gases (including chemically unstable gases)

Not applicable.

8.3 Oxidising gases

Not applicable.

8.4 Gases under pressure

Not applicable.

8.5 Flammable liquids

Table 9: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EC A.9	99°C	-	Benton (2011)

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

The flashpoint of the liquid is determined to be 99°C.

8.5.2 Comparison with the CLP criteria

Flashpoint > 60°C. The substance is therefore concluded to be non-flammable.

8.5.3 Conclusion on classification and labelling for flammable liquids

No classification is proposed.

8.6 Flammable solids

Not applicable.

8.7 Self-reactive substances

No endpoint determined, however based on the executed tests with the active substance and the structure of the active substance, no indication of self-reacting properties are demonstrated and therefore no classification is proposed.

8.8 Pyrophoric liquids

No endpoint determined, however based on the flashpoint (99°C) and self-ignition temperature (275°C), no indication of pyrophoric properties is demonstrated and therefore no classification is proposed.

8.9 Pyrophoric solids

Not applicable.

8.10 Self-heating substances

Table 10: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC A.15	275°C	-	Benton (2011)

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

The self-ignition temperature of the liquid is determined to be 275°C.

8.10.2 Comparison with the CLP criteria

Self-ignition temperature > 140°C.

8.10.3 Conclusion on classification and labelling for self-heating substances

No classification is proposed.

8.11 Substances which in contact with water emit flammable gases

Not applicable.

8.12 Oxidising liquids

Table 11: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
n.a.	Not oxidising	Expert statement	Neumans (2011)

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

According to UN RTDG, (3E)-dec-3-en-2-one exhibits no oxidizing properties at screening based on its chemical structure.

8.12.2 Comparison with the CLP criteria

No chemical groups associated with oxidising properties.

8.12.3 Conclusion on classification and labelling for oxidising liquids

No classification is proposed.

8.13 Oxidising solids

Not applicable.

8.14 Organic peroxides

(3E)-3-decen-2-one is not an organic peroxide.

8.15 Corrosive to metals

Based on the structure of (3E)-dec-3-en-2-one (aliphatic ketone) the substance is not considered to be corrosive to metals. Also a 1% dilution has a pH of 4.33, which does not indicate corrosive properties.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosive

No decomposition or breakdown was observed (peak at 226.28 °C with 271.106 J/g) at up to 400 °C (Benton, 2011). (3*E*)-dec-3-en-2-one is not considered to be explosive (Neumans, 2011); the structure of the substance also corroborates this. Hence, no classification was proposed.

Flammable liquid

In a study (EC A.9), the flash point of the liquid is determined to be 99 °C (> 60 °C). The criteria for classification as a flammable liquid are not met and no classification was proposed.

Self-reactive substance or mixture

No test data presented. The available physical-chemical information and the structure of the substance indicates no self-reacting properties; thus, no classification was proposed.

Pyrophoric liquid

No specific studies are available. However, no indication of pyrophoric properties is demonstrated based on the flashpoint (99 °C) and self-ignition temperature (275 °C). Hence the classification procedure has not been applied.

Self-heating substance or mixture

A study conducted in accordance with EC A.15 is available (Benton, 2011). In this study, the self-ignition temperature of the liquid is determined to be 275 °C. No classification was proposed.

Oxidising liquid

(3*E*)-dec-3-en-2-one is not considered to have oxidising properties (Neumans, 2011); the structure of the substance also corroborates this. Hence, no classification was proposed.

Substance or mixture corrosive to metals

No specific test data available. Based on the structure of (3*E*)-dec-3-en-2-one (aliphatic ketone) the substance is not considered to be corrosive to metals. A 1 % dilution has a pH of 4.33, which does not indicate corrosive properties. Thus, no classification was proposed.

Comments received during consultation

One Company-Manufacturer commented and agreed to propose no classification for any of the physical hazards.

Assessment and comparison with the classification criteria

Explosive

The dossier submitter (DS) proposed no classification based on expert judgement relying on the structure of the substance and the lack of observation of decomposition or breakdown at up to 400 °C. RAC notes that the exothermic decomposition energy is below 500 °C; hence, no classification is applicable.

Flammable liquid

The DS presented data on the determined flash point of 99 °C. RAC agrees with the DS that data are conclusive and that classification is not warranted.

Self-reactive substance or mixture

RAC notes that no specific test data are available. RAC agrees with the DS that classification is not warranted based on the decomposition data and the structure of the substance.

Pyrophoric liquid

RAC notes that no specific test data are available. RAC agrees with the DS to not classify (3*E*)-dec-3-en-2-one into the pyrophoric liquid category based on flash point (99 °C) and self-ignition temperature (275 °C) as this shows no potential to ignite spontaneously on coming into contact with air.

Self-heating substance or mixture

RAC agrees with the DS that classification is not warranted based on the determined self-ignition temperature of 275 °C.

Oxidising liquid

The DS proposed no classification based on expert judgement relying on the structure of the substance. RAC agrees with the DS proposal for no classification as the classification procedure for this hazard class shall not apply.

Substance or mixture corrosive to metals

RAC notes that no specific test data is available. However, RAC agrees with the DS that classification is not warranted based on the structure of the substance and the low pH of the dilution solution.

Overall, RAC agrees with the rationale of the DS and that **no classification and labelling for the physical hazards is warranted for (3*E*)-dec-3-en-2-one**.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 12: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
OECD 417 Sprague-Dawley rats 1000 mg/kg bw single oral dose or 1 mg/kg bw single intravenous dose	Oral administration: Bioavailability 91% $T_{1/2}$ dec-3-en-2-one: 7.97 hours Intravenous administration: $T_{1/2}$ dec-3-en-2-one: 0.0631 hours Extensively metabolised	-	CA 5.1.1-02, (2017)
OECD 417 (bone marrow samples taking from study CA 5.1.1-02, 2017)	dec-3-en-2-one was detected in bone marrow up to 24 hours after exposure.	-	CA 5.1.1-03, (2018)

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

The blood and plasma kinetics of (3E)-dec-3-en-2-one (batch 362-104-0581-A-20160329-DRE, purity 99.7%) were evaluated after a single oral and intravenous administration of dec-3-en-2-one in male Sprague-Dawley rats at a dose level of 1000 mg/kg bw for the oral administration and 1 mg/kg bw for the intravenous administration (CA 5.1.1-02). The study was carried out in accordance with OECD 417. Blood and plasma samples were collected at selected times up to 24 hours post-dose for measurement of the test item and radioactivity concentrations.

The systemic bioavailability of total [14C] after a single high, oral dose of [14C]-dec-3-en-2-one to rats was approximately 91% (calculated based on AUCt). Dec-3-en-2-one accounted for approximately 0.3 – 1.5% of the total exposure to radioactivity (in terms of AUCt) following both oral and intravenous administration indicating that a substantial proportion of the radioactivity in plasma was present as metabolites. Following oral administration, [14C]-dec-3-en-2-one was extensively metabolised with up to at least 10 radioactive metabolites being detected. The major metabolite detected was polar fraction P1 which increased with time in the samples analysed to a maximum of 52.4% plasma radioactivity (114 µg equiv./mL) at 24 hours. Low levels of 2-decanone (2.7% plasma radioactivity, 1.87 µg equiv./mL) were tentatively identified. [14C]-2-decanol was not detected in any of the analysed samples. The terminal half-life of dec-3-en-2-one after oral administration was less than 8 hours. Following intravenous administration, the terminal half-life of dec-3-en-2-one was estimated to be 0.0631 hours.

The concentration of radioactivity in bone marrow collected in the previous study were analysed by liquid scintillation counting to show proof of exposure for the micronucleus study. These results demonstrate significant exposure of the bone marrow to dec-3-en-2-one and its metabolites following oral as well as intravenous exposure up to 24 hours after exposure.

10 EVALUATION OF HEALTH HAZARDS

All studies were carried out under GLP unless indicated otherwise. Other than the public literature studies all studies reported in this section were carried out in accordance with OECD guidelines. Minor deviations were noted in some cases, but these did not affect the overall reliability of the studies. The deviations are included in the summaries where relevant.

Acute toxicity

10.1 Acute toxicity - oral route

Table 13: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 425	Rat, Sprague-Dawley, females, 4/dose	(3E)-dec-3-en-2-one (purity 98.57%)	Single dose, gavage at 5000 mg/kg bw	> 5000 mg/kg bw	CA 5.2.1-01, 2009a

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In an acute oral toxicity study (CA 5.2.1-01) four females were treated with a single gavage dose of (3E)-dec-3-en-2-one (batch KB 147-36-1, purity 98.57%) at a dose level of 5000 mg/kg bw. The study was carried out in accordance with OECD 425. An initial limit dose of 5000 mg/kg bw was administered to one female Sprague-Dawley rat. Due to the absence of mortality in this animal, two additional females received the same dose. As one animal died a fourth female was tested. All animals were observed for mortality, signs of gross toxicity and behavioural changes at least once daily for 14 days. Body weight was recorded prior to administration and on days 7 and 14. Necropsies were performed on all animals.

One out of 4 females died on day 2 of the study. In the female that died hypoactivity, anogenital staining, hunched posture and soft faeces was observed. In the surviving animals clinical signs consisted of anogenital staining, hypoactivity with hunched posture, piloerection, reduced faecal volume, soft faeces and facial stains in all animals. The animals were fully recovered by day 6. No effect on bodyweight occurred. Red intestines were observed in the animal that died.

The acute oral LD_{50} was >5000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

According to the Regulation EC No 1272/2008 a substance should be classified for acute oral toxicity when:

Category 1: ATE ≤ 5 mg/kg bw

Category 2: 5 < ATE ≤ 50 mg/kg bw

Category 3: 50 < ATE ≤ 300 mg/kg bw

Category 4: 300 < ATE ≤ 2000 mg/kg bw

Based on the LD_{50} of >5000 mg/kg bw, (3E)-dec-3-en-2-one does not have to be classified for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

No classification proposed.

10.2 Acute toxicity - dermal route

Table 14: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
OECD 402	Rat, Sprague-Dawley, males and females, 5/sex/dose	(3E)-dec-3-en-2-one (purity 98.57%)	5000 mg/kg bw, 24 hours (semi-occlusive)	> 5000 mg/kg bw	CA 5.2.2-01, 2009

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In an acute dermal toxicity study (CA 5.2.2-01) groups of 5 male and 5 females were treated with a single topical application of (3E)-dec-3-en-2-one (batch KB 147-36-1, purity 98.57%) at a dose level of 5000 mg/kg bw for 24 hours. The study was carried out in accordance with OECD 402. The animals were observed for mortality, signs of gross toxicity and behavioural changes at least once daily for up to 14 days. Body weights were recorded prior to application and again on Days 7 and 14 (termination) or after death. Necropsies were performed on all animals.

One out of 5 females died on day 2 of the study, no mortality occurred in males. In the female that died hypoactivity and prone posture was observed. In the surviving animals clinical signs consisted of hypoactivity (recovered by day 2), dermal irritation in all animals (between days 1 and 14). No effect on body weight occurred. Extremely red intestines were observed in the female that died during the study.

The acute dermal LD_{50} was >5000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

According to the Regulation EC No 1272/2008 a substance should be classified for acute dermal toxicity when:

Category 1: ATE ≤ 50 mg/kg bw

Category 2: 50 < ATE ≤ 200 mg/kg bw

Category 3: 200 < ATE ≤ 1000 mg/kg bw

Category 4: 1000 < ATE ≤ 2000 mg/kg bw

Based on the LD_{50} value of >5000 mg/kg bw (3E)-dec-3-en-2-one does not have to be classified for acute dermal toxicity.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification is proposed.

10.3 Acute toxicity - inhalation route

Table 15: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, , form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
OECD 403 Deviation: strain not reported	Rat, males and females, 5/sex/dose	(3E)-dec-3-en-2-one (purity 98.57%) MMAD: 2.6 µm and 2.95 µm	0.52 and 2.04 mg/L, 4-hours (nose-only)	LC_{50} male >1 mg/L LC_{50} female >2.04 mg/L	CA 5.2.3-01, 2009

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In an acute inhalation study (CA 5.2.3-01) groups of five male and five female rats were exposed to 0.52 mg/L or 2.04 mg/L (3E)-dec-3-en-2-one (batch KB 147-36-1, purity 98.57%) for 4 hours, nose-only exposure (mist, MMAD of 2.6 and 2.95 um at 0.52 and 2.04 mg/L, respectively). The study was carried out in accordance with OECD 403 with the deviation that the strain was not reported. In the oral and dermal toxicity studies from the same laboratory, study director and period, the source of the rats was the same as in this inhalation study, so it is likely that Sprague Dawley rats were used.

Details on mortality are reported in the Table below.

Table 16: Summary of observed mortality in the acute inhalation study

Dose (mg/L)	**Males**	**Females**	**Combined**
0.52	1/5	-	1/5
2.04	3/5	0/5	3/10

At 0.52 mg/L the observed clinical signs included hypoactivity, irregular respiration and moist rales, hunched posture, reduced faecal volume, facial and/or anogenital staining in males. At 2.04 mg/L clinical signs included hypoactivity, abnormal respiration, hunched posture, reduced faecal volume, nasal and oral discharge and/or facial staining. Three animals lost weight by day 7. Oedema and discolouration of the lungs, discolouration of the liver and yellow distended intestines in males. In females no gross abnormalities were observed.

The acute inhalation LC_{50} of the test substance was found to be between 0.52 - 2.04 mg/L for male rats and >2.04 mg/L for female rats. Given the incidence of mortality at these dose levels, it is apparent that the LC_{50} in males is close to 2 mg/L and >1 mg/L.

10.3.2 Comparison with the CLP criteria

According to the Regulation EC No 1272/2008 a substance should be classified for acute inhalation toxicity when:

Category 1: ATE ≤ 0.05 mg/L

Category 2: 0.05 < ATE ≤ 0.5 mg/L

Category 3: $0.5 < ATE \leq 1.0$ mg/L

Category 4: $1.0 < ATE \leq 5.0$ mg/L

Based on the LC_{50} value of >1 mg/L (3E)-dec-3-en-2-one should be classified as Acute Tox. 4: H332. As no LC_{50} could be estimated, it is proposed to apply the converted acute toxicity estimate of 1.5 mg/L (dusts or mists) as included in table 3.1.2 in Annex I of CLP.

For substances classified for acute inhalation toxicity and for which the available data indicates that the mechanism of toxicity is corrosivity, labelling with EUH071 'corrosive to the respiratory tract' is required (CLP 3.1.2.3.2). Given the clear macroscopic changes of the lungs in the dead rats in the acute inhalation study (lung oedema and discoloration), and the presence of erosion and ulceration of the respiratory tract in the 5-day inhalation study, this additional label is considered justified.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

(3E)-dec-3-en-2-one should be classified as Acute Tox. 4: H332 with an ATE of 1.5 mg/L and with an additional EUH071 labelling.

RAC evaluation of acute toxicity

ACUTE ORAL TOXICITY

Summary of the Dossier Submitter's proposal

Based on data for acute oral toxicity with an oral LD_{50} value higher than 5000 mg/kg bw in the rat, the DS proposed no classification for acute oral toxicity.

Comments received during consultation

One comment supporting the proposal was received from a Member State Competent Authority (MSCA).

Assessment and comparison with the classification criteria

***Table**: Summary table of animal study on acute oral toxicity*

Method, guideline	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD TG 425	Rat, Sprague-Dawley, females, 4/dose	(3*E*)-3-decen-2-one purity 98.57 %	Single dose, gavage at 5000 mg/kg bw	> 5000 mg/kg bw	CA 5.2.1-01, 2009a

In an acute oral toxicity study, 4 females were treated with a single gavage dose of (3*E*)-3-decen-2-one at a dose level of 5000 mg/kg bw. The study was carried out in accordance with OECD TG 425.

One out of 4 females died on day 2 of the study. Hypoactivity, anogenital staining, hunched posture and soft faeces were observed. In the surviving animals, clinical signs consisted of anogenital staining, hypoactivity with hunched posture, piloerection, reduced faecal volume,

soft faeces and facial stains in all animals. The animals were fully recovered by day 6. No effect on bodyweight occurred. Red intestines were observed in the animal that died.

Based on the result of the study with an acute oral LD_{50} was > 5000 mg/kg bw, RAC agrees with the DS that **no classification for acute oral toxicity is warranted**.

ACUTE DERMAL TOXICITY

Summary of the Dossier Submitter's proposal

Based on data for acute dermal toxicity with a dermal LD_{50} value higher than 5000 mg/kg bw in the rat, the DS proposed no classification for acute dermal toxicity.

Comments received during consultation

Support for the DS proposal was received from one MSCA.

Assessment and comparison with the classification criteria

***Table**: Summary table of animal study on acute dermal toxicity*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
OECD TG 402	Rat, Sprague-Dawley, males and females, 5/sex/dose	(3*E*)-3-decen-2-one purity 98.57 %	5000 mg/kg bw, 24 hours (semi-occlusive)	> 5000 mg/kg bw	CA 5.2.2-01, 2009

In an acute dermal toxicity study, groups of 5 male and 5 females were treated with a single topical application of (3*E*)-3-decen-2-one at a dose level of 5000 mg/kg bw for 24 hours. The study was carried out in accordance with OECD TG 402. The animals were observed for mortality, signs of gross toxicity and behavioural changes at least once daily for up to 14 days. Body weights were recorded prior to application and again on days 7 and 14 (termination) or after death. Necropsies were performed on all animals.

One out of 5 females died on day 2 of the study, no mortality occurred in males. In the female that died, hypoactivity and prone posture were observed. In the surviving animals, clinical signs consisted of hypoactivity (recovered by day 2), and dermal irritation in all animals (between days 1 and 14). No effect on body weight occurred. Extremely red intestines were observed in the female that died during the study.

Based on the result for acute dermal toxicity with an LD_{50} > 5000 mg/kg bw, RAC agrees with the DS that **no classification for acute dermal toxicity is warranted**.

ACUTE INHALATION TOXICITY

Summary of the Dossier Submitter's proposal

The acute inhalation LC_{50} of the test substance was found to be between 0.52 and 2.04 mg/L for male rats and > 2.04 mg/L for female rats. Given the incidence of mortality at these dose levels, it is apparent that the LC_{50} in males is close to 2 mg/L and > 1 mg/L.

Based on the LC_{50} value of > 1 mg/L, the DS proposed classification as Acute Tox. 4; H332 for (3*E*)-3-decen-2-one. As no LC_{50} could be estimated, the DS proposed to apply the converted acute toxicity estimate (ATE) of 1.5 mg/L (dusts or mists) as included in table 3.1.2 in Annex I of CLP.

For substances classified for acute inhalation toxicity and for which the available data indicates that the mechanism of toxicity is corrosivity, labelling with EUH071 'Corrosive to the respiratory tract' is required (CLP 3.1.2.3.2). Given the clear macroscopic changes of the lungs in the dead rats in the acute inhalation study (lung oedema and discoloration), and the presence of erosion and ulceration of the respiratory tract in the 5-day inhalation study, this additional label is considered justified by the DS.

Comments received during consultation

One MSCA supported the proposal, and they also proposed to use the geometric mean for estimating an approximate LC_{50}. The geometric mean would be somewhere around 1.7 mg/L which is close to the ATE of 1.5 mg/L and therefore is well supported. The company-manufacturer agreed with the proposed classification and the selection of the ATE of 1.5 mg/L. They, however, did not agree with the proposed additional EUH071 labelling "Corrosive to the respiratory tract" as the mechanism of toxicity is irritation and not corrosivity. Additionally, the substance is classified as Skin Irrit. 2 and not as corrosive to skin and has no classification for eye irritation.

Assessment and comparison with the classification criteria

***Table**: Summary table of animal study on acute inhalation toxicity*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value LC_{50}**
OECD TG 403 Deviation: strain not reported	Rat, males and females, 5/sex/dose	(3*E*)-3-decen-2-one purity 98.57 % MMAD: 2.6 µm and 2.95 µm	0.52 and 2.04 mg/L, 4 hours (nose-only)	LC_{50} male > 1 mg/L LC_{50} female > 2.04 mg/L

In an acute inhalation study, groups of five male and five female rats were exposed to 0.52 mg/L or 2.04 mg/L (3*E*)-3-decen-2-one for 4 hours, nose-only exposure (mist, MMAD of 2.6 and 2.95 µm at 0.52 and 2.04 mg/L, respectively). The study was carried out in accordance with OECD TG 403 with the deviation that the strain was not reported.

Details on mortality are reported in the table below.

Table: *Summary of mortality*

Dose (mg/L)	Males	Females	Combined
0.52	1/5	-	1/5
2.04	3/5	0/5	3/10

At 0.52 mg/L, the observed clinical signs included hypoactivity, irregular respiration and moist rales, hunched posture, reduced faecal volume, facial and/or anogenital staining in males. At 2.04 mg/L, clinical signs included hypoactivity, abnormal respiration, hunched posture, reduced faecal volume, nasal and oral discharge and/or facial staining. Three animals lost weight by day 7. Oedema and discolouration of the lungs, discolouration of the liver and yellow distended intestines in males were also seen. In females no gross abnormalities were observed.

The acute inhalation LC_{50} of the test substance was found to be between 0.52 and 2.04 mg/L for male rats and > 2.04 mg/L for female rats.

Given the incidences of mortality at these dose levels, RAC estimates that the LC_{50} value in males is close to 2 mg/L and > 1 mg/L which would warrant an Acute Tox. 4 classification. RAC also agrees with the DS and the commenting MSCA to use the converted ATE of 1.5 mg/L (dusts or mists).

At the dose of 0.52 mg/L, extremely red lungs were reported in one male, and at 2.04 mg/L red oedematous lungs were reported in 2 males and dark red extremely oedematous lungs in one additional male. In the 5-day inhalation toxicity study, degeneration, erosion and ulceration of several tissues were reported in animals exposed at 0.531 mg/L and also 0.278 mg/L.

Since data indicate that the mechanism of toxicity is corrosivity, as lung oedema and discoloration of lungs of dead animals and erosion and ulceration of the respiratory tract in the inhalation study were observed, labelling with EUH071 "corrosive to the respiratory tract" is required (CLP 3.1.2.3.2).

Consequently, RAC considers **classification of (3*E*)-3-decen-2-one as Acute Tox. 4; H332, with an ATE of 1.5 mg/L (dusts or mists) and the additional labelling with EUH071, warranted**.

10.4 Skin corrosion/irritation

Table 16: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 404	Rabbit, New-Zealand White, males, 3/dose	(3E)-dec-3-en-2-one (purity 98.57%)	0.5 mL, 4 hours (semi-occlusive)	Erythema (grade 1-4) from 0.5 hours after treatment. Oedema (grade 1-3) from 0.5 hour after treatment Mean score erythema: 3.2 Mean score oedema: 2.1	CA 5.2.4-01, 2009d

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
				Reversible by day 14	
OECD 402	Rat, Sprague-Dawley, males and females, 5/sex/dose	(3E)-dec-3-en-2-one (purity 98.57%)	5000 mg/kg bw, 24 hours (semi-occlusive)	Erythema in 4/5 males and 4/5 females Desquamation 5/5 males and 3/5 females Reversible by day 14 in all animals except one male which had desquamation until day 14.	CA 5.2.2-01, 2009b

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

In a skin irritation study (CA 5.2.4-01) 3 male New-Zealand White rabbits were treated with 0.5 mL (3E)-dec-3-en-2-one (batch KB 147-36-1, purity 98.57%) for 4 hours under semi-occlusive dressing. The study was carried out in accordance with OECD 404. The animals were observed and scored for skin irritation according to Draize at 30-60 minutes, 24, 48 and 72 and at 7, 10 and 14 days after patch removal. Animals were observed for signs of gross toxicity and behavioural changes at least once daily during the test period.
The primary irritation scores are summarized in the table below.

Table 17: Summary table of skin irritation scores

Scores observed after	0.5 hour	24 hrs	48 hrs	72 hrs	7 days	10 days	14 days	Mean score 24-72 hrs
Erythema	2, 3, 4	2, 3, 4	3, 3, 4	3, 3, 4	2, 2, 3	1, 0, 1	0, 0, 0	2.7, 3.0, 4.0
Oedema	3, 3, 3	2, 3, 2	2, 2, 2	2, 2, 2	1, 1, 1	0, 0, 1	0, 0, 0	2.0, 2.3, 2.0

10.4.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.2.2 a substance should be classified for skin irritation Category 2 in the case where:
(1) Mean value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or
(3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Since (3E)-dec-3-en-2-one resulted in a mean erythema score above 2.3 in all three animals in the skin irritation study and supported by the observed effects in the acute dermal study, the substance should be classified as a skin irritant cat. 2.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

(3E)-dec-3-en-2-one should be classified as Skin irritant cat. 2: H315.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed classification as Skin Irrit. 2; H315, based on results from an OECD TG 404 in rabbits. A mean value of ≥ 2.3-≤ 4.0 for erythema/eschar or oedema in at least 2 out of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal were observed.

Comments received during consultation

Support was received for the DS proposal by one Company-Manufacture and one MSCA, which also proposed to include the additional pictogram GHS05.

Assessment and comparison with the classification criteria

Two OECD guideline studies were included in the CLH dossier, one in rabbits and one in rats. A summary is presented below:

***Table**: Summary of skin irritation studies*

Method, guideline	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results -Observations and time point of onset -Mean scores/animal -Reversibility**
OECD TG 404	Rabbit, New-Zealand White, males, 3/dose	(3*E*)-3-decen-2-one purity 98.57 %	0.5 mL, 4 hours (semi-occlusive)	Erythema (grade 1-4) from 0.5 hours after treatment. Oedema (grade 1-3) from 0.5 hour after treatment Mean score erythema: 3.2 Mean score oedema: 2.1 Reversible by day 14
OECD TG 402	Rat, Sprague-Dawley, males and females, 5/sex/dose	(3*E*)-3-decen-2-one purity 98.57 %	5000 mg/kg bw, 24 hours (semi-occlusive)	Erythema in 4/5 males and 4/5 females Desquamation 5/5 males and 3/5 females Reversible by day 14 in all animals except one male which had desquamation until day 14

In a skin irritation study, 3 male New-Zealand White rabbits were treated with 0.5 mL (3*E*)-3-decen-2-one for 4 hours under semi-occlusive dressing. The study was carried out in accordance with OECD TG 404. The animals were observed and scored for skin irritation according to Draize at 30-60 minutes, 24, 48 and 72 hours and at 7, 10 and 14 days after patch removal. Animals were observed for signs of gross toxicity and behavioural changes at least once daily during the test period.

The primary irritation scores are summarised in the table below.

*Table**: Summary of results for primary skin irritation***

Scores observed after	**0.5 hour**	**24 hrs**	**48 hrs**	**72 hrs**	**7 days**	**10 days**	**14 days**	**Mean score 24-72 hrs**
Erythema	2, 3, 4	2, 3, 4	3, 3, 4	3, 3, 4	2, 2, 3	1, 0, 1	0, 0, 0	2.7, 3.0, 4.0
Oedema	3, 3, 3	2, 3, 2	2, 2, 2	2, 2, 2	1, 1, 1	0, 0, 1	0, 0, 0	2.0, 2.3, 2.0

According to Regulation (EC) No 1272/2008 (CLP Regulation) Table 3.2.2, a substance should be classified for skin irritation in Category 2 if it fulfils the following criteria:

(1) Mean value of ≥ 2.3-≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

(3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Since (3*E*)-3-decen-2-one resulted in a mean erythema score above 2.3 in all three animals in the skin irritation study in rabbits, RAC concludes that **(3*E*)-3-decen-2-one warrants classification as Skin Irrit. 2; H315**.

Supplemental information - In depth analyses by RAC

Regarding inclusion of the additional pictogram **GHS05**:

A proposal from one MSCA was referring to CLP Regulation Annex I 3.1.2.3.3 in conjunction with Note 1 of Table 3.1.3. The pictogram GHS05 for corrosivity may also be used in addition to the appropriate pictogram for acute toxicity (in this case GHS07). Until now, substances with a harmonised classification, included in Annex VI of the CLP Regulation labelled with EUH071 were already classified as Skin Corr. 1, 1A, 1B or 1C (H314), i.e., skin corrosive. Consequently, these substances are then already labelled with GHS05, so the consideration of assigning the pictogram GHS05 in addition to EUH071 was never discussed. In this classification proposal, the substance (3*E*)-dec-3-en-2-one is not considered corrosive to skin. However, respiratory tract effects (oedema, discoloration, irritation, erosion and ulceration of the lungs) were observed. Since classification as STOT SE 3; H335 is not considered to avoid double classification, the additional pictogram GHS05 (corrosivity) should be considered. RAC acknowledges the proposal from the MSCA. As no classification for corrosiveness to the skin was proposed for this substance, GHS05 was not proposed. The regulation is clear on the addition of EUH071, but not GHS05 in this case.

Normally, GHS05 is required in case of corrosivity to metals or classification for skin/eye corrosion. This is not the case for (3*E*)-dec-3-en-2-one. GHS07 applies to respiratory tract irritation (STOT SE 3) and skin irritation (proposed for this substance). However, GHS07 is already applied but may not sufficiently reflect the corrosiveness in the respiratory tract.

Note 1 below Table 3.1.3 of the CLP Regulation states that: '*In addition to an appropriate acute toxicity pictogram, a corrosivity pictogram (used for skin and eye corrosivity) may be*

added together with the statement 'corrosive to the respiratory tract'. This could be sufficient basis to apply GHS05. The addition of GHS05 based on the proposed classification with EUH071 'Corrosive to the respiratory tract' can be considered. However, this could also lead to an over-classification. The effects seen in the respiratory tract is covered by the classification for acute toxicity via inhalation and by EUH071, and therefore **RAC does not support the suggestion from the MSCA for the additional pictogram GHS05**.

10.5 Serious eye damage/eye irritation

Table 18: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405	Rabbit, New-Zealand White, females, 3/dose	(3E)-dec-3-en-2-one (purity 98.57%)	0.1 mL	Mean score corneal opacity: 0.1 Mean score iritis: 0 Mean score conjunctival redness: 1.4 Mean score conjunctival chemosis: 0 Reversible by day 10	CA5.2.5-01, 2009e

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

In an eye irritation study (CA 5.2.5-01) 3 female New-Zealand White rabbits were treated with 0.1 mL (3E)-dec-3-en-2-one (batch KB 147-36-1, purity 98.57%) in the conjunctival sac. The left eye remained untreated and served as a control. Ocular irritation was evaluated according to Draize at 1, 24, 48 and 72 hours and at 4, 7, and/or 10 days post instillation. At 24 hours, a fluorescein dye evaluation procedure was used to verify the absence of corneal damage. Observations for signs of gross toxicity and behavioural changes were made at least once daily during the test period.

The primary irritation scores are summarized in the table below.

Table 20: Summary table of eye irritation scores

Scores observed after	1 hr	24 hrs	48 hrs	72 hrs	4 days	7 days	10 days	Mean scores 24-72 hrs
Cornea/opacity	1, 1, 1	1, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0	0.3, 0, 0
Iris	0, 1, 1	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0	0, 0, 0
Conjunctiva redness	2, 2, 2	2, 2, 2	1, 2, 1	1, 1, 1	1, 0, 0	1, 0, 0	0	1.3, 1.7, 1.3
Conjunctiva chemosis	1, 2, 2	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0	0, 0, 0
Conjunctiva discharge	2, 3, 3	1, 2, 2	1, 2, 2	1, 1, 1	1, 0, 1	1, 0, 0	0	1.0, 1.7, 1.7

10.5.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.3.2.1.2 a substance should be classified for Eye irritation Category 2 in the case where:
Substances that produce in at least in 2 of 3 tested animals, a positive response of:
(a) corneal opacity ≥ 1 and/or
(b) iritis ≥ 1, and/or
(c) conjunctival redness ≥ 2 and/or
(d) conjunctival oedema (chemosis) ≥ 2
calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

The mean score was <1 in all animals for corneal opacity and iritis, and <2 in all animals for conjunctival redness and chemosis. Further, all effects were reversible within 10 days. Therefore, (3E)-dec-3-en-2-one does not need to be classified for eye irritation.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

No classification proposed.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for serious eye damage/irritation. Based on an OECD TG 405 study in rabbits, the mean score was < 1 in all animals for corneal opacity and iritis, and < 2 for conjunctival redness and chemosis. Further, all effects were reversible within 10 days.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Summary of the submitted study on eye damage/irritation:

***Table**: Summary of the submitted study on eye damage/irritation*

Method, guideline	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**
OECD TG 405	Rabbit, New-Zealand White, females, 3/dose	(3*E*)-3-decen-2-one purity 98.57 %	0.1 mL	Mean score corneal opacity: 0.1 Mean score iritis: 0 Mean score conjunctival redness: 1.4 Mean score conjunctival chemosis: 0 Reversible by day 10

In an eye irritation study, 3 female New-Zealand White rabbits were treated with 0.1 mL (3*E*)-3-decen-2-one in the conjunctival sac. The left eye remained untreated and served as a

control. Ocular irritation was evaluated according to Draize at 1, 24, 48 and 72 hours and at 4, 7, and/or 10 days post instillation. At 24 hours, a fluorescein dye evaluation procedure was used to verify the absence of corneal damage. Observations for signs of gross toxicity and behavioural changes were made at least once daily during the test period.

According to CLP Regulation Table 3.3.2.1.2, a substance should be classified for eye irritation in category 2 if it fulfils the following criteria:

Substances that produce in at least in 2 of 3 tested animals, a positive response of:

(a) corneal opacity ≥ 1 and/or

(b) iritis ≥ 1, and/or

(c) conjunctival redness ≥ 2 and/or

(d) conjunctival oedema (chemosis) ≥ 2

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

In line with the DS proposal, RAC concludes that **no classification for serious eye damage/irritation is warranted** since the mean score was < 1 in all animals for corneal opacity and iritis, and < 2 for conjunctival redness and chemosis. Further, all effects were reversible within 10 days.

10.6 Respiratory sensitisation

No data available.

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Not relevant.

10.6.2 Comparison with the CLP criteria

Not relevant.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification proposed, data lacking.

10.7 Skin sensitisation

Table 19: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD 406, Buehler test	Guinea pig, Hartley albino	(3E)-dec-3-en-2-one (purity 98.57%)	100% first four inductions 75% remaining inductions	Topical induction caused very faint to moderate erythema (0.5 – 2), following challenge very faint erythema (0.5) was noted.	CA 5.2.6-01, 2009f

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
			Three times a week for 3 weeks 1% challenge, 27 days after first induction		

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

In a skin sensitisation study (CA 5.2.6-01) a group of 20 male and female guinea pigs (Hartley-albino) were treated with 9 topical inductions and 1 topical challenge of (3E)-dec-3-en-2-one (batch KB 147-36-1, purity 98.57%). An addition group of 10 animals served as control. The study was performed in accordance with OECD 406. For the first four inductions 100% w/w of the test material was used while a 75% w/w mixture was used for the remaining challenges. Twenty-seven days after the first induction dose, a challenge dose of the test substance at its highest non-irritating concentration (1% w/w mixture in mineral oil) was applied to a naïve site on each guinea pig. The doses were based on the results of a range-finding study. Approximately 24 hours and 48 hours after each induction and challenge dose, the animals were scored for erythema.

Topical induction caused very faint to moderate erythema (0.5-2). Following challenge with 1% w/w, very faint erythema (0.5) was noted for seven of twenty sites 24 hours after challenge.

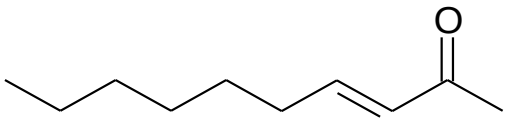
Table 20: Results challenge phase

	Incidence of positive response	
	Hours	
	24	**48**
Test animals	7/20 (severity 0.5)	1/20 (severity 0.5)
Naïve control animals	1/10 (severity 0.5)	0/10

Similar irritation persisted at one site through 48 hours. The very faint erythema (score 0.5) was not considered a positive reaction by the study authors. However, an increase in this score was observed in the treatment group compared to control. Furthermore, the score of 0.5 is not in line with OECD 406 which requires either a score of 0, 1, 2 or 3. Therefore no clear conclusion can be drawn on the skin sensitising properties. However, there is an indication that (3E)-dec-3-en-2-one is a skin sensitiser.

Since no clear conclusion could be drawn on the basis of the study a read-across analysis was carried out.

Skin Sensitization: Read-Across evaluation of (3E)-dec-3-en-2-one



MW = 154.3

Log Kow = 3.16

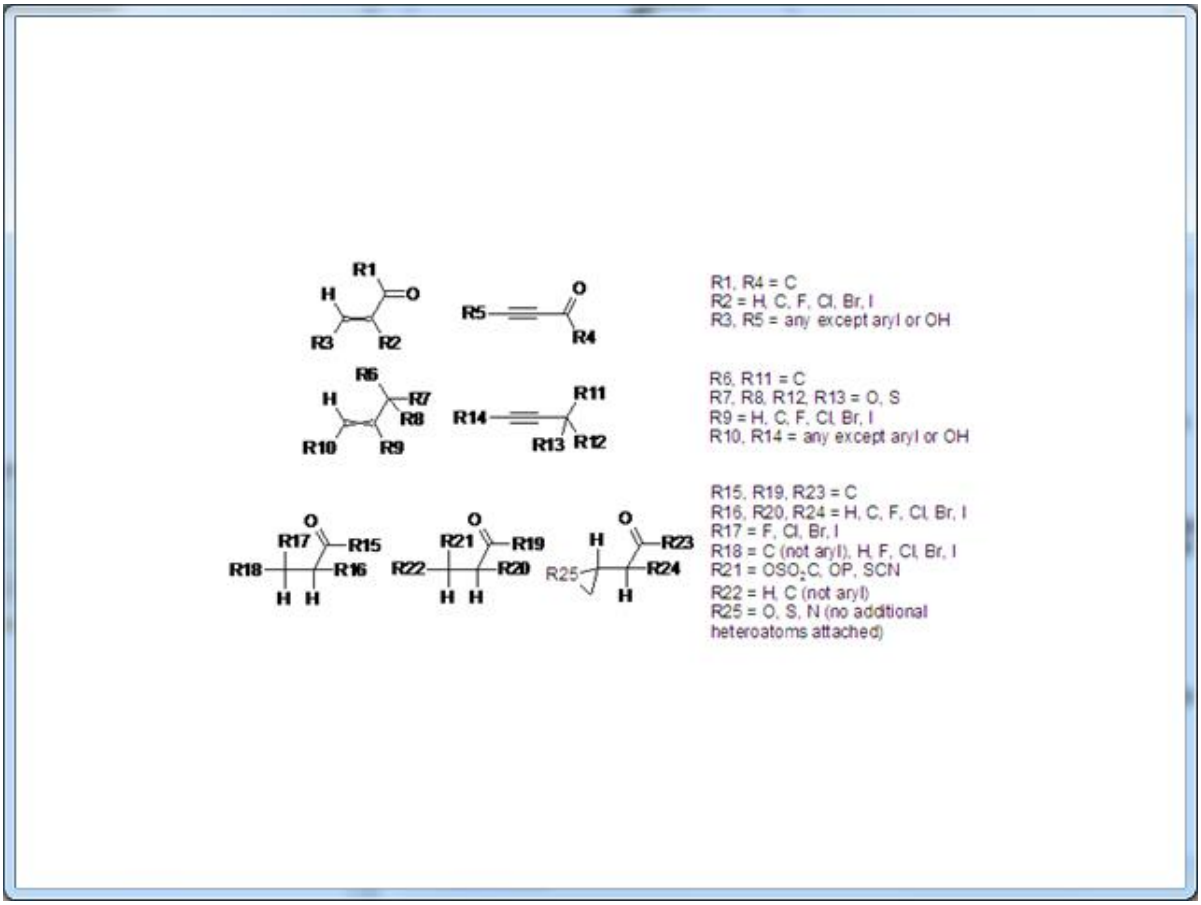
log Kp = -1 .42 (estimated using Potts & Guy with given log Kow and MW)

A prediction of the skin sensitising potential of (3E)-dec-3-en-2-one was made using DEREK (DEREK Nexus v.3.0.1) for all sensitization and irritation endpoints. The prediction for Skin Sensitization in Mammals was: PLAUSIBLE.

"PLAUSIBLE" is the qualitative ranking which indicates that, based on experimental evidence for similar substances, it is predicted that this substance will be a sensitizer, in mammals (which in actual fact almost always means humans). In this case the prediction is based on positive human patch test results for a structurally relevant analogue. "CERTAIN" would indicate there is clear evidence for the substance itself, in humans; "PROBABLE" would indicate there is experimental *animal* data showing sensitization effect (a GPMT, or LLNA test for example) for similar substances, "EQUIVOCAL" would indicate contradicting evidence.

This prediction is based on the presence of the following, mechanistically based, structural alert in the structure:

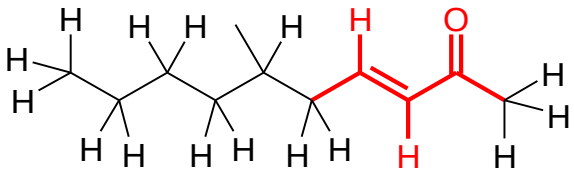
Alert 480: alpha,beta-unsaturated ketone or precursor



This alert describes the skin sensitisation potential of alpha,beta-unsaturated ketones and precursors which interact with skin proteins via a Michael addition mechanism [Nilsson et al, 2001].

The presence of a skin sensitisation structural alert within a molecule indicates the molecule has the potential to cause skin sensitisation. Whether or not the molecule will be a skin sensitiser will also depend upon its percutaneous absorption. Generally, small lipophilic molecules are more readily absorbed into the skin and are therefore more likely to cause sensitisation. It should be noted that (3E)-3-decen-2-one can be considered, in terms of log Kow, log Kp, MW, solubility, a good bioavailable substance, which is expected to be absorbed into the skin sufficiently to be able to cause skin sensitization effects.

The place of the alert in (3E)-dec-3-en-2-one is shown in the following structure representation, where also the H-atoms are indicated:

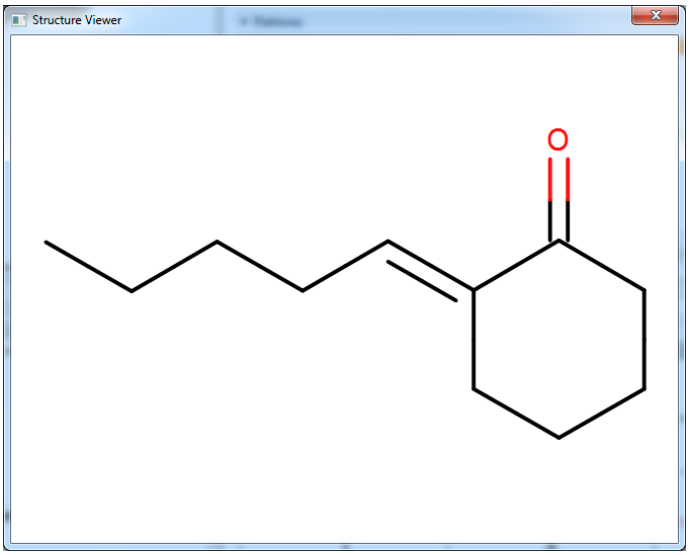


One of the structural requirements for this substructure to be reactive (towards skin proteins) is that one of the substituents of the double bond, farthest away from the (activating) ketone group (this is the beta-position), is a hydrogen; two alkyl substituents in this place would decrease the reactivity (of the double bond, towards electrophilic sites in the skin proteins).

The substituents on the double bond, closest to the ketone group (the alpha-position) are both allowed to be non-hydrogens. A non-hydrogen substituent in this position is also influencing reactivity, but apparently not sufficiently to make the substance a non-sensitizer. In the case of (3E)-dec-3-en-2-one the double bond (the alpha, beta-unsaturated ketone) has a hydrogen substituent in both the alpha and the beta-position, making it very plausible that this double bond will be reactive (towards electrophilic sites in e.g. skin proteins, but also towards DNA).

Based on the structural and physical/chemical properties, (3E)-3-decen-2-one is expected to be a skin sensitiser.

Most relevant analogue with experimental evidence for human skin sensitization having this alert:



2-pentylidene cyclohexanone.

CAS: 25677-40-1

Mw = 166.3

Log Kow = 5.06

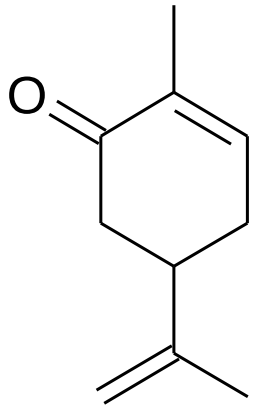
Log Kp = -0.41 (estimated using Potts&Guy with given log Kow and MW)

Skin sensitization result: Strong sensitizer in a Human maximization test [Opdyke, 1998].

The double bond in this molecule is (in contrast to (3E)-dec-3-en-2-one) double substituted in the alpha-position to the ketone. However, the beta-position on the double bond, which is thought to be the site reactive towards proteins (activated by the ketone group next to the double bond) is single-substituted, identical to (3E)-dec-3-en-2-one. The physicochemical properties of this substance are comparable to (3E)-dec-3-en-2-one. (3E)-dec-3-en-2-one can (based on its log Kow and log Kp) is actually expected to be *more* bioavailable in the skin. This makes read-across from the strong sensitization result in humans for 2-pentylidene cyclohexanone conservative, as both the substitution pattern (less steric hindrance expected for reactivity of (3E)-dec-3-en-2-one) as well as the physicochemical properties (lower log Kow, lower log Kp) are expected to lead to higher reactivity and higher absorption in the skin for (3E)-dec-3-en-2-one compared to 2-pentylidene cyclohexanone.

Other relevant analogues:

The substance which led to definition of the alert, and for which the mechanism of reactivity has been studied extensively [Nillson, 2001] is Carvone,



CARVONE

CAS: 99-49-0

CLH: Skin Sens. 1 (Index no: 606-148-00-8, ATP 2015/1221)

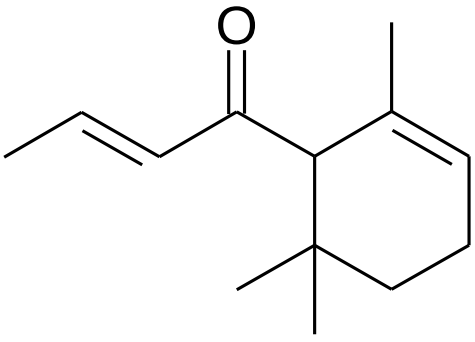
MW = 150.22

Log Kow = 2.2

log Kp = -2.07 (estimated using Potts&Guy with given log Kow and MW)

Carvone is tested as a strong sensitizer in the GPMT [Nillson, 2001]. This substance has the (reactive) double bond which is considered responsible for reactivity with skin proteins and subsequent sensitization reaction, present within a ring structure. The beta-position to the ketone group is single-substituted (has one H-substituent), similar to (3E)-3-decen-2-one. The physicochemical properties of Carvone are very similar to (3E)-dec-3-en-2-one.

Another sensitizing structure, where the reactive double bond is in an aliphatic non-cyclic carbon chain would be alpha-damascone:



DAMASCONE

CAS: 24720-09-0

MW = 192.3

Log Kow = 3.82

Log Kp = -1.18 (estimated using Potts&Guy with given log Kow and MW)

Alpha-Damascone was tested as a strong sensitizer in the GPMT test [Cronin, 1994]

Overall summary and discussion of skin sensitisation

Seven out of twenty sites showed very faint (0.5) erythema 24 hours after challenge, which was not considered a positive reaction by the study authors. Given this doubtful reaction and the known limited sensitivity of the Buehler assay, no conclusion can be drawn regarding the skin sensitising properties of this substance based on this study. Therefore, information from similar substances were collected to assess the possibilities for grouping and read-across. This substance is an alpha,beta-unsaturated ketone that interacts with skin proteins via a Michael addition mechanism. All substances with a high structural similarity are known to induce skin sensitisation.

10.7.2 Comparison with the CLP criteria

The only available study in guinea pigs shows doubtful reactions and as such does not allow a conclusion regarding the skin sensitising properties of this substance. The CLP criteria for animal studies (CLP Annex I 3.4.2.2.3.1-3) are not fulfilled, but there is no human data available either. Therefore, the specific considerations in chapter 3.4.2.2.4 were taken into account. The criteria in 3.4.2.2.4.1 were not met as no such data is available. According to 3.4.2.2.4.3, if a combination of two or more of five indicators are positive, classification could be applied based on a case by case basis. In our opinion indicator e (positive results from close structural analogs) is fulfilled as three close analogs are positive. In addition, indicator c (data from animal tests, performed according to existing guidelines, which do not meet the criteria for a positive result described in section 3.4.2.2.3, but which are sufficiently close to the limit to be considered significant) could be considered as fulfilled as the response in the Buehler test of 0.5 (grading scale) is not considered positive but sufficiently close to the limit of a positive result (a grading scale of 1). The other three indicators are not met as there is no human data or information from non-standard tests. Taken together, as two indicators are fulfilled, classification as a skin sensitiser is considered appropriate. As there is no information on the potency, classification as Skin Sens. 1 is proposed.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Classification as Skin Sens. 1 is proposed.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed classification as Skin Sens. 1 for (3*E*)-3-decen-2-one, based on the results of a Buehler study and on information from similar substances that were collected to assess the possibilities for grouping and read-across.

In their assessment of the Buehler study, performed according to OECD TG 406 on Guinea pigs, the DS concluded that the reactions seen in the study were not clear and as such do not allow a conclusion regarding the skin sensitising properties of this substance.

(3*E*)-3-decen-2-one is an alpha,beta-unsaturated ketone that interacts with skin proteins via Michael addition mechanism. All substances with a high structural similarity are known to induce skin sensitisation. The skin sensitisation prediction made by the DS using the DEREK

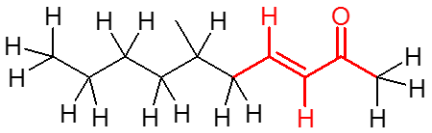
database is based on the presence of the following, mechanistically based structural alert in the structure:

Alert 480: alpha,beta-unsaturated ketone or precursor.

This alert describes the skin sensitisation potential of alpha,beta-unsaturated ketones and precursors which interact with skin proteins via a Michael addition mechanism.

The presence of a skin sensitisation structural alert within a molecule indicates that the molecule has the potential to cause skin sensitisation. Whether or not the molecule will be a skin sensitiser will also depend on its percutaneous absorption. Generally, small lipophilic molecules are more readily absorbed into the skin and are therefore more likely to cause sensitisation. It should be noted that (3*E*)-3-decen-2-one can be considered, in terms of $\log K_{ow}$, $\log K_p$, molecular weight, solubility, a substance with a good bioavailability, which is expected to be absorbed into the skin sufficiently to be able to cause skin sensitisation effects.

The place of the alert in (3*E*)-3-decen-2-one is shown in the structure representation below, where also the hydrogen atoms are indicated:



Comments received during consultation

One Company-Manufacturer did not agree with the use of read-across in this case. They stated that despite the very faint erythema scored as 0.5, the Buehler test should be considered negative as supported by a statement from the study director; thus, the substance has no potential for sensitisation.

One MSCA supported the use of read across and the use of the DEREK prediction (substance is a PLAUSIBLE skin sensitiser) and support the conclusion by the DS.

Assessment and comparison with the classification criteria

One OECD TG 406, Buehler test, was included in the CLH dossier with the following results.

***Table**: Summary of the submitted Buehler test*

Method, guideline	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results**
OECD TG 406, Buehler test	Guinea pig, Hartley albino	(3*E*)-3-decen-2-one purity 98.57 %	100 % first four inductions 75 % remaining inductions Three times a week for 3 weeks 1 % challenge, 27 days after first induction (in mineral oil)	Topical induction caused very faint to moderate erythema (0.5-2), following challenge very faint erythema (0.5) was noted.

In a skin sensitisation study, a group of 20 male and female Guinea pigs were treated with 9 topical inductions and 1 topical challenge of (3*E*)-3-decen-2-one. An additional group of 10 animals served as control. For the first four inductions, 100 % w/w of the test material was

used while a 75 % w/w mixture was used for the remaining challenges. Twenty-seven days after the first induction dose, a challenge dose of the test substance at its highest non-irritating concentration (1 % w/w mixture in mineral oil) was applied to a naïve site on each Guinea pig. The doses were based on the results of a range-finding study. Approximately 24 hours and 48 hours after each induction and challenge dose, the animals were scored for erythema.

Topical induction caused very faint to moderate erythema (0.5-2). Following challenge with 1 % w/w, very faint erythema (0.5) was noted for seven of twenty sites 24 hours after challenge.

***Table**: Results of the challenges*

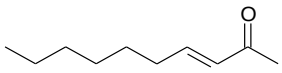
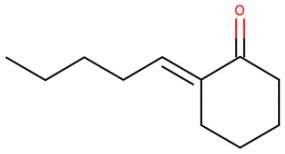
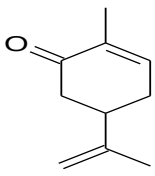
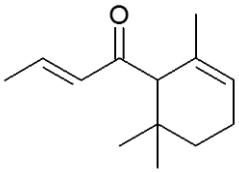
	Incidence of positive response	
	Hours	
	24	**48**
Test animals	7/20 (severity 0.5)	1/20 (severity 0.5)
Naïve control animals	1/10 (severity 0.5)	0/10

Similar irritation persisted at one site through 48 hours. The very faint erythema (score 0.5) was not considered a positive reaction by the study authors. However, an increase in this score was observed in the treatment group compared to control. Furthermore, the score of 0.5 is not in line with OECD TG 406 which requires either a score of 0, 1, 2 or 3. Therefore, no clear conclusion can be drawn from this study on the skin sensitising properties.

Since no clear conclusion could be drawn on the basis of the study, a read-across analysis was carried out.

Read Across approach by the DEREK database

***Table**: Results of the DEREK prediction and structure of the analogues*

Name of the substance	**Structural**	**LogK$_{ow}$ / logK$_p$**	**Results**
Substance to be evaluated (3*E*)-3-decen-2-one		3.16 / 1.42	DEREK; Plausible
2-pentylidene cyclohexanone		5.06 / -0.41	Strong Sensitisation results in human maximization test
Carvone		2.2 / -2.07	Skin Sens. 1 in GPMT
Damascone		3.83 / -1.18	Strong sensitiser in GPMT

This prediction made by using DEREK database, is based on the presence of alpha,beta-unsaturated ketone structural alert in the structure.

This alert describes the skin sensitisation potential of alpha,beta-unsaturated ketones and precursors which interact with skin proteins via a Michael addition mechanism.

Read Across approach by QSAR, VEGA-CEASAR and VEGA-IRFMN/JRC

(3*E*)-3-decen-2-one is predicted positive for skin sensitisation potential via Michael addition in QSAR Toolbox (within applicability domain). In the VEGA–(CEASAR) databases, the substance falls within the applicability domain and the model gives a positive prediction which is, however, based on data from aldehydes. The PRED SKIN provided positive skin sensitisation potential for AOP key events, including LLNA, DPRA, Keratinocyte responses and h-LCAT. However, the prediction for human maximization test and HRIPT were negative.

Assessment and conclusion by RAC

The only available study is a Buehler Guinea pigs study that shows reactions with grading 0.5. There are no human data available either. In their proposal for skin sensitisation, the DS referred to the specific consideration in the CLP Regulation, Annex I 3.4.2.2.4.3: if a combination of two or more of the listed indicators is positive, classification could be applied based on a case-by-case basis. In the DS assessment, indicator e) (positive results from close structural analogues) is fulfilled as close analogues are positive. In addition, indicator c) (data from animal tests, performed according to existing guidelines, which do not meet the criteria for a positive result described in section 3.4.2.2.3, but which are sufficiently close to the limit to be considered significant) is also considered as fulfilled based on the response in the Buehler test of 0.5 (grading scale), which is not considered positive but sufficiently close to the limit of a positive result (a grading scale of 1). The other three indicators are not met as there is no human data or information from non-standard tests.

The only available study for assessing the skin sensitisation potential is a Buehler Guinea pig study. The study director stated in a separate position paper, that the result of the Buehler test is negative, and the substance should not be classified as a skin sensitiser, despite the increased incidence of the 0.5 scores seen in the test animals compared to the controls. Although such increased incidence may indicate a too low concentration chosen for the challenge, the concentration of 1 % used by the study director is considered justified, based on the results of the preliminary irritation study.

RAC agrees that reactions of grade 0.5 in dermal sensitisation studies does not necessarily mean that they are positive for sensitising potential. There is no equivalent grading of 0.5 in the OECD TG 406 and it is important to note that there is no consensus that such responses are directly equivalent to an OECD TG 406 grade 1 scored reaction. Buehler (1994) discussed the scoring system in a 1994 paper and noted that the most controversial aspect was the 0.5 grade for patchy erythema. According to Buehler "this designation covers a wide range of slight reactions from an effect due to hydration to a more substantive erythema that is still patchy". Buehler only considered grades 1, 2 and 3 to be indicative of a clear positive response.

Buehler (1994) noted that in cases where experimental results were not clear a rechallenge would be necessary. If any one of the test animals showed greater reactivity at rechallenge, then the test material could be designated as a sensitiser.

Unfortunately, a rechallenge was not performed in this case. RAC considers that a score of 0.5 therefore seems to imply a doubtful/negligible erythema; hence, the result may be considered negative or the study equivocal and inconclusive for skin sensitisation potential.

The predictions from databases DEREK, CEASAR, QSAR and PREDSKIN indicates positive predictions for skin sensitisation potential. However, the CEASAR database applicability domain is mostly based on data from aldehydes, and the proposed read across substances as weight of evidence for classification are not fit for purpose because of the differences in the chemical structures. PREDSKIN also added uncertainty for the hole read across approach as the Bayesian Outcome shows negative prediction for human maximization and human repeated insult patch test (HRIPT and HMT).

The conclusion by RAC is therefore that **no classification for skin sensitisation is warranted based on inconclusive data**.

10.8 Germ cell mutagenicity

Table 21: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
OECD 471, Ames test	(3E)-dec-3-en-2-one (purity 98%)	Test system: TA98, TA100, TA102, TA1535, TA1537 Test concentrations: 0.0316-5.0 µL/plate Positive controls: 4-NOPD, NaN_3, MMS (-S9); 2-AA (+S9)	Negative with and without S9	CA 5.4.1-01, 2009
OECD 476 gene mutation test, mouse lymphoma cells	(3E)-dec-3-en-2-one (purity 98%)	Test system: mouse lymphoma cells L5178Y Test concentrations: Exp I: 0.05-0.37 mM (+S9), 0.005 – 0.16 mM (- S9) Exp II: 0.10-0.38 mM (+S9), 0.0001-0.04 mM (-S9) Positive controls: BaP (+S9), EMS and MMS (-S9)	Negative with S9 Positive without S9	CA 5.4.1-02, 2009

Table 22: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD 474, micronucleus	(3E)-dec-3-en-2-one (purity 98%)	Test animal: mouse, NMRI 5/sex/dose Single ip dose Dose levels: 1 MTD (50% solution/kg bw), 0.5 MTD (25% solution/kg bw), 0.2 MTD (10% solution/kg bw)	Negative	CA 5.4.2-01, 2009
OECD 486, UDS test	(3E)-dec-3-en-2-one (purity 98.6%)	Rat, Wistar Han 4/sex/dose Dose levels: 1000 and 2000 mg/kg bw	Negative	CA 5.4.2-02, 2011
OECD 489 in vivo Comet assay	(3E)-dec-3-en-2-one (purity 99.5%)	Rat, Wister Han 6 males/dose Dose levels: 500, 1000 and 2000 mg/kg bw/day Two consecutive doses, 24 hours apart	Negative (duodenum and liver)	CA 5.4.2-03, 2016

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro studies

An Ames test (CA 5.4.1-01) was carried according to OECD 471 with (3E)-dec-3-en-2-one (batch 3D2-2009/01, purity 98%). The tested strains were TA 98, TA 100, TA 102, TA 1535 and TA 1537. The concentration range covered two logarithmic decades: 0.0316, 0.100, 0.316, 1.0, 2.5, and 5.0 µL/plate with and without S9. A plate incorporation method was used followed by a pre-incubation method.

No precipitation of the test item was observed in any of the five tester strains used in experiment I and II, with and without metabolic activation. No toxic effects were observed in experiment I. In experiment II toxic effects of the test item were noted in all tester strains at a dose of 5.0 µL/plate (without metabolic activation) and in TA102 at a dose of 5000 µg/plate (with metabolic activation). Under the test conditions, (3E)-dec-3-en-2-one did not induce point mutations in S. typhimurium. See tables below for details.

Table 23: Plate incorporation assay with (3E)-3-decen-2-one – Mean number of revertants

Strain	TA 98		TA 100		TA 102		TA 1535		TA1537	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9

Table 23: Plate incorporation assay with (3E)-3-decen-2-one – Mean number of revertants

Strain	TA 98		TA 100		TA 102		TA 1535		TA1537	
Neg. control (A. dest.)	23	29	109	140	253	319	9	6	8	12
Dec-3-en-2-one										
0.0316 µg/plate	29	34	115	116	253	313	11	10	8	8
0.100 µg/plate	29	36	122	123	263	277	8	8	6	8
0.316µg/plate	29	34	126	114	253	321	10	10	11	9
1.0 µg/plate	27	30	126	116	269	322	11	10	7	11
2.5 µg/plate	18	37	116	117	236	305	11	8	6	9
5.0 µg/plate	23	37	100	123	243	319	7	8	5	8
Pos. control										
4-NOPD	447	-	-	-	-	-	-	-	71	-
NaN_3	-	-	1276	-	-	-	1420	-	-	-
MMS	-	-	-	-	1419	-	-	-	-	-
2-AA	-	3033	-	2655	-	1559	-	235	-	378

Table 24: Pre-incubation assay with (3E)-3-decen-2-one – Mean number of revertants

Strain	TA 98		TA 100		TA 102		TA 1535		TA 1537	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control (A. dest.)	27	32	119	102	175	301	14	8	11	13
Dec-3-en-2-one										
0.0316 µg/plate	29	34	121	100	178	247	11	10	10	11
0.100 µg/plate	27	27	122	106	179	255	11	9	12	10
0.316µg/plate	22	31	117	113	202	242	6	9	12	11
1.0 µg/plate	26	26	131	105	222	233	12	7	11	11
2.5 µg/plate	29	31	110	95	178	236	12	8	9	11
5.0 µg/plate	16	33	0	92	0	139	0	9	0	10
Pos. control										
4-NOPD	459	-	-	-	-	-	0	-	97	-
NaN_3	-	-	1106	-	-	-	1418	-	-	-
MMS	-	-	-	-	795	-	-	-	-	-
2-AA	-	2042	-	1430	-	897	-	120	-	252

A mammalian gene mutation test (CA 5.4.1-02) was carried out in mouse lymphoma cells L5178Y. The study was carried out according to OECD 476. Selection of exposure concentrations was based on data from a pre-experiment.

The results of the study are shown in the table below.

Table 25: Gene mutation in mammalian cells – 1st experiment

Test group	RSG (%)	RCE (%)	RTG (%)	Mutants/10^6 cells	MF	No. Large Colonies	No. Small Colonies
With metabolic activation; 4-hour exposure period							
NC1	94.32	103.5	97.19	121.78		84	22
NC2	91.36	106.10	96.93	147.76		111	25
S1	100	100	100	162.23		98	30
S2				168.06		93	31
dec-3-en-2-one							
0.05	97.41	103.05	10.38	142.69	0.86	No data	No data
0.15	90.97	98.17	89.31	191.81	1.16	No data	No data
0.22	78.42	107.93	84.64	146.18	0.89	No data	No data
0.25	70.58	106.71	75.32	163.45	0.99	No data	No data
0.28	51.20	103.66	53.07	192.50	1.17	No data	No data
0.31	36.61	101.83	37.28	225.79	1.37	95	73
0.34	21.19	100.61	21.32	244.20	1.48	100	73
0.37	13.09	93.29	12.22	315.01	1.91	101	78
Pos. control							
BaP 3.5 µg/mL	42.03	103.66	43.57	432.60	2.62	133	132
Without metabolic activation; 4-hour exposure period							
NC1	115.95	105.04	121.79	96.88		74	28
NC2	111.95	105.64	118.26	88.96		78	19
S1	100	100	100	127.92		108	13
S2				158.58		105	15
dec-3-en-2-one							
0.005	93.55	90.80	84.94	178.64	1.25	No data	No data
0.01	95.89	99.70	95.60	124.27	0.87	No data	No data
0.02	88.87	104.45	92.83	97.12	0.68	No data	No data
0.05	71.75	93.77	67.28	141.04	0.98	No data	No data
0.10	38.14	96.14	36.67	200.76	1.40	No data	No data
0.12	27.05	97.92	26.49	193.38	1.35	91	54
0.14	18.60	93.18	17.33	234.81	1.64	100	51
0.16	13.96	94.36	13.17	265.61	1.85	90	80
Pos. control							
EMS 500 µg/ml	77.55	100.89	78.24	738.31	5.15	No data	No data
MMS 10 µg/ml	86.60	100.30	86.86	388.56	2.71	103	144

Table 26: Gene mutation in mammalian cells – 2nd experiment

Test group	RSG (%)	RCE (%)	RTG (%)	Mutants/10^6 cells	MF	No. Large Colonies	No. Small Colonies
With metabolic activation; 4-hour exposure period							
NC1	111.68	95.91	107.11	168.48		107	21
NC2	103.73	100.58	104.33	151.80		115	19
S1	100.00	100.00	100.00	138.41		111	32
S2				190.20		116	23
dec-3-en-2-one							
0.10	97.06	100.00	97.06	188.45	1.15	No data	No data
0.15	98.39	94.74	93.22	222.77	1.36	No data	No data
0.19	85.13	95.32	81.15	181.65	1.11	No data	No data
0.23	74.59	99.42	74.15	219.37	1.34	No data	No data
0.27	53.95	95.91	51.74	307.97	1.87	No data	No data

Test group	RSG (%)	RCE (%)	RTG (%)	Mutants/10^6 cells	MF	No. Large Colonies	No. Small Colonies
0.31	42.47	85.96	36.51	365.24	2.22	125	61
0.35	21.44	97.66	20.93	246.30	1.50	110	69
0.38	10.42	88.30	9.20	353.79	2.15	135	55
Pos. control							
BaP 3.5 µg/mL	42.03	93.74	33.30	1322.40	8.05	183	105
Without metabolic activation; 24-hour exposure period							
NC1	141.19	95.43	134.73	179.57		119	22
NC2	120.38	92.57	111.44	176.43		115	14
S1	100.00	100.00	100.00	122.40		96	23
S2				117.46		92	23
dec-3-en-2-one							
0.0001	100.59	98.86	99.44	162.56	1.36	No data	No data
0.007	97.97	99.43	97.41	115.30	0.96	No data	No data
0.004	98.53	95.43	94.03	163.74	1.37	No data	No data
0.007	80.10	95.43	76.44	153.03	1.28	No data	No data
0.014	61.51	95.43	58.70	177.95	1.48	No data	No data
0.028	33.36	89.71	29.93	340.70	2.84	141	57
0034	20.43	85.71	17.51	435.14	3.63	104	112
0.04	15.05	87.43	13.16	495.78	4.13	132	109
Pos. control							
EMS 500 µg/ml	82.51	80.57	66.48	1172.73	9.78	dng	dng
MMS 10 µg/ml	54.50	52.57	28.65	1986.63	16.57	123	185

RSG = relative suspension growth, RCE = relative cloning efficiency, RTG = relative total growth

Cytotoxicity: In experiment I the relative total growth (RTG) was 12.22% and 13.71% for the highest concentrations (0.37 and 0.16 mM) evaluated with and without metabolic activation respectively. In experiment II the relative total growth (RTG) was 9.20% and 13.16% for the highest concentrations (0.38 and 0.04 mM) evaluated with and without metabolic activation respectively.
Mutagenicity: In experiment I with metabolic activation all mutant values found were within the historical control data of the test facility BSL BIOSERVICE, no dose-relationship was observed and the mutation frequencies found in the treated groups did not show a biologically relevant increase compared to solvent controls. Without metabolic activation all mutant values found were within or slightly above the historical control data of the test facility. Even though a slight dose-response relationship was observed, the higher mutant value at 0.16 mM was considered as biologically not relevant due to the lack of mutagenicity. However, this should be verified in an independent repetition experiment. In experiment II with metabolic activation some of the mutant values found were within the historical control data of the test facility. Some of the mutant values (at doses of 0.27, 0.31 and 0.38 mM) clearly exceeded the range of historical control data. Two dose groups (0.31 and 0.38 mM) the threshold value of 2 for the mutation factor was slightly exceeded and a slight dose response relationship could be observed. However, since in experiment I no mutagenicity was evaluated up to an RTG of 12.22% these results are considered to be equivocal. In experiment II without metabolic activation all mutant values found up to the dose of 0.0014 mM were within the historical control data of the test facility. At doses from 0.028 mM the data exceeded the historical control range. In addition, in these dose groups the threshold value of 2 for the mutation factor was exceeded and a dose-response relationship could be observed.

Relationship of large to small colonies: Colony sizing was performed for the highest concentrations of the test item and for the controls. A mutation frequency above 2 in combination with an increased

occurrence of small colonies is an indication for potential clastogenic effects and/or chromosomal aberrations. In experiment I with metabolic activation in the highest dose groups tested and increased number of small colonies was noted. However, no clear corresponding mutagenicity was found in these dose groups so no clear conclusion could be drawn. To clarify the findings an independent repetition was performed. In experiment I without metabolic activation all dose groups were considered not clastogenic. In experiment II with metabolic activation the increases in the number of small colonies noted at doses of 0.31 mM and 0.38 mM showed clastogenicity since in this experiment for these dose groups the threshold value for mutagenicity was exceeded. Without metabolic activation an increase in small colonies noted at a dose of 0.034 mM suggests clastogenicity since corresponding mutagenicity was found in this dose group.

In vivo studies

A mouse micronucleus test (CA 5.4.2-01) was performed with (3E)-dec-3-en-2-one (batch 3D2-2009/01, purity 98%). The dose groups were as follows: 1 maximum tolerable dose (MTD) (50% solution/kg bw), 0.5 MTD (25% solution/kg bw), 0.2 MTD (10% solution/kg bw) at 10 ml/kg bw via single ip administration. The dose levels were based on a preliminary study. At a 100% solution/kg bw the treated animal showed severe toxic signs such as catalepsis, loss of weight and constricted abdomen, This dose was therefore conducted to be beyond the MTD. At 50% solution/kg bw animals showed clinical signs including reduction of spontaneous activity, prone position, ataxia, piloerection, closed or half closed eyes and slight weight loses (6-10%). Therefore, this dose was selected as MTD. Peripheral blood was sampled at 44 and 68 hours after dosing.

All animals treated with the highest dose group (1 MTD) showed toxic effects (reduction of spontaneous activity, prone position, clonic convulsion, ataxia, constricted abdomen, piloerection, half eyelid closure, diarrhea, cramps and loss of weight). The animals treated with 25% solution/kg bw (0.5 MTD) showed slight toxic effects after the treatment with the test item. No abnormalities were detected in the animals treated with 10% solution/kg bw (0.2 MTD). The relative PCE values measured for negative control animals were within historical controls. For the 0.2 MTD dose group male PCE values were within the control range, females were reduced compared to controls but the reduction was not statistically significant. The 0.5 MTD and 1 MTD dose groups showed reduced and increased values compared to control in males and females respectively, the differences were not statistically significant. The micronucleated polychromatic erythrocyte values obtained for the negative control were within historical control data. For the 0.2 and 0.5 MTD dose groups the values were within the range of corresponding negative controls. For the 1 MTD dose group values were within negative control values 44 hours after dosing. By 68 hours after dosing male values were reduced compared to control values and females were increased, but the difference was not statistically significant. No biologically relevant increase of micronuclei was found after treatment with the test item in any of the dose groups evaluated. On the basis of the study it is unclear if bone marrow has been sufficiently exposed. In the ADME study radioactivity was measured in bone marrow samples which showed that sufficient exposure occurred up to 24 hours after treatment of an oral dose at 1000 mg/kg bw or an intravenous dose of 1 mg/kg bw. It should be noted that this ADME study was carried out in the rat while the micronucleus study was carried out in mice. CPA used as a positive control demonstrated the validity of the assay (see table below).

Table 27: Summary of the results from the *in vivo* micronucleus test

Test group	Males		Females	
	MN (% mean)	Rel. PCE (mean)	MN (% mean)	Rel. PCE (mean)
44 h interval				
Neg. control (cottonseed oil)	0.31	2.90	0.21	2.36
dec-3-en-2-one				
10% solution/ kg/bw	0.23	2.92	0.22	2.09

25% solution/ kg/bw	0.23	2.32	0.21	3.59
50% solution/ kg/bw	0.30	2.52	0.19	3.69
Pos. control				
CPA 40 mg/kg bw	3.76	1.06	2.47	1.50
68 h interval				
Neg. control (cottonseed oil)	0.31	2.78	0.22	2.30
dec-3-en-2-one				
50% solution/kg/bw	0.28	2.00	0.27	3.00

Rel. PCE: (quotient of polychromatic (immature) erythrocytes to total erythrocytes) x 100

Genotoxicity testing was performed in rat, according to OECD 486, UDS-test (CA5.4.2-02). In Experiment 1, initially two groups of four male rats were dosed orally at a dose volume of 10 mL/kg with the test item at 2000 and 1000 mg/kg and two groups of four male rats were dosed with distilled water and 2AAF (50 mg/kg) as vehicle and positive controls respectively. Perfusion of livers commenced approximately 16 hours after dosing. The viability counts obtained from the initial experiment were lower than expected and therefore, at the request of the sponsor, an exact repeat of Experiment 1 was performed. Experiment 2 was performed in exactly the same way as Experiment 1 except that the positive control was NDHC and liver perfusion started approximately 4 hours after dosing.

There were no premature deaths in any of the dose groups. No clinical signs were observed with the test item at either of the dose levels. For a number of animals in Experiment 1 the cell viability was substantially less than 50%, this was considered to be due to high collagenase potency, and though the cells were processed and scored this experiment was repeated. For the repeated Experiment 1 and Experiment 2 cell viability was considered acceptable. The test item did not induce any marked increases in the incidence of cells in repair at either dose level as no significant increase compared to control was observed in the treatment groups. In Experiment 1 and Experiment 2 the net nuclear gain counts (N-C) were outside the typical range of -2 to -6 in the vehicle and test item dose groups, however these values were considered to be due to experimental variation and therefore acceptable. The positive controls induced a marked increase in the percentage of cells in repair thus demonstrating the viability of the test.

In conclusion, under the test conditions (3E)-dec-3-en-2-one was considered to be non-genotoxic.

Table 28: Summary of the results from the *in vivo* UDS test

Test group	**Net nuclear grain count (N-C)**		**Net nuclear grain count of cells in repair**		**Percentage of cells in repair (N-C ≥5)**	
Experiment 1						
	Mean	SD	Mean	SD	Mean	SD
Neg control (distilled water)	-0.3	0.2	6.6	1.1	1.5	1.3
dec-3-en-2-one						
1000 mg/kg	-0.4	0.6	5.5	0.8	2.5	2.4
2000 mg/kg	-0.5	0.3	9.3	5.8	0.8	0.5
Pos. control						
2-AAF 50 mg/kg	5.6	0.6	10.5	2.7	44.3	11.4
Experiment 1 (repeat)						

Neg control (distilled water)	-2.4	0.5	5.0	-	0.2	0.5
dec-3-en-2-one						
1000 mg/kg	-1.7	0.2	5.7	-	0.2	0.5
2000 mg/kg	-1.6	0.6	5.3	0.0	0.8	1.0
Pos. control						
2-AAF 50 mg/kg	7.6	2.0	11.4	1.4	60.8	9.6
Experiment 2						
Neg control (distilled water)	-0.6	0.2	5.7	-	2.0	2.2
dec-3-en-2-one						
1000 mg/kg	-0.7	0.4	6.3	-	0.2	0.5
2000 mg/kg	-0.4	0.1	5.4	0.4	1.3	0.6
Pos. control						
NDHC 40 mg/kg	5.3	4.3	10.5	3.7	42.8	24.9

An *in vivo* Comet assay (CA 5.4.2-03) was carried out in which (3E)-dec-3-en-2-one (batch HA-2013/02, purity 99.5%) was administered to groups of 6 male Wistar Han rats for two consecutive doses 24 hours apart at dose levels of 500, 1000 and 2000 mg/kg bw/day. The vehicle control group received purified water and the positive control group ethyl methanesulphonate at 200 mg/kg. For the positive control group 3 males were included instead of 6 in the other dose groups. Cell suspensions from the tissues (liver and duodenum) were obtained from male animals in the vehicle control group and in each of the test substance groups 3 hours after administration of the second dose. Cell suspensions from male animals in the positive control group were obtained approximately 3 hours after a single dose. 150 morphologically normal cells were analysed for the presence of comets per animal per tissue. DNA strand breaks were assessed by comparing the mean and median % tail intensities (% TI) from 3-decen-2-one treated animals compared with vehicle control values. The slides were also examined for any overt toxicity, *e.g.* an increase in background debris and/or an increase in the incidence of excessively damaged cells (*i.e.* 'hedgehog' or 'ghost' cells).

No statistically significant increases in the median % TI were observed in either the duodenum or liver of male Wistar Han rats administered dec-3-en-2-one at any dose level, compared to vehicle control values. The positive control compound, ethyl methanesulphonate, produced statistically significant increases in the median % TI in the duodenum and liver ($p<0.001$) when compared to vehicle control values. No "hedgehog" or "ghost" cells were observed in either the duodenum or liver of male Wistar Han rats administered 3-decen-2-one at any dose level.

It is concluded that dec-3-en-2-one did not demonstrate any evidence of causing an increase in DNA strand breaks or cytotoxicity in either the duodenum or liver of male Wistar Han rats when administered orally in this *in vivo* comet test.

Table 29: *In vivo* Comet assay – Liver data

Treatment	Dose (mg/kg/day)	Number of cells scored	Group mean tail intensity%[#] (SD)	Group mean of median tail intensity%[#] (SD)
Vehicle	-	900	3.55 (0.58)	1.05 (0.81)
dec-3-en-2-one	500	900	3.12 (0.49)	1.21 (0.32)
dec-3-en-2-one	1000	900	3.18 (0.36)	1.37 (0.58)
dec-3-den-2-one	2000	900	3.16 (0.54)	1.76 (0.74)
EMS	200	450	50.17 (8.70)	**49.88 (9.49)*****[L+]

Vehicle: Purified water
EMS: Ethyl methanesulphonate (positive control), dosed once only, approx. 3 hours prior to termination
SD: Standard deviation
[#] Occasional apparent errors of ± 1% may occur due to rounding of values for presentation in the table
Statistical analysis performed on median tail intensity values only. p values for comparisons with control using Williams' test, unless indicated otherwise (+t-test)
*** $p < 0.001$ (significant), otherwise $p > 0.05$ (not significant)
L-Analysis performed upon logarithmically transformed data

Table 30: *In vivo* Comet assay – Duodenum data

Treatment	Dose (mg/kg/day)	Number of cells scored	Group mean tail intensity%[#] (SD)	Group mean of median tail intensity%[#] (SD)
Vehicle	-	900	4.43 (0.55)	1.00 (0.36)
dec-3-en-2-one	500	900	4.10 (0.54)	1.00 (0.55)
dec-3-en-2-one	1000	900	3.60 (0.18)	0.80 (0.22)
dec-3-en-2-one	2000	900	3.75 (0.52)	1.19 (0.95)
EMS	200	450	61.63 (6.92)	**64.03 (8.23)*****[L+]

Vehicle: Purified water
EMS: Ethyl methanesulphonate (positive control), dosed once only, approx. 3 hours prior to termination
SD: Standard deviation
[#] Occasional apparent errors of ± 1% may occur due to rounding of values for presentation in the table
Statistical analysis performed on median tail intensity values only. p values for comparisons with control using Williams' test, unless indicated otherwise (+t-test)
*** $p < 0.001$ (significant), otherwise $p > 0.05$ (not significant)
L-Analysis performed upon logarithmically transformed data

10.8.2 Comparison with the CLP criteria

(3E)-dec-3-en-2-one does not cause gene mutation in bacterial strains tested. (3E)-dec-3-en-2-one is considered to be mutagenic in the *in vitro* mouse lymphoma thymidine kinase locus in the cell line L5178Y without metabolic activation. However, (3E)-dec-3-en-2-one is considered to be non-mutagenic with respect to clastogenicity and/or aneugenicity in the *in vivo* mammalian erythrocyte micronucleus test and non-genotoxic in the UDS assay and *in vivo* Comet assay in duodenum and liver.

On the basis of the results of these studies it can be concluded that (3E)-dec-3-en-2-one is not genotoxic.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification proposed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for germ cell mutagenicity based on results from the *in vitro* and *in vivo* experiments.

(3*E*)-3-decen-2-one did not cause gene mutation in bacterial strains tested. It is considered to be mutagenic in the *in vitro* mouse lymphoma thymidine kinase locus in the cell line L5178Y without metabolic activation. However, (3*E*)-3-decen-2-one is considered to be non-mutagenic with respect to clastogenicity and/or aneugenicity in the *in vivo* mammalian erythrocyte micronucleus test and non-genotoxic in the UDS assay and *in vivo* Comet assay in duodenum and liver.

On the basis of the results of these studies, the DS concluded that (3*E*)-3-decen-2-one is not genotoxic.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

A summary of the studies included in the CLH dossier are presented below.

***Table**: Summary of submitted in vitro studies*

Method, guideline.	Test substance	Relevant information about the study	Observations
OECD TG 471, Ames test	(3*E*)-3-decen-2-one purity 98 %	Test system: TA98, TA100, TA102, TA1535, TA1537 Test concentrations: 0.0316-5.0 µL/plate Positive controls: 4-NOPD, NaN_3, MMS (-S9); 2-AA (+S9)	Negative with and without S9
OECD TG 476, gene mutation test, mouse lymphoma cells	(3*E*)-3-decen-2-one purity 98 %	Test system: mouse lymphoma cells L5178Y Test concentrations: Exp I: 0.05-0.37 mM (+S9), 0.005-0.16 mM (- S9) Exp II: 0.10-0.38 mM (+S9), 0.0001-0.04 mM (-S9) Positive controls: BaP (+S9), EMS and MMS (-S9)	Negative with S9 **Positive without S9**

***Table**: Summary of submitted in vivo studies*

Method, guideline	Test substance,	Relevant information about the study (as applicable)	Observations
OECD TG 474, micronucleus	(3*E*)-3-decen-2-one purity 98 %	Test animal: mouse, NMRI 5/sex/dose Single i.p. dose Dose levels: 1 MTD, 0.5 MTD, 0.2 MTD	Negative
OECD TG 486, UDS test	(3*E*)-3-decen-2-one purity 98.6 %	Rat, Wistar Han 4/sex/dose Dose levels: 1000 and 2000 mg/kg bw	Negative
OECD TG 489, *in vivo* Comet	(3*E*)-3-decen-2-one	Rat, Wister Han 6 males/dose	Negative (duodenum and

assay	purity 99.5 %	Dose levels: 500, 1000 and 2000 mg/kg bw/d Two consecutive doses, 24 hours apart	liver)

MTD: maximum tolerated dose

Summary of in vitro studies

An Ames test was carried according to OECD TG 471 in five strains. No precipitation of the test item was observed in any of the tested strains used in experiment I and II, with and without metabolic activation. No toxic effects were observed in experiment I.

Under the test conditions, (3*E*)-3-decen-2-one did not induce point mutations in *S. typhimurium*.

A mammalian gene mutation test was carried out in mouse lymphoma cells L5178Y. The study was carried out according to OECD TG 476. Selection of exposure concentrations was based on data from a pre-experiment.

In experiment I with metabolic activation, all mutant values found were within the historical control of the test laboratory, no dose-relationship was observed, and the mutation frequencies found in the treated groups did not show a biologically relevant increase compared to solvent controls. However, this should be verified in an independent repetition experiment. In experiment II with metabolic activation, some of the mutant values observed were within the historical control data of the test laboratory. Some of the mutant values (at doses of 0.27, 0.31 and 0.38 mM) clearly exceeded the range of historical control data. In two dose groups, (0.31 and 0.38 mM) the threshold value of 2 for the mutation factor was slightly exceeded and a slight dose-response relationship could be observed. However, since in experiment I no mutagenicity was evaluated up to a relative total growth of 12.22 %, these results are considered to be equivocal.

In experiment II without metabolic activation, all mutant values recorded up to the dose of 0.0014 mM were within the historical control data of the test facility. At doses from 0.028 mM, the data exceeded the historical control range. In addition, in these dose groups the threshold value of 2 for the mutation factor was exceeded and a dose-response relationship could be observed. An increase in small colonies noted at a dose of 0.034 mM suggests clastogenicity since corresponding mutagenicity was observed in this dose group.

Summary of in vivo studies

A **mouse micronucleus test** was performed with (3*E*)-3-decen-2-one. The dose groups were as follows: 1 MTD (50 % solution/kg bw), 0.5 MTD (25 % solution/kg bw), 0.2 MTD (10 % solution/kg bw) at 10 mL/kg bw via single i.p. administration.

All animals treated with the highest dose group (1 MTD) showed toxic effects. The animals treated with 25 % solution/kg bw (0.5 MTD) showed slight toxic effects after the treatment with the test item. No abnormalities were detected in the animals treated with 10 % solution/kg bw (0.2 MTD). The relative polychromatic erythrocytes (PCE) values measured for negative control animals were within historical controls. For the 0.2 MTD dose group, male PCE values were within the range of the study controls, females were reduced compared to controls, but the reduction was not statistically significant. The 0.5 MTD and 1 MTD dose groups showed reduced and increased values compared to control in males and females, respectively; the differences were not statistically significant. The micronucleated PCE values obtained for the negative control were within historical control data. For the 0.2 and 0.5 MTD dose groups, the values were within the range of corresponding negative controls. For the

1 MTD dose group, the values were within negative control values 44 hours after dosing. By 68 hours after dosing, the male values were reduced compared to the control values while the female values were increased, but the difference was not statistically significant. No biologically relevant increase of micronuclei was found after treatment with the test item in any of the dose groups evaluated.

Genotoxicity testing was performed in rat, according to OECD TG 486, UDS-test. In experiment I, initially two groups of four male rats were dosed orally at a dose volume of 10 mL/kg bw with the test item at doses of 2000 and 1000 mg/kg bw and two groups of four male rats were dosed with distilled water and N-2-fluorenylacetamide (2AAF, 50 mg/kg bw) as vehicle and positive controls respectively. Perfusion of livers commenced approximately 16 hours after dosing. The viability counts obtained from the initial experiment were lower than expected, and therefore, at the request of the sponsor, an exact repeat of experiment I was performed.

Experiment II was performed in exactly the same way as experiment I except that the positive control was N,N'-dimethylhydrazine dihydrochloride (NDHC) and liver perfusion started approximately 4 hours after dosing.

There were no premature deaths or any clinical signs in any of the dose groups. For some animals in experiment I, the cell viability was substantially less than 50 %, which was considered to be due to high collagenase potency, and though the cells were processed and scored, the experiment was repeated. In both experiments, cell viability was considered acceptable. The test item did not induce any marked increases in the incidence of cells in repair at either dose level as no significant increase compared to control was observed in the treatment groups. In the two experiments, the net nuclear gain counts (N-C) were outside the typical range of -2 to -6 in the vehicle and test item dose groups; however, these values were considered to be due to experimental variation and therefore acceptable. The positive controls induced a marked increase in the percentage of cells in repair, thus demonstrating the viability of the test. Under the test conditions the substance was considered to be non-genotoxic.

An *in vivo* Comet assay was carried out in which (3*E*)-3-decen-2-one was administered to groups of 6 male Wistar rats in two consecutive doses 24 hours apart at dose levels of 500, 1000 and 2000 mg/kg bw/d. The mean and median % tail intensity (% TI) from (3*E*)-3-decen-2-one treated animals compared with vehicle control values were used to assess the DNA strand breaks. No statistically significant increases in the median % TI were observed in either the duodenum or liver of male Wistar rats at any dose level, compared to vehicle control values. The positive control compound, ethyl methane sulphonate, produced statistically significant increases in the median % TI in the duodenum and liver ($p < 0.001$) when compared to vehicle control values. Consequently, (3*E*)-3-decen-2-one did not demonstrate any evidence of causing an increase in DNA strand breaks or cytotoxicity in either the duodenum or liver of male Wistar rats when administered orally.

Conclusion by RAC

(3*E*)-3-decen-2-one did not cause gene mutation in bacterial strains tested. The substance was considered to be mutagenic in the *in vitro* mouse lymphoma thymidine kinase locus in the cell line L5178Y without metabolic activation.

However, (3*E*)-3-decen-2-one was considered to be non-mutagenic with respect to clastogenicity and/or aneugenicity in the *in vivo* mammalian micronucleus test and non-genotoxic in the UDS assay and *in vivo* Comet assay in duodenum and liver.

On the basis of the results of these studies, and in line with the DS proposal, RAC concludes that **no classification is warranted for (3*E*)-3-decen-2-one**.

10.9 Carcinogenicity

No data available.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Not relevant.

10.9.2 Comparison with the CLP criteria

Not relevant.

10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification proposed based on absence of data.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No data were included in the CLH report.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC concludes that **no classification can be concluded based on absence of data**.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

No data available as no reproductive toxicity study is available. No investigation of reproductive organs was conducted in the repeated dose inhalation study.

10.10.2Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Not relevant.

10.10.3 Comparison with the CLP criteria

Not relevant.

10.10.4 Adverse effects on development

Table 31: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 414, prenatal developmental toxicity study Rat, Sprague-Dawley, female,	(3E)-dec-3-en-2-one (batch AMV-1018, purity 99.81%) 0, 100, 300, 1000 mg/kg bw/day GD6-19	NOAEL maternal: 300 mg/kg bw/day based on slightly reduced bodyweight gain (-12%) NOAEL developmental: 1000 mg/kg bw/day	CA 5.6.2-02, 2012

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In a developmental study (CA 5.6.2-02) groups of 24 female Sprague-Dawley rats (0, 100, 300 and 1000 mg/kg bw/day, gavage) were treated with (3E)-dec-3-en-2-one (batch HA-2011/08, purity 99.81%) at gavage doses of 0, 100, 300 and 1000 mg/kg bw/day during gestation day 6 to 19. The study was carried out according to OECD 414.

No mortality or clinical signs of toxicity were observed. The only significant effect was a slight decrease in maternal body weight and food consumption, and a reduced body weight gain at the high dose. No effects on macropathology, pregnancy outcome, foetal body weight or foetal development was observed. The developmental NOAEL was set at 1000 mg/kg bw/day and the maternal NOAEL at 300 mg/kg bw/day based on the reduction in body weight gain.

Dose (mg/kg bw/day)	0	100	300	1000
Maternal effects				
Mortality	No mortality occurred			
Clinical signs	No substance-related findings.			

Dose (mg/kg bw/day)	**0**	**100**	**300**	**1000**
Pregnant animals Body weight (gain)	24	24	24	24
BW day 6	293	286 (-2.3%)	288 (-1.7%)	286 (-2.3%)
Terminal BW	421	414 (-1.7%)	415 (-1.4%)	**400** (-5%)**
Gravid Uterine Weight	89	88 (-1.1%)	88 (-1.1%)	87 (-2.2%)
Adjusted BW day 20	332	326 (-1.8%)	327 (-1.5%)	**313** (-5.7%)**
BW change				
- day 0-6	36	32 (-11.1%)	32 (-11.1%)	33 (-8.3%)
- day 6-20	128	128 (-)	127 (-0.8%)	**114** (-11.6%)**
- day 6-8	7	9	6	5
- day 6-18	95	95	97	90
- day 18-20	33	33	30	**24****
Food consumption	Slightly reduced at 1000 mg/kg bw/day (see table below)			
Pathology				
Macroscopy	At 1000 mg/kg bw/day one female had pale areas on the liver.			
Litter response				
Number of dams examined	24	24	24	24
Resorptions	No substance-related findings.			
Corpora lutea/dam	No substance-related findings.			
Dams with live foetuses	24	24	24	24
Live foetuses/dam				
-females (mean)	7.5	7.7	7.4	7.7
-males (mean)	7.5	7.0	7.3	7.1
Foetal weight	No substance-related findings.			
Placental weight	No substance-related findings.			
Post implantation loss (mean %)	4.6	4.1	4.8	3.2
Sex ratio m/f (%)	49.9/50.1	52.0/48.0	50.4/49.6	52.0/48.0
Examination of the foetuses				
External observations	No substance-related findings.			

Dose (mg/kg bw/day)	**0**	**100**	**300**	**1000**
Skeletal findings (incidence)	No substance-related findings.			
Visceral findings	No substance-related findings.			

10.10.6 Comparison with the CLP criteria

The developmental study in rats did not show effects on macropathology, pregnancy outcome, foetal body weight or foetal development was observed. The developmental NOAEL was set at 1000 mg/kg bw/day, the highest dose tested. Classification is not warranted based on this study in rats.

10.10.7 Adverse effects on or via lactation

No data available.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

Not relevant.

10.10.9 Comparison with the CLP criteria

Not relevant.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

No classification proposed based on absence of data (fertility and lactation).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for adverse effects on sexual function and fertility, due to the lack of data: no reproductive toxicity study is available, and no investigation of reproductive organs was conducted in the repeated dose inhalation study.

No classification on developmental toxicity was proposed by the DS based on results from a developmental study in rats where the NOAEL was found to be 1000 mg/kg bw/d.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

RAC concludes that **no classification can be concluded for adverse effects on sexual function and fertility based on absence of data**.

Adverse effects on development

The summary of the study on developmental toxicity included in the CLH dossier on developmental toxicity.

***Table**: Summary of submitted developmental toxicity study*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**
OECD TG 414, prenatal developmental toxicity study Rat, Sprague-Dawley, 24 females	(3*E*)-3-decen-2-one (batch AMV-1018), purity 99.81 % 0, 100, 300, 1000 mg/kg bw/d GD6-19	NOAEL maternal: 300 mg/kg bw/d based on slightly reduced bodyweight gain (-12 %) NOAEL developmental: 1000 mg/kg bw/d

In this developmental study, groups of 24 female Sprague-Dawley were treated with (3*E*)-3-decen-2-one at gavage doses of 0, 100, 300 and 1000 mg/kg bw/d during GD6 to 19 according to OECD TG 414.

No mortalities or clinical signs of toxicity were observed. The only significant effect was a slight decrease in food consumption and corrected body weight gain at the high dose. No effects on macropathology, pregnancy outcome, foetal body weight or foetal development were observed. The developmental NOAEL was set at 1000 mg/kg bw/d and the maternal NOAEL at 300 mg/kg bw/d based on the reduction in body weight gain.

Conclusion by RAC

Developmental toxicity was assessed with a prenatal developmental toxicity study according to OECD TG 414. In this study, neither mortality nor clinical signs of toxicity and no effects on macropathology, pregnancy outcome, foetal body weight or foetal development were observed. The only significant effect was a reduced corrected body weight gain (-5.7 %) at the high dose (1000 mg/kg bw/d). Consequently, RAC agrees with the DS that a **classification for adverse effects on development is not warranted**.

Effects on or via lactation

No data are available to assess this hazard class, therefore RAC concludes that **no classification can be concluded based on absence of data**.

10.11 Specific target organ toxicity-single exposure

Table 32: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
OECD 425 Rat, Sprague-Dawley Females, 4/dose	(3E)-dec-3-en-2-one (purity 98.57%) Single oral dose at 5000 mg/kg bw	Mortality in one animal at 5000 mg/kg bw Clinical signs: hypoactivity, anogenital staining, hunched posture, piloerection, reduced faecal volume, soft faeces and facial stains. Red intestines in the animals that died.	CA 5.2.1-01, 2009a
OECD 402 Rat, Sprague-Dawley Males and females, 4/dose	(3E)-dec-3-en-2-one (purity 98.57%) Single dermal dose at 5000 mg/kg bw (semi-occlusive)	Mortality in one female, no mortality in males. Clinical signs: hypoactivity, prone posture, dermal irritation. Red intestines in the female that died.	CA 5.2.2-01, 2009b
OECD 403 Rat Males and females 5/sex/dose	(3E)-dec-3-en-2-one (purity 98.57%) MMAD: 2.6 µm and 2.95 µm 0.52 and 2.04 mg/L, 4-hours (nose-only)	Mortality in 3 out of 5 males at 2.04 mg/L and in 1 out of 5 males at 0.52 mg/L. Clinical signs: hypoactivity, abnormal respiration, hunched posture, reduced faecal volume, nasal and oral discharge and/or facial staining. Loss in body weight. Oedema and discolouration of the lungs, discolouration of the liver, yellow distended intestines in males.	CA 5.2.3-01, 2009c
OECD 486 Rat Males and females 4/sex/dose	(3E)-dec-3-en-2-one (purity 98.6%) Single oral dose at 1000 and 2000 mg/kg bw	No clinical signs observed	CA 5.4.2-02, 2011
OECD 489 Rat Males *6/sex/dose*	(3E)-dec-3-en-2-one (purity 99.5%) Single oral dose at 500, 1000 and 5000 mg/kg bw	Clinical signs at 1000 and 2000 mg/kg bw: salivation, flattened posture, decreased activity, unsteady gait, chin rubbing and piloerection	*CA 5.4.2-03*, 2016

10.11.1Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

In the oral acute toxicity studies at the limit dose of 5000 mg/kg bw, a female rat died on day 2 and at postmortem was reported to have reddened intestines, suggesting severe irritation at the site of first contact. In the *in vivo* Comet assay clinical signs included salivation, flattened posture, decreased activity, unsteady gait, chin rubbing and piloerection at 1000 and 2000 mg/kg bw.

Similarly, in the acute dermal toxicity studies (24hr; semi-occlusive exposure), irritation at the site of first contact was reported in all animals at 5000 mg/kg bw.

In the nose-only inhalation exposure (4-hour) study observed effects at postmortem included oedema and discolouring of the lungs at 2.04 mg/L. The clinical signs observed (hypoactivity, irregular respiration, moist rales, oral and nasal discharge) were considered to be associated with the local respiratory tract irritation. A subsequent exposure level of 0.52 mg/L (6.73 mg/L nominal) in 5 male rats resulted in one death and clinical signs evident for all animals similar to those seen at 2.04 mg/L and are considered to be indicative of respiratory tract irritation *i.e.* a consequence of severe local irritant effect rather than systemic toxicity.

10.11.2 Comparison with the CLP criteria

According to the CLP Regulation, substances should be classified for STOT SE when:

- They produce significant toxicity in animals (relevant for humans) or humans following single exposure at certain dose levels: Cat 1
- They have the potential to be harmful to animals (relevant for humans) or humans following single exposure at certain dose levels: Cat 2
- They have transient narcotic effects or cause transient respiratory tract irritation: Cat 3

(3E)-dec-3-en-2-one does not fulfil these criteria as the effects observed at 5000 mg/kg bw in the acute oral and dermal studies were limited and above 2000 mg/kg bw. In the *in vivo* Comet assay clinical signs, including salivation, flattened posture, decreased activity, unsteady gait, chin rubbing and piloerection were observed at 1000 and 2000 mg/kg bw. However, these do not represent significant or specific toxicity and are not considered to be sufficient for classification.

The respiratory irritation observed in the acute inhalation study would warrant classification with STOT SE. However, this would be a double classification as the substance is already classified for acute inhalation toxicity. In addition the label EUH071 is proposed. Therefore, no STOT SE classification is warranted for the inhalation route.

10.11.3Conclusion on classification and labelling for STOT SE

No classification proposed.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on the information from the acute oral and dermal studies. The respiratory irritation observed in the acute inhalation study would warrant classification with STOT SE; however, this would be an additional classification as the

substance is classified for acute inhalation toxicity. Moreover, the addition of the label EUH071 was proposed.

Comments received during consultation

Comments agreeing with the DS proposal were received from one Company-Manufacturer and one MSCA.

Assessment and comparison with the classification criteria

According to the CLP Regulation, substances should be classified for STOT SE when:

- They produce significant toxicity in animals (relevant for humans) or humans following single exposure at certain dose levels: Cat. 1
- They have the potential to be harmful to animals (relevant for humans) or humans following single exposure at certain dose levels: Cat. 2
- They have transient narcotic effects or cause transient respiratory tract irritation: Cat. 3

(3*E*)-3-decen-2-one does not fulfil these criteria as the effects observed at 5000 mg/kg bw in the acute oral and dermal studies were limited and seen above 2000 mg/kg bw. In the *in vivo* Comet assay, clinical signs, including salivation, flattened posture, decreased activity, unsteady gait, chin rubbing, and piloerection were observed at 1000 and 2000 mg/kg bw. However, these do not represent significant or specific toxicity and are not considered to be sufficient for classification.

The respiratory irritation observed in the acute inhalation study would warrant classification with STOT SE. However, this would be a double classification as the substance is already classified for acute inhalation toxicity and the label EUH071 is proposed.

Therefore, RAC concludes that **no classification is warranted for STOT SE, in line with the DS proposal**.

10.12 Specific target organ toxicity-repeated exposure

Table 33: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
None guideline, dose-range finding inhalation study Rat Crl:CD(SD) 3/sex/dose	(3E)-dec-3-en-2-one, batch HA-2013/02 purity 99.4% Inhalation (vapour), snout only, 6-hours daily on 5 consecutive days Concentration: 139, 278, 531 and 816/1103* µg/L *Dose exceeded target of 750 µg/L. 1 animal additionally dosed at 816 µg/L, but stopped due to excessive clinical signs.	816/1103 µg/L: Elevated/unsteady gait, slow breathing, irregular breathing, wet râles, gasping, decreased activity hunched posture. Body weight loss, reduced food consumption. Incomplete deflation and abnormal colour in lungs, enlarged lymph nodes, distended gastro-intestinal tract. Due to excessive clinical signs study was stopped at day 2. 531 µg/L: Body weight loss, reduced food consumption, increased lung weight, increased spleen weight. Incomplete deflation, abnormal colour and firm lungs, enlarged lymph nodes. Histopathological finding (degeneration, inflammation, ulceration, metaplasia, fibrosis, hyperplasia) in olfactory, respiratory and transitional epithelium, nasopharynx, trachea, tracheal bifurcation, larynx, lungs and bronchi. 278 µg/L: Body weight loss, reduced food consumption. Histopathological finding (degeneration, inflammation, ulceration) in olfactory, respiratory and transitional epithelium, nasopharynx, trachea, tracheal bifurcation, larynx, lungs and bronchi. 139 µg/L: No treatment related adverse findings.	CA 5.3.2-01, 2014

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

A waiver was requested by the notifier for short-term oral studies based on the following arguments:

- (3E)-dec-3-en-2-one occurs naturally in the diet
- (3E)-dec-3-en-2-one is approved as a direct food additive
- (3E)-dec-3-en-2-one is not reported to be associated with sub-chronic toxicity
- (3E)-dec-3-en-2-one is metabolized to innocuous components
- Structurally similar compounds did not cause adverse effects in rats exposed for 90-days
- (3E)-dec-3-en-2-one is of very low toxicity potential based on developmental toxicity data

For the comparison with structurally similar compounds the notifier limits the studies to those described in JECFA 2003. However, in the EFSA evaluation (EFSA, 2010) a number of other studies using chemicals within flavouring groups 5 are described, that were evaluated at the 51st and 59th JECFA meeting (see Table below). While the majority of these substances indicate a low oral toxicity a few do seem to induce adverse effects. For example, for 2-heptanone a NOAEL of 20 mg/kg bw/day and methyl-5-heptan-3-one a NOAEL of 82 mg/kg bw/day were found. In the US EPA hazard characterization of 2-heptanone (June, 2010) a maternal NOAEL of 250 mg/kg/day is indicated from an oral prenatal developmental toxicity study in rats. In comparison, the developmental toxicity study provided for (3E)-dec-3-en-2-one indicates a low oral toxicity

with a NOAEL of 300 mg/kg bw/d based on a slight reduction in bodyweight gain. Considering the natural occurrence in the diet, the metabolic pathways and the low toxicity in the developmental toxicity it is agreed that repeated dose oral toxicity studies are not required.

Chemical name	Species; sex; no. per Group	Route	Dose levels (mg/kg bw/day)	Duration	NOAEL (mg/kg bw/d)	Reference	Comments
Acetone [07.050]	Rat; M.F. 10	Drinking water	0, 250, 500, 1000, 2000, 5000	13 weeks	1000[1]	Dietz, 1991 (NTP TOX 3)	3 NTP study. http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox003.pdf
	Mouse; M.F. 10	Drinking water	M: 0, 312.5, 625, 1250, 2500, 5000 F: 0, 625, 1250, 2500, 5000,	13 weeks	2500[1]	Dietz, 1991 (NTP TOX 3)	3 NTP study. http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox003.pdf

Chemical name	Species; sex; no. per Group	Route	Dose levels (mg/kg bw/day)	Duration	NOAEL (mg/kg bw/d)	Reference	Comments
			12500				
	Rat; M.F. 30	Gavage	0, 100, 500, 2500	90 days	100	Sonawane et al. 1986	3 Meeting abstract
	Rat; NR 3	Drinking water	1000	4 weeks	1000[1,2]	Spencer et al. 1978	3 Examinations were limited to specific neurotoxic effects. No other parameters were monitored.
Isopropyl alcohol [02.079]	Human; M 8	Oral	0, 2.6, 6.4	6 weeks	6.4[2]	Wills et al. 1986	3 Paper published in a peer reviewed journal.
	Rat; M 22	Drinking water	0, 870, 1280, 1680, 2520	12 weeks	870	Pliegaard & Ladefoged 1993	3 Good quality study.
Pentan-3-one [07.084]	Rat; F 5	Drinking water	0, 1860	120 days	Not detected (< 1860)	Union Carbide Corp 1977	Good quality unpublished report. Focused on neurtotoxic effect.
2-heptanone [07.002])	Rat; M.F. 15	Gavage (dissolved in corn oil)	0, 20, 100, 500	13 weeks	20	Gaunt et al. 1972a	3 Good quality study-peer reviewed journal.
	Rat; NR 5	Drinking water	0, 500	12 weeks	500[1,2]	Spencer et al. 1978	3 Good quality study-peer reviewed journal.
4-heptanone [07.058]	Rat; M 8	Gavage	0, 1000	90 days	Not detected (<1000)	O'Donoghue& Krasavage, 1980	3 Good quality Unpublished report.
	Rat; M 3	Gavage	0, 1000, 2000, 4000	3 weeks	Not detected (<1000)	Krasavage & O'Donoghue, 1979	3 Good quality Unpublished report.
2-nonanone [07.020]	Rat; M 3	Gavage (undiluted)	0, 1000, 2000, 4000	3 weeks	Not detected (<1000)	Krasavage & O'Donoghue, 1979	3 Good quality Unpublished report.
	Rat; M 8	Gavage (undiluted)	0, 2000	90 days	Not detected (<2000)	O'Donoghue & Krasavage 1980	3 Good quality Unpublished report.
4-methyl-2-pentanone [07.017]	Rat; M.F. 5	Drinking water	0, 1040	120 days	Not detected (<1040)	Union Carbide Corp, 1977	Good quality unpublished report. Focused on neurotoxic effect.
	Rat; F NR	Drinking water	1000	120 days	1000[2]	Homan & Maronpot, 1978	3 Meeting abstract
Methyl-5-	Rat; M	Gavage	82, 410,	13 weeks	82	(IBM Corp,	Good quality unpublished

Chemical name	Species; sex; no. per Group	Route	Dose levels (mg/kg bw/day)	Duration	NOAEL (mg/kg bw/d)	Reference	Comments
heptan-3-one [07.181]	5	(in distilled water)	820	(5 days/week)		1989)	Report – submitted to EPA
2,6-dimethyl-4-heptanone [07.262]	Rat; M 8	Gavage	0, 2000	90 days	Not detected (<2000)	O'Donoghue & Krasavage 1980	3 Good quality Unpublished report.
5-methyl-5-hexen-2-one [07.100]	Rat; M.F 5	Diet	0, 10	14 days	10[2]	Gil & Van Miller, 1987	4 GLP study-unpublished report
2,6,10-Trimethyl-2,6,10-pentadecatrien-14-one [07.114]	Rat; M. F 5	Oral (gavage in maize oil)	0, 0.35, 3.5	14 days	3.5[5]	De Groot et al. 1974	4 TNO Unpublished report
9-decen-2-one [07.262]	Rat; M.F 5	Oral (gavage in corn oil)	0, 250, 500, 1000	28 days	1000[5]	Flavour Industry, 2009	

NR= sex not reported, M = male, F= female

1. Concentration converted to mg/kg bw/day using conversion table for test chemical treatment doses used in PAFA (FDA, 1993)

2 This study was performed at a single dose that produced no adverse effect

3 Summarised by JECFA, 51st meeting (JECFA, 1999)

4 Summarised by JECFA, 59th meeting (JECFA, 2003)

5 The highest dose tested.

A repeated dose inhalation study was carried out in which (3E)-dec-3-en-2-one (batch HA-2013/02, purity 99.4%) was administered to groups of 3 male and 3 female Sprague-Dawley rats at concentrations of 139 µg/L, 278 µg/L, 531 µg/L and 819/1103 µg/L (achieved concentration) for 5 consecutive days for 6 hours a day. The high dose were exposed higher than the target concentration of 750 µg/L. Treatment-related findings were evident in the respiratory tract at 139, 278 and 531 µg/L with the incidence and severity for animals significantly lower for animals exposed to 139 µg/L. A pilot phase consisting of 1 male and 1 female at the high dose was initiated, but as the achieved exposure level of 816 µg/L was not tolerated a lower exposure at 500 µg/L was selected for a new high dose group.

Clinical signs consisting of elevated/unsteady gait, slow breathing, irregular breathing, wet râles, gasping, decreased activity hunched posture were observed in the high dose group of 816/1103 µg/L. Body weight loss was observed at 278 µg/L and higher. Microscopic finding consistent with the irritation properties of the substance were observed from 278 µg/L and above. At 531 µg/L the microscopic findings seen in the lungs correspond to the increased macroscopic finding and the increased lung weight. Test article-related histopathological findings at 139 µg/L were confined to a single incidence of a localised, low grade, squamous metaplasia in the transitional epithelium of the frontal section of the nose, and therefore in a region that was dissimilar to that at higher exposure levels, and this was considered not adverse. Consequently, 139 µg/L is considered the no observed adverse effect level (NOAEL) in this study.

10.12.2Comparison with the CLP criteria

No repeated dose oral studies were conducted with dec-3-en-2-one. The available data for structurally similar compounds indicate a low oral toxicity with the exception of 2-heptatnone with a NOAEL of 20 mg/kg bw/day and methyl-5-heptan-3-one with a NOAEL of 82 mg/kg bw/day. For methyl-5-heptan-3-one, the NOAEL of 82 mg/kg bw/day was based on neurotoxic effects. This substance may undergo

oxidation to a neurotoxic gamma-diketone. This is not the case for dec-3-en-2-one as the gamma spacing of the two ketone moieties is a prerequisite for these neurotoxic effects, thus only ketones with this structural feature may yield neurotoxic metabolites. Therefore, the neurotoxic effects observed with methyl-5-heptan-2-one are not considered relevant for dec-3-en-2-one. Regarding heptan-2-one, the effects seen at 100 mg/kg bw/day (the LOAEL) after 90-day exposure were limited to significantly increased number of cells excreted in urine in both sexes which is not sufficient for classification. It should also be pointed out that heptan-2-one has a shorter alkyl chain (C7) than dec-3-en-2-one (C10) and thus a lower molecular weight. Generally, the toxicity of aliphatic molecules tends to decrease with increasing chain length. Therefore, based on the read-across to oral toxicity data from structurally similar compounds no classification is required. However, it is noted that some uncertainty remains as no short-term oral study is available for 3-decen-2-one itself.

The effects observed in the 5-day inhalation study at the highest dose level of approximately 1 mg/L could be considered sufficient for classification with STOT RE 1. In addition, some respiratory irritation such as olfactory and respiratory epithelium degeneration was also observed at 278 and 531 µg/ml. However, the substance is already classified as Acute Tox inhalation Cat 4. Lethality was observed at the same or a somewhat higher concentration (males 0.5 -2.0 mg/L) as the airway irritation in the repeated dose study. Further, the label EUH071 "corrosive to the respiratory tract is already proposed. To avoid double classification no STOT RE classification is warranted on the basis of the respiratory tract effects observed in the repeated dose inhalation study. However, as no longer repeated dose study by inhalation is available, it remains difficult to assess whether effects could occur at even lower concentration. Therefore, no classification based on absence of data is proposed for both oral and inhalation repeated dose toxicity.

10.12.3Conclusion on classification and labelling for STOT RE

No classification proposed based on absence of data.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed no classification for STOT RE based on absence of data.

The notifier waived the request for short-term oral studies based on the argument that (3*E*)-3-decen-2-one occurs naturally in the diet and is approved as a direct food additive. The substance is not reported to be associated with sub-chronic toxicity nor is it metabolised to innocuous components. Structurally similar compounds did not cause adverse effects in rats exposed for 90 days and the developmental data shows very low toxicity potential.

Comments received during consultation

Comments in agreement with the DS proposal were received from one Company-Manufacturer and from one MSCA.

Assessment and comparison with the classification criteria

No repeated dose toxicity studies (28 or 90 days) were conducted with (3*E*)-3-decen-2-one.

For the comparison with structurally similar compounds, the notifier referred to studies described in the Joint FAO/WHO Expert Committee on Food Additives document (JECFA, 2003).

In the EFSA evaluation (EFSA, 2010), a number of other studies using chemicals within flavouring groups 5 are described, that were evaluated at the 51st and 59th JECFA meeting (see the CLH report). While the majority of these substances indicate a low oral toxicity, a few do seem to induce adverse effects. For example, for 2-heptanone a NOAEL of 20 mg/kg bw/d and methyl-5-heptan-3-one a NOAEL of 82 mg/kg bw/d were found. In the US EPA hazard characterisation of 2-heptanone (June, 2010) a maternal NOAEL of 250 mg/kg bw/d is indicated from an oral prenatal developmental toxicity study in rats.

In comparison, the developmental toxicity study provided for (3*E*)-3-decen-2-one indicates a low oral toxicity with a NOAEL of 300 mg/kg bw/d based on a slight reduction in bodyweight gain; however, the dosing period was short (gestation day (GD) 9 to 16). Considering the natural occurrence in the diet, the metabolic pathways, and the low toxicity in the developmental toxicity study, it has been agreed that repeated dose oral toxicity studies are not required.

Therefore, RAC supports the proposal from the DS that **no classification can be concluded based on absence of data**.

10.13 Aspiration hazard

Viscosity	Kinematic viscosity: 2.21 mm^2/s (25°C) 1.76 mm^2/s (45°C) Dynamic viscosity: 1.858 mPa.s (25°C) 1.475 mPa.s (45°C)	Bradbury (2010)[a]	Measured

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

No information is available regarding aspiration in humans (cases).

The kinematic viscosity of dec-3-en-2-one at 40°C is expected to be between 1.76 and 2.21 mm^2/s.

The molecule dec-3-en-2-one contains mainly carbon and hydrogen atoms but also one oxygen atom.

Seen the skin irritating and lung irritating/corrosive properties after dermal and inhalation exposure (mist), it can be expected that also aspiration of liquid dec-3-en-2-one will cause lung irritation or corrosivity.

10.13.2 Comparison with the CLP criteria

Classification is required when there is reliable and good quality human data or when the substance is a hydrocarbon with a kinematic viscosity of 20.5 mm^2/s or less. The first criterion is not fulfilled as no human cases of aspiration are known. The second criterion is only partially fulfilled as the kinematic viscosity of dec-3en-2-one is below the cut-off. However, dec-3-en-2-one is not a hydrocarbon in the strict sense as it contains also on oxygen atom. However, seeing the irritating or corrosive properties to the lung after inhalation of dec-3en-2-one, it is considered likely that aspiration of the liquid will also result in lung irritation or corrosion. Therefore, classification with Asp. Tox. 1 is considered justified. Classification for the hazard class for substances fulfilling the kinematic viscosity criterion but containing not only hydrogen and carbon atoms is in line with previous RAC conclusions for several alkyl amines

(CAS 61788-46-4, 61790-33-8, 112-90-3, 124-30-1). Also, the "Substances in Category 1 include but are not limited to certain hydrocarbons, turpentine and pine oil." also shows that other compounds than strictly hydrocarbons can be classified as an aspiration hazard (H304).

10.13.3Conclusion on classification and labelling for aspiration hazard

Classification with Asp. Tox. 1 is proposed.0

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

The DS proposed classification as Asp. Tox. 1 based on results from measured kinematic viscosity. The measured kinematic viscosity is 2.21 mm^2/s (25 °C) and 1.76 mm^2/s (45 °C). The criterion for classification is 20.5 mm^2/s or below.

Comments received during consultation

One Company-Manufacturer disagreed with the decision, indicating that (3*E*)-3-decen-2-one actually does not meet criterion 1 (no reliable and good quality human evidence is available) and only partially criterion 2 (kinematic viscosity). However, the substance is not a pure hydrocarbon. The substance should therefore not be classified.

Assessment and comparison with the classification criteria

The following measurements are noted in the CLH report.

Table: *Summary of the submitted data on aspirational hazard*

Property	Value	Reference	Comment (e.g., measured or estimated)
Kinematic viscosity	2.21 mm^2/s (25 °C) 1.76 mm^2/s (45 °C)	Bradbury (2010)[a]	Measured
Dynamic viscosity	1.858 mPa.s (25 °C) 1.475 mPa.s (45 °C)	Bradbury (2010)[a]	Measured

[a] As summarised in the DAR (Volume 3, annex B.2)

No information is available regarding aspiration in humans (cases).

The kinematic viscosity of (3*E*)-3-decen-2-one at 40°C is between 1.76 and 2.21 mm^2/s. The substance contains mainly carbon and hydrogen atoms but also one oxygen atom. Based on the skin irritating and lung irritating/corrosive properties after dermal and inhalation exposure, it can be expected that also aspiration of liquid (3*E*)-3-decen-2-one will cause lung irritation or corrosivity.

Classification is required when there is reliable and good quality human data or when the substance is a hydrocarbon with a kinematic viscosity of 20.5 mm^2/s or less.

The first criterion is not fulfilled as no human cases of aspiration are known. The second criterion is only partially fulfilled as the kinematic viscosity of (3*E*)-3-decen-2-one is below the cut-off but, (3*E*)-3-decen-2-one is not a hydrocarbon in the strict sense as it also contains one oxygen atom. However, seeing the irritating or corrosive properties to the lung after inhalation exposure of (3*E*)-3-decen-2-one, it is considered likely that aspiration of the liquid will also result in lung irritation or corrosion.

Therefore, RAC agrees with the DS that **classification as Asp. Tox. 1 is warranted**.

Supplemental information - In depth analyses by RAC

The DS added this information in its reply to the consultation comments:

Classification for the hazard class for substances fulfilling the kinematic viscosity criterion but containing not only hydrogen and carbon atoms is in line with previous RAC conclusions for several alkyl amines (ECHA, 2011). In addition, the note below Table 3.10.1 in the CLP Regulation, Annex I states: "*Substances in Category 1 include but are not limited to certain hydrocarbons, turpentine and pine oil*". Consequently, other compounds than strictly hydrocarbons can be classified as an aspiration hazard.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 34: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Hydrolysis OECD 111 (EEC C.7) (3E)-dec-3-en-2-one (Purity 98.6%) pH 4, 5 and 9 at 50˚C	pH 4: 33.9% pH 7: 41.3% pH 9: 62.5% Extrapolated DT_{50s} at 20°C pH 4 = 144 days pH 7 = 112 days pH 9 = 61 days	Tier 1 test only Percentage of active ingredient degraded after 5 days at 50˚C. Losses were due to volatilisation, not hydrolysis Study considered reliable with restrictions (Klimisch: 2); question for additional information was set. **Key study** together with statement by Freedlander (2014).	Benton, 2011
Hydrolysis Method: statement in DAR	Substance is completely unreactive to hydrolysis under the normal conditions employed within the OECD study guideline	Accepted in DAR. Study considered reliable (Klimisch: 1) **Key study** together with study by Benton (2011).	Freedlander, 2014
Hydrolysis Method: statement on ECHA's dissemination website	Waived under REACH as the substance is readily biodegradable.	-	ECHA dissemination site, 2021
Photo-degradation in air AOP v1.92	Hydroxyl Half-life = 2.23 hour (cis-isomer) Half-life = 1.99 hour (trans-isomer) Ozone Half-life = 17.91 hour (cis-isomer) Half-life = 8.96 hour (trans-isomer)	Accepted in DAR. Study considered reliable (Klimisch: 1). **Key study**	Benton, 2011

Method	Results	Remarks	Reference
Ready biodegradability OECD 301F GLP: Yes Purity (98.6%) Duration: 28 day	Mean percentage biodegradation by the end of 28-day exposure = 60%	Readily biodegradable, not meeting the ten-day window condition The active substance is trans (E) (3E)- 3-decen-2-one. This isomer was tested in the ready biodegradability study. The cis (Z) isomer is only a minor component in technical grade (3E)- 3-decen-2-one. Precautions were taken to limit volatilization of the test substance from the test flasks, e.g. gas-tight test flasks were used, and at test start pH was measured in separately prepared test flask. Study considered reliable (Klimisch 1). **Key study**	Feil, 2011 ECHA dissemination site, 2021

11.1.1 Ready biodegradability

Substance	Water type	T [°C]	pH	Duration [d]	Transformation at end [%]	Classification
Dec-3-ene-2-one, purity 98.6%	Test water	22±1	7.5	28	60	readily biodegradable*

* Failing the 10 days window, conclusion reported in the DAR

The test item dec-3-en-2-one was investigated for its ready biodegradability in a manometric respirometry test (OECD TG 301F) over a period of 28 days. The biodegradation was followed by the oxygen uptake of the micro-organisms during exposure. Sodium benzoate was tested simultaneously as reference substance and also functioned as a procedure control. The loading of the test item was 105 mg/L corresponding to an oxygen demand of about 305 mg/L ($ThOD_{NH4}$). The loading of the reference item sodium benzoate was 103 mg/L corresponding to an oxygen demand of about 172 mg/L ($ThOD_{NH4}$). Aerobic activated sludge dissolved in test water (deionised water plus analytical grade salts) was incubated at 21-22ºC during 28 days in closed flasks. The change of pressure in the test flasks was measured by means of a manometric method (BSB/BOD-Sensor-System). The test flasks were closed gas-tight by a measuring head. The preparation of the test solutions included dec-3-en-2-one (duplicate), inoculum control (duplicate), procedure (single), abiotic (single) and toxicity controls (single). Temperature was measured daily in the climatized room. The pH was determined at test start and test end in the control, the procedure control and test vessels. At test start, the pH was determined in a separately prepared test flask to prevent loss of test item in the test flasks.

The $ThOD_{NH4}$ of dec-3-en-2-one was calculated to be 2.904 mg O_2/mg test item. The validity criteria of the study were met. The oxygen demand of the inoculum control was 30 mg O_2/L and thus not greater than 60 mg O_2/L within 28 days. The pH at the end of the test was 7.5-7.6 and therefore within the range of pH 6-8.5 as required. The percentage degradation was more than 60% after 4 days of incubation. The difference between duplicate values of degradation at the end of the test was less than 20%.

The reference item sodium benzoate was sufficiently degraded to 93% at day 14 and 28 of incubation. In the toxicity control 34% biodegradation was noted within 14 days and 46% after 28 days. According to the test guidelines the test item can be assumed to be not inhibitory to aerobic activated sludge because degradation was >25% within 14 days. The oxygen demand in the abiotic control was zero.

The mean percentage biodegradation for dec-3-en-2-one at the end of the 28-day exposure was 60% ($ThOD_{NH4}$); duplicate 1= 60% and duplicate 2 = 59%. Considering that 10% biodegradation was already reached at day 3 (duplicate 1 =12%; duplicate 2= 17%), but that the pass level of 60% was only reached at day 28, it was concluded that the 10-day window was not met. Therefore, dec-3-en-2-one is considered to be readily biodegradable but failing the 10-day window.

Comments by RMS
The study was performed adequately, and the results are used for further assessment. During the peer review process there was a request for the isomer composition of the test substance. The active substance is *trans* (E) dec-3-en-2-one, while the test was conducted with technical grade dec-3-en-2-one. Dec-3-en-2-one was tested in all studies, including ready biodegradability, and contains the *cis* (Z) isomer as minor component (% not specified). No information is available on potential differences in biodegradability of the cis/trans isomers of dec-3-en-2-one. Overall, it can be concluded that dec-3-en-2-one is readily biodegradable but failing the 10-day window. These data can be used for the classification of the active substance (3E)-dec-3-en-2-one.

11.1.2 BOD_5/COD

No data.

11.1.3 Hydrolysis

The hydrolytic stability of (3E)-dec-3-en-2-one was assessed in a preliminary test (Tier 1) at pH 4, 7, and 9 at 50˚C according to OECD TG 111. The screening test showed that (3E)-dec-3-en-2-one was unstable, with losses of 34, 41 and 63% at a pH of 4, 7 and 9 respectively. No DT50 values were presented.

The decline observed in the Tier 1 test with non-radiolabelled (3E)-dec-3-en-2-one was not a result of the hydrolysis, but rather volatilization from the test system given the compound's volatile nature. According to the registrant, a tier 2 study will not bring relevant scientific information because of (3E)-dec-3-en-2-one low emission to water and its quick dissipation from water by volatilization (Henry's law constant = 473.8 $Pa.m^3.mol^{-1}$). No further hydrolysis testing was performed.

As an alternative, a scientific justification was provided in the DAR addressing the hydrolysis for (3E)-dec-3-en-2-one. The justification is based on literature information that supports the line of reasoning that the molecule would be completely unreactive to hydrolysis under the normal conditions employed within the OECD study guideline. (3E)-dec-3-en-2-one is considered hydrolytically stable.

Comments RMS:

The RMS agreed with the applicant that no OECD guideline-based hydrolysis study is required to further address the hydrolytical behaviour of (3E)-dec-3-en-2-one.

11.1.4 Other convincing scientific evidence

None.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data.

11.1.4.2 Inherent and enhanced ready biodegradability tests

None.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

There are no simulation data for the water, the water-sediment or the soil compartments

11.1.4.4 Photochemical degradation

Photodegradation in water

No studies were submitted for this endpoint, nor deemed necessary. (3E)-dec-3-en-2-one does not show any appreciable absorption above 270 nm and therefore does not constitute a significant route of degradation/dissipation in the aquatic environment.

Photodegradation in air

Calculations were done using the method of Atkinson for indirect photo-oxidation in the atmosphere through reaction with hydroxyl radicals using the model US EPA AOP v1.92. The OH radical concentration assuming an atmospheric hydroxyl radical concentration of 1.5×10^{6} radicals cm^{-3} was used.

Table 37 Results

Determination	Units	Cis-Isomer	Trans-Isomer
Overall OH Rate Constant	cm^3.molecule-sec^{-1}	$57.57\times10^{-12-}$	$64.41\times10^{-12-}$
Half-Life (12-hr day)	Hours (1.5×10^{6} radicals cm^{-3})	2.23	1.99
Overall Ozone Rate Constant	cm^3.molecule-sec^{-1}	$1.536\times10^{-17-}$	$3.071\times10^{-17-}$
Half-Life	Hours (7×10^{11} mol cm^{-3})	17.91	8.96

The calculations using the method of Atkinson for indirect photo-oxidation in the atmosphere through reaction with hydroxyl radicals (most relevant in EU assessment) resulted in an atmospheric half-life estimated at 2 hours for both the cis and trans isomers indicating the volatilized (3E)-dec-3-en-2-one would be unlikely to be subject to long range atmospheric transport.

Comments RMS:

The RMS agreed with the results regarding the photochemical degradation, presented above.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.2.1 Summary of data/information on environmental transformation

Hydrolysis is not considered as a route of degradation for (3E)-dec-3-en-2-one in the aquatic environment. A screening study with (3E)-dec-3-en-2-one showed that losses of the substance were not due to hydrolysis but rather volatilization from the test system given the compound's volatile nature. The likelihood of hydrolysis was provided in the DAR, which shows that the molecule would be unreactive to hydrolysis under normal conditions employed within the OECD study guideline.

(3E)-dec-3-en-2-one is considered indirectly photodegradable using the model AOPWIN (v1.92). A half-life of 2 hours (with hydroxyl radicals) was estimated for the substance. Under environmentally relevant conditions, e.g. deeper and more turbid waters with lower light intensities, photolysis might be a less relevant degradation route.

In a standard OECD ready biodegradability test, technical grade dec-3-en-2-one was found to be 60 % biodegraded to CO_2 over a test period of 28 days. The test material (purity 98.6%) contained both the active

substance, i.e. *trans* (3E)-dec-3-en-2-one, as well as the *cis* (Z) isomer. The latter was only present as a minor component (exact amount could not be elucidated). Nevertheless, this is not considered a major issue, as both isomers are expected to show similar biodegradation behaviour. The criterion for ready biodegradability is that 60 % degradation is observed during a 10-day window, starting when at least 10 % degradation has been achieved. (3E)-dec-3-en-2-one did not meet the latter criterion and therefore is considered to be "readily biodegradable, but failing the 10-day window".

There are no simulation degradation data for (3E)-dec-3-en-2-one for the water, the water-sediment or the soil compartments.

Following the decision scheme of the CLP guidance in Annex II (Section 4 point a), (3E)-dec-3-en-2-one is considered **not rapidly degradable**. The pass level of the test of 60% (theoretical oxygen demand) was not achieved within 10 days from the onset of biodegradation.

11.3 Environmental fate and other relevant information

Sorption:

Regarding adsorption of the substance to organic matter, three QSAR estimates are presented below.

USES: Koc (L/kg) = 1.26* $Kow^{0.81}$. K_{oc} (L/kg) = 785 L/kg, based on $logK_{ow}$ = 3.45.

EpiSuite™ (KocWin): Koc : 165.2 L/kg (MCI method)

EpiSuite™ (KocWin) Koc : 1069 L/kg (Kow method)

Depending on the QSAR model, the proposed K_{oc} for dec-3-en-2-one varies between the lowest value of **165.2 L/kg** and the highest value of **1069 L/kg**.

Vapour pressure:

The vapour pressure of dec-3-en-2-one was measured as 430 Pa at 25 °C and the Henry's law constant was estimated to be 473.8 Pa x m^3. mol^{-1} (see Section 1, Point 2.3.1 and 2.3.2). The active substance is considered to be highly volatile.

Photodegradation in air:

Reference/notifier	:	Benton, J.R., 2011,	GLP statement	:	Not applicable
Type of study	:	Computer modelling study. No laboratory work was conducted. US EPA AOP v.1.92	Guideline	:	Not applicable
Year of execution	:	2011	Acceptability	:	Acceptable
Test substance	:				

Calculations were done using the method of Atkinson for indirect photo-oxidation in the atmosphere through reaction with hydroxyl radicals using the model US EPA AOP v1.92. The OH radical concentration assuming an atmospheric hydroxyl radical concentration of 1.5×10^6 radicals cm^{-3} was used.

Determination	**Units**	**Cis-Isomer**	**Trans-Isomer**
Overall OH Rate Constant	cm^3.molecule-sec^{-1}	$57.57\times10^{-12-}$	$64.41\times10^{-12-}$
Half-Life (12-hr day)	Hours (1.5×10^6 radicals cm^{-3})	2.23	1.99
Overall Ozone Rate Constant	cm^3.molecule-sec^{-1}	$1.536\times10^{-17-}$	$3.071\times10^{-17-}$
Half-Life	Hours (7×10^{11} mol cm^{-3})	17.91	8.96

The calculations using the method of Atkinson for indirect photo-oxidation in the atmosphere through reaction with hydroxyl radicals (most relevant in EU assessment) resulted in an atmospheric half-life estimated at 2 hours for both the cis and trans isomers indicating the volatilized (3E)-dec-3-en-2-one would be unlikely to be subject to long range atmospheric transport.

11.4 Bioaccumulation

Table 35: Summary of relevant information on bioaccumulation

Method	**Results**	**Remarks**	**Reference**
OECD 107; shake flask method.	log P_{ow}: 3.45 ± 0.02 at 24°C (98.1%) at pH 5.8	K_{ow} below the threshold of log K_{ow} ≥4 **Key study**	Wo, 2009 ECHA dissemination site, 2021
OECD 107; shake flask method.	pH 4: 3.45 at 22°C pH 7: 3.47 at 22°C pH 9: 3.43 at 22°C		Benton, 2011
QSAR Method: BCFBAF* (v3.01)	BCF: 87.8 L/kg wwt (regression-based method) BCF: 138 L/kg wwt (Arnot-Gobas (upper trophic) method)	QSAR Based on a log K_{ow} of 3.45, that was experimentally determined in this dossier (KOWWIN estimates log K_{ow} as 3.28). Reliable **Supportive data**	EPA, 2012
QSAR Method: theoretical relations * Chemical classes: - Pesticides: logBCF = 0.85 logKow – 0.70 - Various organic chemicals logBCF = 0.76 logKow – 0.23	BCF = 171 L/kg BCF = 247 L/kg	QSAR based on a log Kow of 3.45 Reliable **Supportive data**	Veith et al. 1979
QSAR Method: theoretical relations * logBCF = logKow – 1.32	BCF = 135 L/kg	QSAR based on a log Kow of 3.45 Reliable **Supportive data**	Mackay, Don 1982

* For details see section 11.4.1

11.4.1 Estimated bioaccumulation

Using the experimentally determined log Kow of 3.45, a BCF of 171 L/kg was estimated in the DAR using the following relation as defined by Veith et al. 1979 (based on chemical class: pesticides): logBCF = 0.85 logKow – 0.70. In the DAR, two other QSARs were also used to calculate the BCF, i.e. logBCF = 0.76 logKow – 0.23 (Veith et al. 1979, based on chemical class: various organic chemicals) producing a BCF of 247 L/kg and logBCF = logKow – 1.32 (Mackay, Don 1982) producing a BCF of 135 L/kg. Additionally, in this proposal BCF values were also calculated using the BCFBAF (v3.01) module provided in the EPI Suite™-Estimation Program Interface developed by EPA. BCFBAF estimates fish bioconcentration factor and its logarithm using two different methods. By default, BCFBAF uses the log K_{ow} calculated by the KOWWIN module. However, the assessor used for this proposal the higher experimentally determined value of 3.45 instead of the KOWWIN estimated value of 3.28, which resulted in slightly higher BCF values of 87.8 L/kg wwt (regression-based method) and 138 L/kg wwt (Arnot-Gobas (upper trophic) method). Considering that all QSAR estimated values are below the trigger value of 500 L/kg, ***t*he estimated BCF values indicate a low bioaccumulation potential.**

11.4.2 Measured partition coefficient and bioaccumulation test data

There are no experimentally determined bioaccumulation data available for (3E)-dec-3-en-2-one.

There are two studies available that experimentally determined the log K_{ow} of (3E)-dec-3-en-2-one using the shake-flask methodology (OECD TG 107). Wo (2009) reported a log K_{ow} of 3.45 ± 0.02 at pH 5.8 and 24°C, while Benton (2011) reported log K_{ow} values of 3.45, 3.47 and 3.43 at pH 4, 7 and 9 at 22°C, respectively. It should be noted that according to EC A.8 *"The shake-flask method applies only to essentially pure substances soluble in water and n-octanol. It is not applicable to surface active materials (for which a calculated value or an estimate based on the individual n-octanol and water solubilities should be provided)."* As the surface tension of the pure active substance is < 60 mN/m (~30 mN/m) it is considered surface active and therefore the experimentally determined values should be considered with care. Based on the separate solubiliesin water (0.14 g/L) and n-octanol (expected > 250 g/L), the log Pow can be estimated to be ~3.25. Estimated values using BioLoom v1.5 and Kowwin v1.68, are 3.16 and 3.28, respectively. Considering above, the experimental log K_{ow} value of 3.45 is considered acceptable and used for this proposal.

As the experimentally determined log K_{ow} values and the estimated log K_{ow} values are below the threshold of log Kow ≥4, (3E)-dec-3-en-2-one is **considered to have a low potential for bioaccumulation**.

11.5 Acute aquatic hazard

Ecotox studies were performed with dec-3-en-2-one, lot HA-2010/01 (purity 98.6%) and AMV-1018 (purity 99.4%) which primarily consist of the [3E]-dec-3-en-2-one (trans-isomer) and contain minor amounts of cis-isomer. These studies are usable for classification of the trans-isomer. There is no information available regarding differences in toxicity between isomers.

Table 36: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Acute toxicity to fish semi-static OECD 203	Rainbow trout (*Oncorhynchus mykiss*)	dec-3-en-2-one, lot HA-2010/01 Purity: 98.6%	96h-LC50: 1.50 mg/L	mean measured concentrations Acceptable **Key study**	IIA 8.2.1 and 8.2.1.1 Anonymous. 2011a ECHA dissemination site, 2021
Acute toxicity to aquatic invertebrates	*Daphnia magna*	dec-3-en-2-one, lot HA-2010/01	48h-EC50 : 1.68 mg/L	mean measured concentrations	IIA 8.3.1 and 8.3.1.1

semi-static OECD 202		Purity: 98.6%		Acceptable **Key study**	Hoffman, K., Deierling, T. 2011b ECHA dissemination site, 2021
Lemna growth inhibition semi-static OECD 221	*Lemna gibba*	dec-3-en-2-one, batch HA-2010/01 Purity: 98.6%	7d-ErC50: 2.84 mg/L 7-d EyC50: 1.69 mg/L	mean measured concentrations Acceptable **Supportive study**	IIA 8.6 Hoffman, K., Deierling, T. 2012 ECHA dissemination site, 2021
Algal growth inhibition Static OECD 201	*Pseudokirchneriella subcapitata*	dec-3-en-2-one (AMV-1018), Lot nr: 411730MS0036 Purity: 98.6%	72h-ErC50: 2.8 mg/L 72h-EyC50: 0.89 mg/L	mean measured concentrations Acceptable **Key study**	CA 8.2.6.1-03 Vryenhoef, H. 2016 ECHA dissemination site, 2021

11.5.1 Acute (short-term) toxicity to fish

Anonymous, 2011a

Characteristics

Reference	:	STUDY IIA, 8.2.1/01	Species	:	Rainbow trout (*Oncorhynchus mykiss*)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	2011	nominal conc.	:	0.13, 0.28, 0.62, 1.36 and 3.0 mg test item/L
GLP statement	:	Yes	dosing method	:	Semi-static
Guideline	:	OECD 203, EC C.1	Acceptability	:	Acceptable
test substance	:	Dec-3-en-2-one, lot HA-2010/01	96-h LC_{50}	:	1.50 mg/L
Purity	:	98.6%			

Methods

A 96-hour acute toxicity test in juvenile rainbow trout (*Oncorhynchus mykiss*) (1 replicate of 7 fish per concentration, not fed for 24 hours prior to testing) was conducted under semi-static conditions (daily renewal) with dec-3-en-2-one (98.6% pure) at test concentrations of 0.13, 0.28, 0.62, 1.36 and 3.0 mg test item/L. The test included an untreated control also tested in 1 replicate of 7 fish. The test solutions were prepared by serial dilution of a stock solution in test medium. The concentrations of dec-3-en-2-one were analytically confirmed by GC-MS in duplicate samples of all test solutions taken on day 0 and 3 (fresh) and day 1 and 4 (aged). Samples were injected directly into the GC-MS. Quantification was based on a (non-linear) calibration curve extending from 0.075-4 mg/L. The method was validated by analysis of samples of test water fortified with the test item at levels of 0.1, 0.2, 1 and 3 mg/L (n=8 at each level). Recovery was acceptable (range 68-123%, mean 96%, RSD 12%) at 0.2-3 mg/L, but at 0.1 mg/L 3 recoveries were not acceptable (38, 45 and 140%). The reported LOD was 0.05 mg/L, the reported LOQ was 0.2 mg/L. The LOD and LOQ are considered to be acceptable.

Results

The measured concentrations in the test solutions are shown in Table 39 below. No accurate mean measured concentrations could be calculated for nominal test concentrations of 0.13 and 0.28 mg test item/L, but this is

acceptable since only the two highest concentrations are relevant for estimation of the LC_{50} value. Test endpoints were based on geometric mean measured concentrations, which is acceptable.

Table 39 Measured concentrations (% of nominal) of 3-decen-2-one in test solutions

	Measured concentrations (% of nominal) at nominal concentration (mg test item/L):				
Day	**0.13**	**0.28**	**0.62**	**1.36**	**3**
0 (fresh)	<LOQ	78	105	90	90
	<LOQ	78	112	92	93
1 (aged)	<LOD	<LOQ	52	43	61
	<LOD	<LOQ	44	40	60
3 (fresh)	<LOQ	97	143	131	n.a.
	<LOQ	96	131	132	n.a.
4 (aged)	<LOD	<LOQ	43	57	n.a.
	<LOD	<LOQ	32	62	n.a.
geometric mean (% nominal)	-	-	72	75	74
geometric mean (mg/L)	-	-	0.45	1.02	2.22

n.a. = not applicable (all fish dead after 24 hours.

Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. Clinical signs of intoxication were observed at the two highest test concentrations and included dark colouration, strongly extended gills, exophthalmus, fish mainly on the bottom, fish lying on side or back on the bottom, hyperventilation, tumbling during swimming. Mortality was 0% in the control and all test solutions, except at the highest concentration, where all fish died (See table 40).

Table 40: Fish mortality:

Geomean exposure concentration (mg a.s./L)	**% mortality**
Control	0
<LOD	0
<LOQ	0
0.45	0
1.02	0
2.22	100

The 96-hour LC_{50}, determined as the geometric mean measured concentration of the two highest test concentrations, is 1.50 mg/L.

Remarks

The study is acceptable. The 96-hour LC_{50} is 1.50 mg/L (based on geometric mean measured concentrations) and can be used for classification purposes.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Hoffman and Deierling, 2011b

Characteristics

Reference	:	STUDY IIA, 8.3.1/01	Species	:	Daphnia magna

type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	2011	nominal conc.	:	0.088, 0.19, 0.43, 0.94, 2.07, 4.55 and 10 mg test item/L
GLP statement	:	Yes	dosing method	:	Semi-static
Guideline	:	OECD 202, EC C.2	Acceptability	:	Acceptable
test substance		dec-3-en-2-one, lot HA-2010/01	48-h EC50	:	1.68 mg/L
Purity	:	98.6%			

Methods

A 48-hour acute toxicity test in Daphnia magna (<24 h old, 4 replicates of five daphnia each for the treatments and the untreated control) was conducted under semi-static conditions (daily renewal) with dec-3-en-2-one (98.6% pure) at nominal test concentrations of 0.088, 0.19, 0.43, 0.94, 2.07, 4.55 and 10 mg test item/L. The test solutions were prepared by serial dilution of a stock solution in test medium.
The concentrations of dec-3-en-2-one were analytically confirmed by GC-MS in duplicate samples of test solutions from 0.19-10 mg test item/L taken on day 0 and 1 (fresh) and day 1 and 2 (aged). Samples were injected directly into the GC-MS. Quantification was based on a (non-linear) calibration curve extending from 0.1-12.5 mg/L. The method was validated by analysis of samples of test water fortified with the test item at levels of 0.2, 1 and 10 mg/L (n=4 at each level). Recovery was slightly high, but still considered to be acceptable (range 108-130%) at 1-10 mg/L, but at 0.2 mg/L the method performance was inadequate (recoveries 162-332%). The reported LOD was 0.053 mg/L and the reported LOQ was 1 mg/L. The LOD and LOQ are considered acceptable.

Results

The measured concentrations in the test solutions are shown in Table 41 below. No accurate mean measured concentrations could be calculated for nominal test concentrations of 0.19 and 0.43 mg test item/L, as these nominal concentrations were below the LOQ of the analytical method. This is acceptable since these two concentrations are not relevant for estimation of the EC_{50} value. Test endpoints were based on geometric mean measured (mm) concentrations, which is acceptable.

Table 41 Measured concentrations (% of nominal) of 3-decen-2-one in test solutions

	Measured concentrations (% of nominal) at nominal concentration (mg test item/L):					
Day	**0.19**	**0.43**	**0.94**	**2.07**	**4.55**	**10**
0 (fresh)	<LOQ	<LOQ	102	86	89	92
	<LOQ	<LOQ	98	89	89	93
1 (aged)	<LOD	<LOD	46	42	50	59
	<LOD	<LOD	44	42	50	58
1 (fresh)	<LOD	<LOQ	76	76	88	88
	<LOD	<LOQ	66	75	84	92
2 (aged)	<LOQ	<LOQ	87	70	72	77
	<LOQ	<LOQ	87	79	72	73
geometric mean (% nominal)	-	-	75	69	73	79
geometric mean (mg/L)	-	-	0.71	1.43	3.32	7.90

Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. Immobility was 0% in the control and the test solutions up to gmm 0.71 mg/L, and 10%, 100% and 100%, respectively, at gmm 1.43, 3.32 and 7.90 mg/L (see table B.9.2.2.1-04).

Table 41: Daphnia immobility:

Geomean exposure concentration (mg a.s./L)	**% immobility**
Control	0
<LOQ (nominal 0.19)	0

<LOQ (nominal 0.43)	0
0.71	0
1.43	10
3.32	100
7.90	100

The 48-hour EC_{50} (Probit analysis) is 1.68 mg/L.

Remarks

The study is acceptable. The 48-hour EC_{50} is 1.68 mg/L (based on geometric mean measured concentrations) and will be used for classification purposes.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Hoffman and Deierling, 2012

Characteristics

Reference	:	STUDY IIA, 8.6/01	Species	:	*Lemna gibba*
Type of study	:	Lemna, growth inhibition	Exposure duration	:	7 days
Year of execution	:	2012	Nominal concn.	:	0.24, 0.76, 2.44, 7.81, 25.0 mg/L
GLP statement	:	Yes	Dosing method	:	Semi-Static; stock solution in test water
Guideline	:	OECD 221	Acceptability	:	Acceptable
Test substance	:	dec-3-en-2-one, batch HA-2010/01	7-d ErC_{50} 7-d EyC_{50} 7-d ErC_{10} 7-d EyC_{10}	: : : :	2.84 mg/L 1.69 mg/L 1.03 mg/L 0.55 mg/L
Purity	:	98.6%			

Methods

A 7-day toxicity test on the growth of duckweed (*Lemna gibba G3*) (3 replicates per concentration, each containing three plants with four fronds each at test initiation) was conducted under semi-static conditions (daily renewal) with dec-3-en-2-one (98.6% pure) at nominal test concentrations of 0.24, 0.76, 2.44, 7.81 and 25 mg test item/L, with untreated control tested in 3 replicates. The test solutions were prepared by serial dilution of a stock solution in test medium.

The concentrations of 3-decen-2-one were analytically confirmed by GC-MS in duplicate samples of all test solutions taken on day 0, 3 and 6 (fresh) and day 1, 4 and 7 (aged). Samples were injected directly into the GC-MS. Quantification was based on a (non-linear) calibration curve extending from 0.075-35 mg/L. The method was validated by analysis of samples of test water fortified with the test item at levels of 0.1, 0.5, 2 and 30 mg/L (n=9 at each level). Recovery was acceptable (range 69-99%) at 0.5-30 mg/L, but at 0.1 mg/L 4 recoveries were in the range 51-69%. The reported LOD was 0.02 mg/L and the reported LOQ was 0.5 mg/L. The LOD and LOQ are considered to be acceptable.

On day 0, 3, 5 and 7, frond number and any change in plant development were recorded. Frond dry weight was recorded on day 0 and 7.

Results

The measured concentrations in the test solutions are shown in Table 42 below. The reported geometric mean concentrations were calculated by setting values <LOQ and <LOD at 0.5xLOQ and 0.5xLOD, respectively. This may not be accurate, as actual values may be lower than 0.5xLOQ or 0.5xLOD. The procedure is however considered to be acceptable since the magnitude of the EC_{50} values is predominantly determined by the dose-response at the 3 highest test concentrations (negligible effects at lower concentrations), and at the 3 highest test concentrations only a few samples were <LOD or <LOQ. The report presented test endpoints based on nominal and geometric mean measured concentrations. Since measured concentrations were <80% of nominal in many samples, test endpoints should be based on geometric mean measured (mm) concentrations.

Table 42: Measured concentrations (% of nominal) of 3-decen-2-one in test solutions

	Measured concentrations (% of nominal) at nominal concentration (mg test item/L):				
Day	**0.24**	**0.76**	**2.44**	**7.81**	**25**
0 (fresh)	<LOQ	37	66	57	81
	<LOQ	34	63	54	81
1 (aged)	<LOQ	<LOQ	30	47	68
	<LOQ	<LOQ	29	46	66
3 (fresh)	92	84	83	80	77
	94	87	84	77	79
4 (aged)	<LOQ	<LOD	<LOD	10	41
	<LOQ	<LOD	<LOD	10	46
6 (fresh)	91	108	95	122	108
	89	102	99	115	109
7 (aged)	<LOD	<LOD	<LOD	<LOD	4
	<LOD	<LOD	<LOD	<LOD	<LOQ
geometric mean (% nom)	<LOQ	16	12	18	40
geometric mean (mg/L)	0.04	0.12	0.29	1.38	9.96

Water quality parameters (light intensity, pH and temperature) were in accordance with the OECD 221 guideline. The doubling time in the control was 1.9 days, hence the validity criterion of OECD 221 (2006) was satisfied (doubling time <2.5 days). Phytotoxic effects were observed at 0.29 mg/L mm (slightly shortened roots), at 1.38 mg/L mm (shorter roots and necrosis) and at 9.96 mg/L mm (shorter roots, necrosis and separated fronds). Other biological test results and test endpoints based on geometric mean measured concentrations are summarised in Table 43 below.

Table 43 The toxicity of 3-decen-2-one to *Lemna gibba*

Geometric mean measured conc. (mg test item/L)	**frond number**		**Biomass**	
	% inhibition growth rate	**% inhibition yield**	**% inhibition growth rate**	**% inhibition yield**
0.04	0.5	1.3	-1.2	-3.3
0.12	1.4	4.1	2.6	6.2
0.29	2.7	7.4	-1.3	-3.3
1.38	18.1*	40.6*	12.5*	26.9*
9.96	94.3*	98.7*	90.2*	96.9*
EC10 (95% CI)	1.03 (0.91-1.16)	0.55 (0.31-)	1.23 (0.91-1.76)	0.84 (0.36-1.35)
EC50 (95% CI)	2.84 (2.54-3.24)	1.69 (1.44-2.06)	3.50 (3.14-3.90)	2.26 (1.97-2.77)
NOEC	0.29	0.29	0.29	0.29

* statistically significant difference from control at 5% level.

Remarks

The test was performed in agreement with OECD 221 and is acceptable. It concerns a semi-static test with verified actual test concentrations. The effect concentrations, expressed as geometric mean measured concentrations, are presented in the table above. The lowest E_rC_{10} of 1.03 mg/L and the E_rC_{50} of 2.84 mg/L both based on frond number will be used for classification purposes.

Vryenhoef, 2016

Characteristics

Reference	:	CA 8.2.6.1-03	Species	:	*Pseudokirchneriella subcapitata*
type of study	:	Algae growth inhibition test	exposure duration	:	72 hours
year of execution	:	2016	nominal conc.	:	1.0, 3.2, 10, 32, 100 % v/v saturated solution
Report Nr	:	JF15FM			

GLP statement	:	Yes	dosing method	:	Static, in test water
Guideline	:	OECD 201 (2006), EEC C.3	Acceptability	:	Acceptable
test substance	:		72 h-ErC_{50}	:	2.8 mg/L
		dec-3-en-2-one (AMV-1018), lot nr: 411730MS0036	72 h-EyC_{50}	:	0.89 mg/L
			72 h-ErC_{10}	:	0.63 mg/L
			72 h-EyC_{10}	:	0.49 mg/L
Purity	:	98.72%			

Methods

A study was performed to assess the effect of dec-3-en-2-one on the growth of the green alga *Pseudokirchneriella subcapitata*. The method followed that described in the OECD Guidelines for Testing of Chemicals (2006) No 201, "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" referenced as Method C.3 of Commission Regulation (EC) No 761/2009.

Following a preliminary range-finding test, *Pseudokirchneriella subcapitata* was exposed to solutions of dec-3-en-2-one at nominal concentrations of 1.0, 3.2, 10, 32 and 100% v/v saturated solution or mg/L (three replicate flasks per concentration) for 72 hours. To address the volatility of the compound the dec-3-en-2-one solutions were prepared by dispersing an excess (100 mg/L, 5 mg in 5L) of dec-3-en-2-one in culture medium with the aid of ultrasonication for approximately 10 minutes. After sonicating, any undissolved dec-3-en-2-one was removed by filtration (0.2 µm) to produce a 100% v/v saturated solution of 3-decen-2-one in culture medium. This saturated solution was then further diluted as necessary, to provide the remaining test solutions. Due to the potentially volatile nature of dec-3-en-2-one, testing was conducted in completely filled, stoppered test vessels in order to minimise possible losses due to volatilisation. Following the recommendations of published data, in order to prevent inhibition of growth due to the restriction of gaseous exchange, additional sodium bicarbonate was added to the culture medium to provide a source of carbon dioxide for algal growth.

Samples of the algal populations were removed daily and cell concentrations determined for each control and treatment group, using a Coulter® Multisizer Particle Counter.

Results

Analysis of the test preparations achieved test concentration up to 64% of nominal at 0 hours and showed a decline in the course of the experiment, down to < LOQ (0.0054%) at 72 hours, the end of the test.
A sample of the 1.0% v/v saturated solution (mg/L) test concentration containing no algal cells was prepared at the start of the test and incubated under test conditions until analysis at 72 hours. A measured concentration of 0.095 mg/L was obtained (15% of the 0-Hour measured concentration) indicating that the test item was unstable, likely due to volatility under test conditions and/or to some extent potentially due to chemical adsorption to the algal cells within the test system present. Given the decline in measured concentrations observed, it was considered appropriate to calculate the results based on the geometric mean measured test concentrations of those samples prepared with the addition of algal cells in order to give a "worst-case" analysis of the data.

The geometric mean measured test concentrations were determined to be:

Table 44

Nominal Test Concentration (% v/v Saturated Solution)	Geometric Mean Measured Test Concentration (mg/L)	Expressed as a Percentage of the 0-Hour Measured Test Concentration (%)
1.0	0.30	46
3.2	0.80	51
10	4.2	68
32	12	74

Nominal Test Concentration (% v/v Saturated Solution)	Geometric Mean Measured Test Concentration (mg/L)	Expressed as a Percentage of the 0-Hour Measured Test Concentration (%)
100	49	76

Exposure of *Pseudokirchneriella subcapitata* to dec-3-en-2-one gave the following results based on the geometric mean measured test concentrations:

Growth rate and yield values for the control and test cultures after 72 hours and percentage inhibition values are given in the following table.

Table 45

Response Variable	EC_{10} (mg/L)	EC_{50} (mg/L)	NOEC (mg/L)	LOEC (mg/L)
Growth Rate	0.63	2.8	0.34	0.86
Yield	0.49	0.89	0.34	0.86

Remarks:

The validity criteria of OECD 201 were fulfilled in the test and an appropriate analytical method was used to verify the exposure concentrations in the test media. The E_rC_{10} of 0.63 mg/L and the E_rC_{50} of 2.8 mg/L (both expressed as geometric mean measured test concentrations) will be used for classification purposes.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.6 Long-term aquatic hazard

Table 46: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Lemna growth inhibition semi-static OECD 221	*Lemna gibba*	dec-3-en-2-one, batch HA-2010/01 Purity: 98.6%	7d-NOE_rC: 0.29 mg/L 7d-E_rC10: 1.03 mg/L 7d-NOE_yC: 0.29 mg/L 7d-E_yC10: 0.55 mg/L	mean measured concentrations Acceptable	Hoffman, K., Deierling, T., 2012
Algal growth inhibition static OECD 201	*Pseudokirchneriella subcapitata*	dec-3-en-2-one (AMV-1018), lot nr: 411730MS0036 Purity: 98.6%	72h-NOE_rC: 0.34 mg/L 72h-E_rC10: 0.63 mg/L 72h-NOE_yC: 0.34 mg/L 72h-E_yC10: 0.49 mg/L	mean measured concentrations Acceptable **Key study**	Vryenhoef, H. 2016 ECHA dissemination site, 2021

11.6.1 Chronic toxicity to fish

No data available.

11.6.2 Chronic toxicity to aquatic invertebrates

No data available.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please see paragraph 11.5.3.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Reliable acute effect concentrations are available for three aquatic trophic levels, i.e. a 96h-LC_{50} of 1.5 mg/L for the fish *O. mykiss*,a 48h-EC_{50} of 1.68 mg/L for the aquatic invertebrate *D. magna* , 72h- E_rC_{50} of 2.8 mg/L for the algae *P. subcapitata*, and a 7d-E_rC_{50} of 2.84 mg/L for the aquatic plant *L. gibba.* All data are based on geometric mean measured test concentrations.

From these data it appears that fish are the most sensitive species, with a 96h-LC_{50} of 1.5 mg/L. In accordance with table 4.1.0(a) (according to CLP guidance V4.1 June 2015, p. 503-505) and considering that the lowest acute effect concentration is above 1 mg/L, (3E)-dec-3-en-2-one is not to be classified for acute aquatic toxicity.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The biodegradation potential of (3E)-dec-3-en-2-one has been evaluated using a ready biodegradability test. Test substance was technical dec-3-en-2-one consisting of the active substance (trans isomer), and minor amounts of the cis-isomer (exact amount not specified). Degradation of dec-3-en-2-one amounted to 60% after 28 days based on oxygen consumption, equalling the pass level of 60% (theoretical oxygen demand). This was, however, not achieved within 10 days from the onset of biodegradation. Therefore, dec-3-en-2-one is considered readily biodegradable but failing the 10-day window. A hydrolysis study is available, but the losses were attributed to evaporation and not hydrolysis. (3E)-dec-3-en-2-one was concluded to be hydrolytically stable under environmentally relevant conditions. There are no simulation data available for the water, water/sediment or soil compartments. Taking above into account, and according to Annex I: 4.1.2.9.5. (CLP guidance V4.1 June 2015, p. 495), (3E)-dec-3-en-2-one is to be considered not rapidly degradable.

The bioaccumulation potential of (3E)-dec-3-en-2-one in fish was not determined experimentally. The log K_{ow} of (3E)-dec-3-en-2-one was experimentally determined to be 3.45 ± 0.02 at 24°C (98.1%), which is below the threshold of log K_{ow} ≥4. QSAR estimated BCF values are in the range 87.8- 247 L/kg wwt. Taking above into account, (3E)-dec-3-en-2-one is considered to have a low potential for bioaccumulation.

Reliable chronic effect concentrations are only available for aquatic primary producers, i.e. a 72h- E_rC_{10} of 0.63 mg/L (mean measured) for the algae *P. subcapitata*, and a 7d-E_rC_{10} of 1.03 mg/L (mean measured) for the aquatic plant *L. gibba*. In accordance with table 4.1.0(b)(i) (according to CLP guidance V4.1 June 2015, p. 503-505) and considering that the lowest chronic effect concentration is below 1 mg/L, (3E)-dec-3-en-2-one is to be classified as Category Chronic 2.

For fish and aquatic invertebrates, only acute aquatic toxicity data are available, i.e. a 48h-EC_{50} of 1.68 mg/L for the aquatic invertebrate *D. magna* and a 96h-LC_{50} of 1.5 mg/L for the fish *O. mykiss*. The surrogate approach is applicable though as (3E)-dec-3-en-2-one is non rapidly degradable. In accordance with table 4.1.0(b)(iii) (according to CLP guidance V4.1 June 2015, p. 503-505) and considering that both acute effect

concentrations are in the range >1 to ≤10 mg/L, (3E)-dec-3-en-2-one is to be classified as Category Chronic 2.

Overall, both approaches support the classification of (3E)-dec-3-en-2-one as Aquatic Category Chronic 2.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

- Acute (short-term) aquatic hazard: classification not warranted.
- Long-term aquatic hazard: Aquatic Chronic 2

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

There is no current entry in Annex VI of the CLP Regulation for the substance (3*E*)-dec-3-en-2-one.

The DS proposed to classify the substance as Aquatic Chronic 2; H411, the substance being not rapidly degradable, based on the **surrogate approach** and the lowest EC_{50} obtained with *Oncorhynchus mykiss* (1.50 mg/L, mean measured (mm)) due to no chronic aquatic toxicity data available for the most acutely sensitive species (fish).

The physico-chemical characteristics show that (3*E*)-dec-3-en-2-one has moderate water solubility (140 mg/L at 24 °C), vapour pressure of 430 Pa at 25 °C and the Henry's law constant was estimated to be 473.8 $Pa \times m^3 \times mol^{-1}$, indicating high volatility.

Degradation

A summary of the relevant information on degradation is provided in Table 34 of the CLH report.

Abiotic degradation

A preliminary OECD TG 111 study has been presented (Benton, 2011). The hydrolytic stability of (3*E*)-dec-3-en-2-one was studied in sterile aqueous buffer solutions at pH 4, 7 and 9 showing that the substance was unstable, with losses of 34, 41 and 63 % at a pH of 4, 7 and 9 respectively. No DT_{50} values were presented. Additional testing was not considered to give relevant scientific information due to quick dissipation from water by volatilisation (Henry's law constant = 473.8 $Pa \times m^3 \times mol^{-1}$).

A scientific justification was additionally provided based on literature information supporting that the substance molecule would not be reactive to hydrolysis under the normal conditions employed within the OECD study guideline thus (3*E*)-dec-3-en-2-one is considered hydrolytically stable. (3*E*)-3-decen-2-one is manifested by the fragmentation of the alkene bond and that reaction only occurs at elevated temperatures and elevated pressures and even that reaction is not highly efficient (Freedlander, 2014).

No studies were submitted for photodegradation in water due to (3*E*)-dec-3-en-2-one not showing absorption above 270 nm and not showing a significant route of degradation/dissipation in the aquatic environment.

Model US EPA AOP v1.92 and Atkinson method were used to calculate the indirect photo-oxidation assuming an atmospheric hydroxyl radical concentration of 1.5×10^6 cm^{-3} and resulting in a DT50 of 2h for both the cis and trans isomers indicating (3*E*)-dec-3-en-2-one would not be subject to long range atmospheric transport (Benton, 2011).

Biodegradation

Ready biodegradation

A valid OECD TG 301F manometric respirometry test has been presented that was conducted under aerobic conditions in activated sludge and run at the loading of 105 mg/L of (3*E*)-dec-3-en-2-one corresponding to an oxygen demand of about 305 mg/L (T_hODNH_4). The preparation of the test solutions included (3*E*)-dec-3-en-2-one (duplicate), inoculum control (duplicate), procedure (single), abiotic (single) and toxicity controls (single). The T_hODNH_4 of (3*E*)-dec-3-en-2-one was calculated to be 2.904 mg O_2/mg test item.

The degradation was more than 60 % after 4 days of incubation. The difference between duplicate values of degradation at the end of the test was less than 20 %. In the toxicity control 34 % biodegradation was noted within 14 days and 46 % after 28 days. The test item was assumed to be not inhibitory to aerobic activated sludge due to degradation > 25 % within 14 days.

The mean percentage biodegradation for (3*E*)-dec-3-en-2-one at the end of the 28-day exposure was 60 % (T_hODNH_4); duplicate 1 = 60 % and duplicate 2 = 59 %. 10 % of biodegradation was already reached at day 3 (duplicate 1 = 12 %; duplicate 2 = 17 %), but the pass level of 60 % was only reached at day 28, therefore it was concluded that the 10-day window was not met. (3*E*)-dec-3-en-2-one was tested in all studies, including ready biodegradability, and contains the cis (Z) isomer as minor component (% not specified). No information was given on the potential differences in biodegradability of the cis/trans isomers.

There are no simulation data available for the water, water-sediment or soil compartments.

Overall, the DS considered the available data adequate for classification purposes and concluded that (3*E*)-dec-3-en-2-one is considered not rapidly degradable in the aquatic environment, according to the CLP criteria.

Bioaccumulation

A summary of the available information on bioaccumulation is provided in Table 35 of the CLH report.

There are two studies available that experimentally determined the $logK_{ow}$ of (3*E*)-dec-3-en-2-one using the shake-flask methodology (OECD TG 107). Wo (2009) reported a $logK_{ow}$ of 3.45 ± 0.02 at pH 5.8 and 24 °C, while Benton (2011) reported $logK_{ow}$ values of 3.45, 3.47 and 3.43 at pH 4, 7 and 9 at 22 °C, respectively. As the surface tension of (3*E*)-dec-3-en-2-one is < 60 mN/m (~30 mN/m) which is considered surface active the experimentally determined values should be considered with care. Based on the solubility in water (0.14 g/L) and in n-octanol (expected > 250 g/L), the $logK_{ow}$ can be estimated to be ~3.25. Estimated values using BioLoom v1.5 and Kowwin v1.68, are 3.16 and 3.28, respectively. The DS considered the experimental $logK_{ow}$ value of 3.45 acceptable.

No experimental studies on bioaccumulation in fish are available.

Using the experimentally determined $logK_{ow}$ of 3.45, a BCF of 171 L/kg was estimated using the following relation as defined by Veith *et al.* 1979 (based on chemical class: pesticides):

logBCF = 0.85 $logK_{ow}$ – 0.70. Two other QSARs were also used to calculate the BCF, i.e. logBCF = 0.76 $logK_{ow}$ – 0.23 (Veith *et al.* 1979, based on chemical class: various organic chemicals) producing a BCF of 247 L/kg and logBCF = $logK_{ow}$ – 1.32 (Mackay, 1982) producing a BCF of 135 L/kg. Additionally, BCF values were calculated using the BCFBAF (v3.01) module provided in the EPI Suite™. BCFBAF estimates fish bioconcentration factor and its logarithm using two different methods. By default, BCFBAF uses the $logK_{ow}$ calculated by the KOWWIN module. However, the assessor used for this proposal the higher experimentally determined value of 3.45 instead of the KOWWIN estimated value of 3.28, which resulted in slightly higher BCF values of 87.8 L/kg wwt (regression-based method) and 138 L/kg wwt (Arnot-Gobas (upper trophic) method).

Three QSAR estimates are also presented: USES Koc (L/kg) = 785 L/kg, based on $logK_{ow}$ = 3.45 and depending on the QSAR model, the proposed Koc for (3*E*)-dec-3-en-2-one varies between 165.2 L/kg and 1069 L/kg using EpiSuite™ (KocWin) and EpiSuite™ (KocWin respectively.

Overall, as the experimentally determined and the estimated $logK_{ow}$ values are below the threshold of $logK_{ow} \geq 4$, and due to the QSAR estimated values being below the trigger value of 500 L/kg, the DS considered **(3*E*)-dec-3-en-2-one to have low bioaccumulation potential**.

Aquatic toxicity

Aquatic acute toxicity

A summary of the relevant information on aquatic acute toxicity is presented in Table 36 of the CLH report.

The DS noted that the aquatic toxicity studies were performed with (3*E*)-dec-3-en-2-one, lot HA-2010/01 (purity 98.6 %) and AMV-1018 (purity 99.4 %) which primarily consisted of the substance itself (3*E*)-dec-3-en-2-one (trans-isomer) and contained minor amounts of the cis-isomer. There is no information available regarding differences in toxicity between isomers.

Aquatic acute toxicity studies are presented for all three trophic levels: fish, invertebrates, and algae and aquatic plants.

One valid OECD TG 203 acute fish toxicity study is available with *Oncorhynchus mykiss* in a semi-static test design. No accurate mean measured concentrations could be calculated for nominal test concentrations of 0.13 and 0.28 mg/L. Test endpoints were based on geometric mean measured concentrations of the two highest test concentrations presenting an LC_{50} of 1.50 mg/L (mm) after 96 h of exposure. (Anonymous, 2011a)

For invertebrates, one reliable acute study has been given. The *Daphnia magna* OECD TG 202 immobilisation test provided an LC_{50} of 1.68 mg/L (mm) after 48h exposure. No accurate mean measured concentrations could be calculated for nominal test concentrations of 0.19 and 0.43 mg/L, as these nominal concentrations were below the LOQ of the analytical method. This is acceptable since these two concentrations are not relevant for estimation of the EC_{50} value (Hoffman and Deierling, 2011b).

One reliable OECD TG 201 study is available with *Pseudokirchneriella subcapitata* showing a 72 h E_rC_{50} value of 2.8 mg/L (mm). A measured concentration of 0.095 mg/L was obtained (15 % of the 0-Hour measured concentration) indicating that the test item was unstable due

to chemical adsorption to the algal cells thus geometric mean measured concentrations were calculated (Vryenhoef, 2016).

Another supportive OECD TG 221 study is available with *Lemna gibba* showing E_rC_{50} of 2.84 mg/L (mm) based on frond number (Hoffman and Deierling, 2012).

According to the provided valid studies, fish are found to be the most sensitive species. The lowest EC_{50} is obtained with *O. mykiss* (1.50 mg/L (mm)) not meeting the CLP classification criteria for aquatic acute hazards so the **DS proposed not to classify (3*E*)-dec-3-en-2-one for acute aquatic hazards, based on the L(E)C_{50} ≥ 1 mg/L in CLP Table 4.1.0 (a).**

Aquatic chronic toxicity

A summary of the relevant information on aquatic acute toxicity is presented in Table 46 of the CLH report.

Valid data for aquatic chronic toxicity is only presented for one trophic level: algae and aquatic plants. No long-term fish and invertebrate tests are available.

The OECD TG 201 study with *P. subcapitata* resulted in a NOE_rC of 0.34 mg/L (mm) and E_rC_{10} of 0.63 mg/L (mm) (Vryenhoef, 2016).

The OECD TG 221 growth inhibition test (static, 7 d) with *L. gibba* resulted in a NOEC of 0.29 mg/L (mm) and E_rC_{10} of 1.03 mg/L (mm) based on based on frond number (Hoffman and Deierling, 2012).

Since no chronic aquatic toxicity data were available for fish and invertebrates but fish species are acutely the most sensitive endpoint, the DS considered based on a surrogate approach and for a not rapidly degradable substance that **(3*E*)-dec-3-en-2-one fulfils the criteria for classification as Aquatic Chronic Category 2; H411 based on the L(E)C_{50} > 1 and ≤ 10 mg/L in CLP Table 4.1.0 (b)(iii).**

Comments received during consultation

One Company-Manufacturer indicated that the proposed classification proposal as Aquatic Chronic 2 is not warranted based on the substance not persisting, in their opinion, in the aquatic environment due to a combination of degradation and very significant volatilisation. A 28-day study on the emergence of *chironomids* demonstrating low toxicity (NOEC = 103 mg/kg sediment/d; AMVAC ref. no. 965-AQU-007) is also mentioned.

With regard to the biodegradability, the DS responded by referring to the CLP Regulation and cases where the 10-day window can be waived. It was indicated that none of these conditions was met and the 10-day window requirement should be fulfilled so that the substance be considered not rapidly degradable. The DS also noted that the study with *Chironomus riparius* was not available until after the CLH report was submitted to the ECHA and has not been evaluated. Despite the fact that a chronic study for fish is not available, the "most stringent" classification would anyway be used warranting the same proposal for classification as Aquatic Chronic 2.

One MSCA agreed with the classification proposal as Aquatic Chronic 2; H411. Further information was requested on the identity and the CAS number of the substance as the CLH dossier and the available DAR refers to different CAS numbers for the same substance.

The DS explained that CAS 18402-84-1 specifically relates to the '3-Entgegen'-isomer (3*E*) 3-decen-2-one that is the active substance and should be preferred, whereas CAS 10519-33-2 refers to '3-decen-2-one', i.e., no stereoisomer in specific, which is inaccurate.

Assessment and comparison with the classification criteria

The CLH report did not include information on the photodegradation in water and behaviour in the water-sediment system.

RAC agrees with the DS to consider volatilisation as the primary route of dissipation based on the Henry's law constant and that the hydrolysis is limited route of degradation for (3*E*)-dec-3-en-2-one in the aquatic environment. Furthermore, the substance can be considered indirectly photodegradable.

According to the available data presented by the DS, the test material (purity 98.6 %) containing both the trans-isomer (3*E*)-dec-3-en-2-one and the cis (Z) isomer was found to be 60 % biodegraded to CO_2 over a test period of 28 days. The cis (Z) isomer was only present as a minor component (exact amount is not specified). Both isomers are expected to show similar biodegradation behaviour but no information is given on the potential differences in biodegradability of the cis/trans isomers.

RAC notes that during the test, the degradation of (3*E*)-dec-3-en-2-one did not meet the 10-day window, thus the substance is not demonstrated to be readily biodegradable in a 28-day test for ready biodegradability as the pass level of the test must be achieved within 10 days from the onset of biodegradation, according to Section 4.1.2.9.5 of the CLP regulation.

The 10-day window condition may be waived as discussed in the CLP regulation Annex II.2.3. If this is not possible, then the pass level should be evaluated within a 14-day window if possible, or after the end of the test. RAC concludes that there is currently not sufficient justification that the 10-day window condition may be waived.

Based on the available data on the hydrolytic behaviour of the substance in the water demonstrating stability, the additional justifications on the unreactive nature of the structure of the substance and due to the degradation of (3*E*)-dec-3-en-2-one amounting to 60 % after 28 days based on oxygen consumption, equalling the pass level of 60 % (theoretical oxygen demand), RAC agrees with the DS to consider (3*E*)-dec-3-en-2-one as **not rapidly degradable** for classification purposes.

There are no experimental bioaccumulation studies available for fish species. However, the available data on the experimentally determined $\log K_{ow}$ and the estimated BCF values can be considered sufficient to come to conclusion on the bioaccumulation potential of the substance. RAC agrees with the DS and concludes that (3*E*)-dec-3-en-2-one has **low potential for bioaccumulation** based on the experimentally determined the $\log K_{ow}$ 3.45 ± 0.02 at 24 °C which is below the threshold of $\log K_{ow} \geq 4$ and the QSAR estimated BCF values in the range 87.8-247 L/kg wwt well below the cut-off value of 500 L/kg.

RAC notes that no data has been provided as part of the CLH dossier showing toxicity of the degradation products so they are not taken into account for classification purposes. RAC also notes that the study with *Chironomus riparius* mentioned during the consultation round has not been evaluated and taken into account as part of this proposal.

RAC agrees with the DS that, based on the most sensitive fish species result (LC_{50} of 1.50 mg/L for *O. mykiss*), together with the other scientifically robust and reliable acute data, **no classification for aquatic acute hazards** is warranted.

Taking into account that reliable chronic data is not available for all trophic levels, RAC agrees with the DS proposal RAC to classify the substance as **Aquatic Chronic 2; H411,** with the substance being not rapidly degradable and based on the application of CLP Table 4.1.0 (b)(i) and (b)(iii) that both lead to Aquatic Chronic 2.

RAC notes that if additional data become available either on the biodegradation, bioaccumulation potential and the degradation products in the environment and acute or chronic toxicity of (3*E*)-dec-3-en-2-one and its metabolites or isomers, the classification could be reconsidered.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

No data.

12.1.2 Comparison with the CLP criteria

Not relevant.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

No classification proposed. Data lacking.

13 ADDITIONAL LABELLING

None.

14 REFERENCES

CA 5.2.1-01, 2009 AMV-1018 Acute Oral Toxicity Up and Down Procedure in Rats. Report Number: 27396, GLP, Unpublished

CA 5.2.2-01, 2009 AMV-1018 Acute Dermal Toxicity in Rats – Limit Test. Report Number 27397, GLP, Unpublished

CA 5.2.3-01, 2009 AMV-1018 Acute Inhalation Toxicity Study in Rats. Report Number 27398, GLP, Unpublished

CA 5.2.4-01, 2009 AMV-1018 Primary Skin Irritation Study in Rabbits. Report number 27400, GLP, Unpublished

CA 5.2.5-01, 2009 AMV-1018 Primary Eye Irritation Study in Rabbits. Report number 27399, GLP, Unpublished

CA 5.2.6-01, 2009 AMV-1018 Dermal Sensitisation Study in Guinea Pigs (Buehler Method). Report number 27401, GLP, Unpublished

CA 5.3.3-01, 2014 AMV-1018: Dose range finding study by inhalation administration to rats. Report No. BDG0214, GLP, Unpublished

CA 5.4.1-01, 2009 Reverse Mutation Assay using Bacteria (*Salmonella typhimurium*) with AMV-1018. Report number BDG0214, GLP, Unpublished

CA 5.4.1-02, 2009 *In vitro* Mammalian Cell Gene Mutation Assay (Thymidine Kinase Locus/TK$^{\pm}$) in Mouse Lymphoma L5178Y Cells with AMV-1018. Report number 091462, GLP, Unpublished

CA 5.4.2-01, 2009 Mammalian Micronucleus Test of Murine Peripheral Blood Cells with AMV-1018. Report number 0091461, GLP, Unpublished

CA 5.4.2-02, 2011 AMV-1018:*In Vivo* Liver Unscheduled DNA Synthesis (UDS) Assay. Report number 41005048, GLP, Unpublished

CA 5.4.2-03, 2016 AMV-1018 Wistar Han Rat In Vivo Comet Test. Report number 965-GEN-006, GLP, Unpublished

CA 5.6.2-02, 2012 AMV-1018: Study for Effects on Embryo-Fetal Development in the CD Rat by Oral Gavage Administration. Report No BDG0146, GLP, Unpublished

Cronin MTD and Basketter DA. 1994. Multivariate QSAR analysis of a skin sensitization database. SAR and QSAR in Environmental Research, Vol: 2, pp: 159-179. Note: dataset available at http://www.inchemicotox.org/results/ , DOI: 10.1080/10629369408029901

ECHA dissemination site (2021). https://echa.europa.eu/registration-dossier/-/registered-dossier/30007 (accessed 06 April, 2021)".

Flavouring Group Evaluation by EFSA (EFSA 2010).

JECFA, 2003a. Safety evaluation of certain food additives. Fifty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 50. IPCS, WHO, Geneva.

Nilsson AM, Gafvert E, Salvador L, Luthman K, Bruze M, Gruvberger B, Nilsson JLG and Karlberg AT. 2001 Mechanism of the antigen formation of carvone and related alpha,beta-unsaturated ketones. Contact Dermatitis, Vol: 44, pp: 347-356 http://dx.doi.org/10.1034/j.1600-0536.2001.044006347.x

Opdyke DLJ and Letizia C. Fragrance raw materials monographs: 2-pentylidenecyclohexanone. 1982. Food and Chemical Toxicology, Vol: 20, pp: 797.

Additional references
Buehler. Occlusive patch method for skin sensitization in guinea pigs: the Buehler method. Food Chem Toxicol. 1994 Feb;32(2):97-101

15 ANNEXES

Relevant summaries:

Hydrolysis studies

The hydrolytic stability of *(3E)*-3-decen-2-one was assessed in a screening study at pH 4, 7 and 9 at 50°C:

Report: B.8.4.1/01

Reference	:	Benton	GLP statement	:	Yes
Type of study	:	*(3E)*-3-decen-2-one physical/chemical tests final report,	Guideline	:	OECD test guideline no. 111
Year of execution	:	2011	Acceptability	:	Acceptable
Test substance	:	3-decene-2-one			

Executive summary

Hydrolysis was determined according to the first part of OECD test guideline no. 111.

Sterile aqueous buffer solutions were treated with the specimen, in duplicate, at three different pH's.

The pH 4 buffer was prepared by accurately weighing the appropriate amount of potassium hydrogen phthalate into a 100 mL volumetric flask.

The pH 7 buffer was prepared by accurately weighing the appropriate amount of potassium hydrogen phosphate into a 100 mL volumetric flask.

The pH 9 buffer was prepared by accurately weighing the appropriate amounts of boric acid and potassium chloride into a 100 mL volumetric flask.

An amount (50 mL) of sterile water was added to each flask and the buffer was dissolved by placing the flasks in an ultrasonic bath.

All the solutions were made up to 100 mL with the sterile water, whilst adjusting the pH using 0.1 M sodium hydroxide in order to obtain the following:

pH 4 buffer [pH measured at 20 ± 1°C = 4.00]

pH 7 buffer [pH measured at 20 ± 1°C = 7.01]

pH 9 buffer [pH measured at 20 ± 1°C = 9.01]

The method specifies that the concentration of active ingredient is below 0.01 M. Therefore, as 3- decen-2-one is insoluble in water, approximately 1.35 g of the technical material was accurately weighed into a 10 mL volumetric flask. The flask was then made up to volume with acetonitrile.

5 µL aliquots of this solution were transferred to the three separate 100 mL volumetric flasks containing buffer solution and a fourth containing sterile water. The samples were shaken to mix. Each of the buffer solutions were then split into two 50 mL portions and transferred to 50 mL brown, screw top, bottles.

The water solution was then analysed for 3-Decen-2-one according to GC Laboratories Analytical Method TM242. 'High Performance Liquid Chromatographic Determination of 3-Decen-2-one in Technical Material and Formulations'.

The brown bottles were placed in an incubator at a temperature of 50 ± 0.5°C, for a period of five days. The samples following storage at 50°C were then analysed for 3-Decen-2-one, using method TM242.

The determinations were performed by Reverse Phase HPLC with UV detection at 221 nm using a 250 x 4.6mm Ace, 5 µm, C18 column.

The following results were obtained:

Storage	pH	3-decen-2-one (g/L)			% drop
		(a)	(b)	Average	
Initial results (prior to storage)		0.00667	0.00664	0.00666	0
Five days at 50 ± 0.5°C	4	0.00441	0.00440	0.00440	33.9
	7	0.00391	0.00391	0.00391	41.3
	9	0.00249	0.00250	0.00250	62.5

RMS comments: No information is given with regard to the mass balance and recoveries. Moreover, based on the results it cannot be concluded that the substance is stable with respect to hydrolysis. However, on April 8, 2014 the following data requirement was set;

A satisfactory sterile hydrolysis test at 20°C with (3E)- 3-decen-2-one is needed. Ideally an experimental design that enables the Arrhenius activation energy for (3E)- 3-decen-2-one to be determined should be completed. If hydrolysis at 20°C occurs any transformation products formed should be identified.

The Q10 of 2.58 was derived for microbially active soil matrices. In FOCUSsw modelling it is also used for microbially active sediment water systems. However it is not a value that is justified to be used for sterile aqueous hydrolysis condition systems.

The applicant responded to this requirement with the following statement:

STUDY B.8.4.1/02 (IIIA, 7.5/01)

Reference	:	Freedlander	GLP statement	:	No, not relevant
Type of study	:	Scientific justification	Guideline	:	Not relevant
Year of execution	:	2014	Acceptability	:	Acceptable
Test substance	:	3-decene-2-one			

In the review of the Draft Assessment Report, it is commented: "*Was it really unstable or was loss from the test system due to volatilisation the reason for any measured rapid decline*"? This assessment is correct as there were no controls utilized in the study to validate the simple screw top bottle assembly and there was no use of radiolabeling to assess the nature of the small eluting peaks in the chromatograms, which are likely due to some dissolution of materials associated with the test system at the higher temperature used within the study.

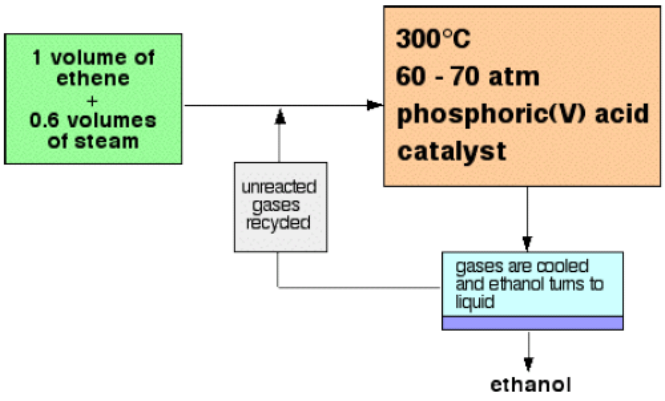
On that basis, the decline observed in this Tier I test at 50°C with non-radiolabeled *(3E)*- 3-decen-2-one was not the result of hydrolysis, but rather the result of volatilization from the test system given the compound's volatile nature.

A data requirement for the conduct of an OECD guideline-compliant hydrolysis study at 20°C was set.

A scientific justification is provided to address the requirement for a hydrolysis study for *(3E)*-3-decen-2-one. The basis for this justification is that there is ample literature information that supports the contention that the molecule would be completely unreactive to hydrolysis under the normal conditions employed within the OECD study guideline.

LIKELIHOOD OF HYDROLYSIS

As gasoline does not decompose in the presence of water, the initial basic organic chemistry principle is clearly understood that nucleophilic or electrophilic displacement of hydrogen atoms on the lengthy hydrocarbon portion of the molecule will not occur under normal environmental conditions. That then leaves two possible locations for hydrolysis to occur: at the double bond or across the conjugated system of the double bond and the carbonyl. Concerning hydrolysis of the double bond, this can be considered in terms of the addition of water to the olefinic structure to produce an alcohol. However, such a reaction is known only to occur under conditions of high pressure and high temperature. This is best illustrated by considering the very common reaction for making ethyl alcohol which entails the addition of water to ethene, the simplest of alkenes. The conditions necessary to enact this particular reaction requires typically a temperature of 300°C and pressures of more than 50 atmospheres and even under these circumstances the yield is very low. Clearly, this reaction would not occur in the environment and for molecules that contain an isolated double carbon bond in its structure, similar reactivity occurs.



Clark, J. extracted from http://www.chemguide.co.uk/organicprops/alkenes/hydration.html on May 14, 2014,

Another example is the industrial process for hydrolytic conversion of butene to butanol. This reaction is also not very efficient and again requires high temperatures or high pressures, or both. Many times catalysts are employed to enhance reactivity and even under these conditions, high temperatures are required. As an example, molecular sieve catalysts are employed at reaction temperatures between 120 and 180°C, resulting in only modest yields. The reaction was studied under different weight hour space velocities (WHSV) which reflects the flow rate of the butene gas over the volume of the catalytic bed. The effect of WHSV on the reactivity of butene at different temperatures is illustrated below.

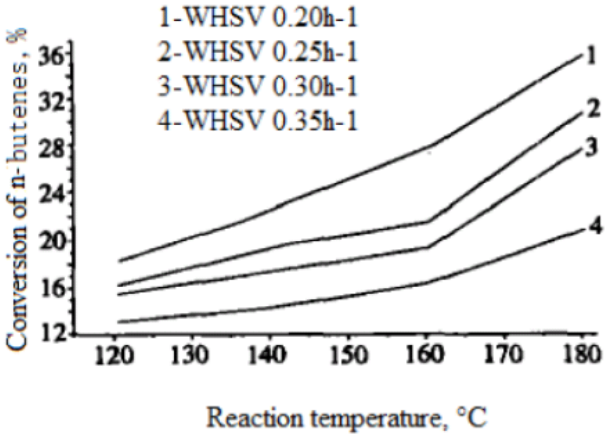


Figure 3. The relationship between the temperature, the WHSV, and the conversion of n-butenes

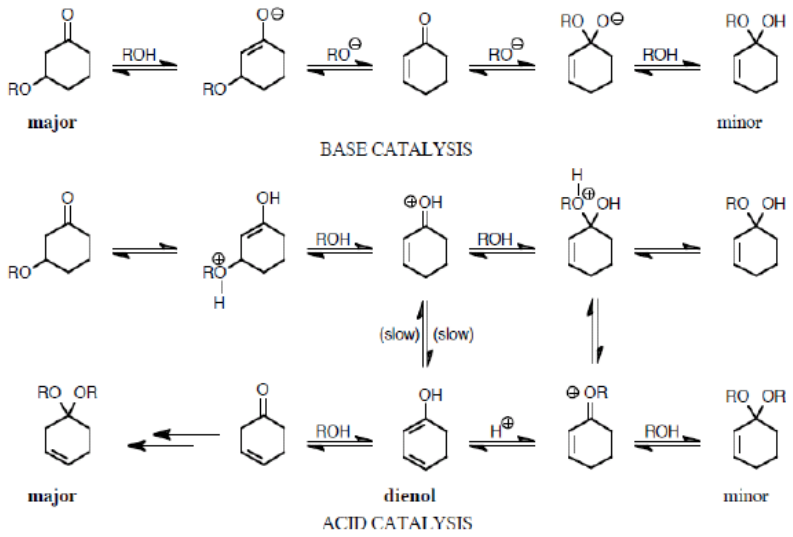
From: Yongmei, Z, Y. Hongjun (2012)

It is recognized that special conditions are required for reacting what are gases at ambient temperature. Still, the examples provided demonstrate the difficulty in reacting water with an olefin. There are fewer examples of efforts to react higher molecular weight olefins with water; but a few examples exist. For example, the hydration of 1-decene in the presence of a wool-palladium complex was performed at 70°C (Xue *et al.*, 2004). Although not investigated, it is reasonable to assume that the use of a higher temperature and an expensive palladium catalyst would not have been employed if the reaction was sufficiently facile to occur at ambient temperature.

The one possibility remaining for hydrolysis of *(3E)*-3-decen-2-one to occur is the potential for reaction of the conjugated carbonyl and alkene moiety, collectively termed the α,β-unsaturated ketone. There are two chemical mechanisms that could result in this type of reaction. First, water addition can fracture the alkene bond. Second, water can be added across the system in what has been classified as a Michael addition reaction.

Chemists have taken advantage of the fact that olefins can be fractured by activating the double bond. One means of activation is the presence of a ketone functionality in conjugation with the double bond. This type of reaction is described as a retro-aldol condensation. A catalyst is required for such a reaction and the reaction will not readily occur solely in the presence of heat (Dolfini & Glinka, 1989) In example VII, 10 grams of *(3E)*- 3-decen-2-one, AMVAC's compound of interest, was placed in an autoclave with 390 grams of water at 275°C at 920 psi for 2 hours. The resulting oil consisted of 3.01 grams of heptanal and 1.49 grams of *(3E)*-3-decen-2-one. The likelihood that the small remainder of material associated with the material balance was some other entity is unlikely.

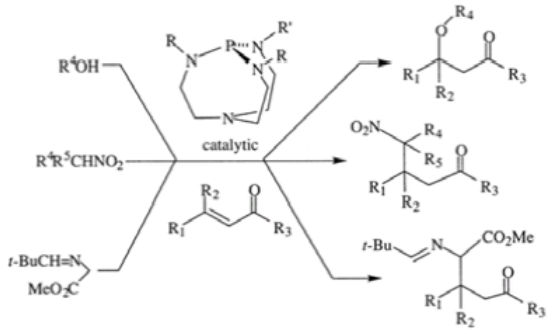
(3E)-3-decen-2-one is unlikely to yield a Michael addition product given the fact that there was so much starting material remaining associated with the fracturing reaction described above. If an addition reaction was to occur, there would have been less of the starting material and the material balance would have been much lower. Still, a quick assessment of the Michael addition reaction can be considered. Based upon past studies, it is understood that the reaction occurs with preference of a 1,4 addition for ketones and a 1,2 addition for aldehydes. There are a few exceptions, but in the case of a reaction with water, one would predict that the reaction would be through 1,4 addition. That being the case, it is recognized that Michael addition of water under acidic conditions would result in nucleophilic attack on the carbonyl, forming an unstable diol, which would simply covert back to the *(3E)*-3-decen-2-one. Under basic conditions, the nucleophile can attack the 1 position, forming an alcohol while still retaining the carbonyl functionality. The mechanism by which water could theoretically react with an α,β-unsaturated ketone, such as *(3E)*-3-decen-2-one, is illustrated below.



Chemistry Department, University of Wisconsin-Eau Claire

available at http://www.uwec.edu/lewisd/chem323/difunctional_carbonyl_cpds.pdf

Although there were no clear references that considered the Michael addition of water to an α,β-unsaturated ketone under mild conditions, there is some information that indicates that the reaction would not likely occur. Studies concerning the addition of primary alcohols to these systems, serve as an surrogate for assessing the reactivity of water to these same substrates. Reactions of certain α,β-unsaturated ketones to primary alcohols have yielded good recoveries but have required the use of catalytic amounts of nonionic strong bases (Kisang *et al.*, 2002). In another example, isopropanol was found to be completely unreactive to methyl acrylate (conditions not specified) and the addition of t-butanol at 60°C for 30 hours yielded no addition product (Fetterly, 2005). Overall these examples support the fact that the Michael addition of water to an α,β-unsaturated ketone such as *(3E)*-3-decen-2-one would not occur under ambient conditions in the absence of a catalyst. This is further supported by the fact that text book examples of the Michael addition reaction to α,β-unsaturated ketones indicates the use of an accompanying strong acid or strong base.



1,4 Addition of Primary Amines to α,β-Unsaturated Substrates (Kisang et al., 2002)

CONCLUSION

In conclusion, a rather complete assessment of the potential for *(3E)*-3-decen-2-one to hydrolyse under the guideline conditions affirms that this would not occur. The most relevant information provided is the previously discussed patent by Dolfini (1989), which using the substrate *(3E)*-3-decen-2-one in the presence of water to demonstrate that hydrolysis of *(3E)*-3-decen-2-one is manifested by the fragmentation of the alkene bond and that reaction only occurs at elevated temperatures and elevated pressures and even that reaction is not highly efficient under the extraordinary conditions of the study.

On this basis, AMVAC requests a waiver from conducting an OECD guideline-based hydrolysis study as the lack of potential for the compound to react with water under environmental conditions is well substantiated based on current information in the literature.

RMS comment

It was decided by EFSA in the Evaluation Table for *(3E)*-3-decen-2-one (November, 2014) that "*The information provided was presented in the revised DAR (August 2014) p. 9-13. In conclusion (3E)-3-decen-2-one will be stable to hydrolysis at 20°C and pH 4, 7 & 9.*" Therefore, no OECD guideline-based hydrolysis study is required to further address the hydrolytic behaviour of 3-decene-2-one.

Photodegradation in water

No studies are submitted under this data point, nor deemed necessary. 3-Decen-2-one does not show any appreciable absorption above 270 nm and photodegradation will therefore not constitute a significant route of degradation/dissipation in the aquatic environment. The primary route of dissipation will be volatilisation, based on the high Henry's law constant.

RMS comment

RMS agrees with the statement, see study by Benton (2011).

Ready biodegradability

Reference/notifier	:	Feil	GLP statement	:	Yes
Type of study	:	Ready Biodegradability of 3-decen-2-one in a Manometric Respirometry Test,	Guideline	:	OECD Guideline for Testing of Chemicals No. 301 F
Year of execution	:	2011	Acceptability	:	Acceptable
Test substance	:	3-decene-2-one Lot no HA-2012/01, purity 98.6%			

Substance	**Water type**	**T [°C]**	**pH**	**Duration [d]**	**Transformation at end [%]**	**Classification**
3-decene-2-one Lot no HA-2012/01, purity 98.6%	Test water	22±1	7.5	28	66	readily biodegradable

Description

The test item 3-decene-2-one was investigated for its ready biodegradability in a manometric respirometry test over a period of 28 days. The biodegradation was followed by the oxygen uptake of the micro-organisms during exposure. Sodium benzoate was tested simultaneously as reference substance and also functioned as a procedure control.

The loading of the test item was 105 mg/L corresponding to an oxygen demand of about 305 mg/L ($ThOD_{NH4}$). The loading of the reference item sodium benzoate was 103 mg/L corresponding to an oxygen demand of about 172 mg/L ($ThOD_{NH4}$). Aerobic activated sludge dissolved in test water (deionised water plus analytical grade salts) was incubated at 21-22ºC during 28 days in closed flasks. The change of pressure in the test flasks was measured by means of a manometric method (BSB/BOD-Sensor-System).

Preparation of test solutions

Treatment	Flask	Test Item mg	Reference Item[1] mg	$HgCl_2$ mL	Activated Sludge[2] mL	Test Water mL	Final Volume mL
3-Decene-2-one	1	25.3	—	—	5	239	244
	2	25.6	—	—	5	239	244
Inoculum Control [3]	3	—	—	—	5	239	244
	4	—	—	—	5	239	244
Procedure Control [4]	5	—	25.2	—	5	239	244
Abiotic Control[5]	6	25.3	—	5	—	239	244
Toxicity Control	7	25.0	25.4	—	5	239	244

[1] Reference item: sodium benzoate
[2] Stock suspension of 1.5 g/L on dry matter base (final concentration: 21 g/L)
[3] The inoculum control was also used for other projects which ran in parallel.
[4] The procedure control was also used for other projects which ran in parallel.
[5] Poisoned with $HgCl_2$ (stock solution of 48.84 mg/mL)
—: Not applicable

Calculation of BOD: the biodegradability (%BOD=mg O_2 per meg test item) exerted after each period was calculated as:

$$BOD = \frac{\text{mg } O_2 \text{ uptake of test item - mg } O_2 \text{ uptake of inoculum control}}{\text{mg test item in flask}}$$

For the toxicity control, the BOD of the treatment is the sum of test item and reference item and is calculated as the sum of test item and reference item concentration in the flask. The percentage biodegradation of the test item and of the reference item sodium benzoate was calculated as:

$$\% \text{ degradation} = \frac{\text{BOD (mg } O_2\text{/mg test item or reference item)}}{ThOD_{NH4} \text{ (mg } O_2\text{/mg test item or reference item)}} \times 100$$

The $ThOD_{NH4}$ of 3-decene-2-one was calculate to be 2.904 mg O_2/mg test item.

Results

The validity criteria of the study were met. The oxygen demand of the inoculum control was 30 mg O_2/L and thus not greater than 60 mg O_2/L within 28 days. The pH at the end of the test was 7.5-7.6 and therefore within the range of pH 6-8.5 as required. The percentage degradation was more than 60% after 4 days of incubation. The difference between duplicate values of degradation at the end of the test was less than 20%.

Percentage biodegradation

The mean percentage biodegradation at the end of the 28-day exposure was 60%($ThOD_{NH4}$). The 10-day window was not passed. Therefore 3-decene-2-one is considered to be readily biodegradable, but failing the 10-day window.

The reference item sodium benzoate was sufficiently degraded to 93% at day 14 and 28 of incubation.

In the toxicity control 34% biodegradation was noted within 14 days and 46% after 28 days. According to the test guidelines the test item can be assumed to be not inhibitory to aerobic activated sludge because degradation was >25% within 14 days.

The oxygen demand in the abiotic control was zero.

RMS comments

The study was performed adequately and the results are used for further assessment. During the peer review process there was a request for the isomer composition of the test substance. The active substance is *trans* (E) *(3E)*- 3-decen-2-one. This isomer was tested in all studies, including ready biodegradability. The *cis* (Z) isomer is only a minor component in technical grade *(3E)*- 3-decen-2-one.