REGULATION (EC) NO 1272/2008 (CLP REGULATION),

ANNEX VI, PART 2

Proposal for Harmonised Classification and Labelling for a biocidal active substance

CLH REPORT

THERMALLY TREATED GARLIC JUICE

EC Number: ---

CAS Number: ---

Index Number:

Applicant: Ecospray Limited UK

Contact details of dossier submitter: Federal Ministry Republic of Austria for Climate Action, Environment, Energy, Mobility, Innovation and Technology, 1010 Vienna, Austria

Version number: 2 **Date:** 06/07/2023

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ASSESSMENT REPORT

SUMMARY

1. PRESENTATION OF THE ACTIVE SUBSTANCE

1.1 IDENTITY OF THE ACTIVE SUBSTANCE

Table 1.1 Main constitiuents

Main constituent(s)	
ISO name	thermally treated garlic juice
IUPAC or EC name	thermally treated garlic juice
EC number	---
CAS number	---
Index number in Annex VI of CLP	N/A
Minimum purity / content	100% (1000g/kg)
Structural formula	R – S_n – R (n=1-7) R1 = CH_3; R = C_3H_5; n = 1-7 Marker compounds: 1. $C_6H_{10}S$ (Diallyl monosulfide, DAS1) 2. $C_6H_{10}S_2$ (Diallyl disulfide, DAS2) 3. $C_6H_{10}S_3$ (Diallyl trisulfide, DAS3) 4. $C_6H_{10}S_4$ (Diallyl tetrasulfide, DAS4)

Table 1.2 Relevant impurities and additives

Relevant impurities and additives		
IUPAC name or chemical name or EC name	Maximum concentration in % (w/w)	**Index number in Annex VI of CLP**
N/A – thermally treated garlic juice is a natural UVCB active substance; the term 'impurity' is not relevant.		

1.2 INTENDED USES AND EFFECTIVENESS

Table 1.3 Use of the biocidal active substance

Product type	
Intended use pattern(s)	Repellent; avoids the excretion of cats at treated places/objects.
Users	Non-professional

Table 1.4 Effectiveness of the biocidal active substance

Function		
Organisms to be controlled		Mammals (Cats, of all ages)
Mode of action		Olfactory repellent

Moreover, thermally treated garlic juice is used as plant protection product.

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE ACCORDING TO THE CLP CRITERIA

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING FOR THE ACTIVE SUBSTANCE

Table 2.1 Proposed harmonised classification and labelling of the substance

	Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Labelling**			**Specific Conc. Limits, M-factors, 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 sub-mitters proposal	TBD	thermally treated garlic juice	---	---	Skin Sens. 1B	H317	Warning GHS07	H317	--	--	**--**
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	TBD	thermally treated garlic juice	---	---	Skin Sens. 1B	H317	Warning GHS07	H317	**--**	**--**	**--**

Table 2.2 Reason for not proposing harmonised classification and labelling and the status under CLH public consultation

Hazard class	Reason for not proposing classification and labelling	Within the scope of 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 and mixtures	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 and mixtures	Hazard class not applicable	No
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	Hazard class not applicable	No
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Data lacking	Yes
Acute toxicity via dermal route	Data lacking	Yes
Acute toxicity via inhalation route	Data lacking	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	Yes
Skin sensitisation	Skin Sens. 1B, H317, harmonised classification proposed	Yes

Germ cell mutagenicity	Data lacking	Yes
Carcinogenicity	Data lacking	Yes
Reproductive toxicity	Data lacking	Yes
Specific target organ toxicity-single exposure	Data lacking	Yes
Specific target organ toxicity-repeated exposure	Data lacking	Yes
Aspiration hazard	Data conclusive but not sufficient for classification	Yes
Hazardous to the aquatic environment	Data conclusive but not sufficient for classification	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

2.2.1 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The active substance thermally treated garlic juice, has been approved as "Garlic extract" under the Plant Protection Products (PPP) Directive 91/414/EEC and this approval has been renewed under PPP Regulation (EC) No 1107/2009 on 01.03.2021 (Commission Implementing Regulation (EU) 2021/129). Under PPP, the active substance "Garlic extract" is associated to the CAS-numbers 8008-99-9 and 8000-78-0. The only identified hazard of this active substance is skin sensitisation potential, proposed to be classified as Skin Sens. 1B.

2.2 PROPOSED CLASSIFICATION AND LABELLING AND PACKAGING FOR THE REPRESENTATIVE PRODUCT(S)

Not applicable for the CLH report.

2.3 DATA SOURCES

ECHA dissemination site:
https://echa.europa.eu/en/substance-information/-/substanceinfo/100.029.426.

The Draft Risk Assessment Report provided by the applicant in course of an application as active substance under the Biocidal Product Regulation (BPR, REGULATION (EU) No 528/2012) including the original study reports served as data source. Moreover, scientific literature was used as information source as well as the public available documents referring to the renewal of Garlic extract as active substance under PPP Regulation (EC) No 1107/2009.
Please see Appendix V: Overall reference list for details.

3. SUMMARY OF THE HUMAN HEALTH RISK ASSESSMENT

Not applicable for the CLH report.

4. SUMMARY OF THE ENVIRONMENTAL RISK ASSESSMENT

Not applicable for the CLH report.

5. ASSESSMENT OF EXCLUSION CRITERIA, SUBSTITUTION CRITERIA AND POP

Not applicable for the CLH report.

A. Assessment of intrinsic properties and effects of the active substance

A.1. General substance information

A.1.1. Identity of the substance

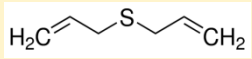
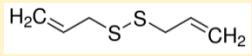
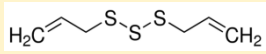
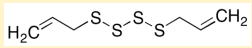
Table A.1 Summary table on substance identity

Summary table on substance identity	
Common name (ISO name, synonyms)	**thermally treated garlic juice** **Synonyms: CLAIL0021, CLAIL0021 Nemguard Liquid, garlic extract**
Chemical name (EC name, CA name, IUPAC name)	thermally treated garlic juice
EC number	---
CAS number	---
other CAS numbers (e.g. deleted, related, preferred, alternate)	---
Molecular formula	R-Sn-R (n=1-7) R1 - Sn - R or R – Sn – R; where: R1 = CH_3; R = C_3H_5; n = 1-7 Marker compounds: 1. $C_6H_{10}S$ (Diallyl monosulfide, DAS1) 2. $C_6H_{10}S_2$ (Diallyl disulfide, DAS2) 3. $C_6H_{10}S_3$ (Diallyl trisulfide, DAS3) 4. $C_6H_{10}S_4$ (Diallyl tetrasulfide, DAS4)
Molecular weight or molecular weight range	N/A – thermally treated garlic juice is a botanical active substance and UVCB. The molar masses of the marker compounds are given below: 1. DAS1: 114.05 g/mol 2. DAS2: 146.27 g/mol 3. DAS3: 178.34 g/mol

	4. DAS4: 210.40 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Thermally treated garlic juice is a botanical active substance and UVCB. Thermally treated garlic juice is characterised by four main marker compounds (DAS1-4). None of the marker compounds are optically active.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Confidential data
Degree of purity (%)*	Thermally treated garlic juice is a botanical active substance and UVCB. The purity is 100%.

Table A.2 Structural formula

Structural formula
N/A – thermally treated garlic juice is a botanical active substance and an UVCB. The structures of the marker molecules are however given below.

Marker molecule	Structural formula
Diallyl monosulfide (DAS1)	
Diallyl disulfide (DAS2)	
Diallyl trisulfide (DAS3)	
Diallyl tetrasulfide (DAS4)	

A.1.2. Composition of the substance (reference specifications)

Table A.3 Main constituent

Main constituent(s)					
Constituent (chemical name)	Typical concentration (%(w/w))	Concentration range (%(w/w))	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	**Remarks / Discussion**
thermally treated garlic juice	100	100	--	Skin Sens. 1B, H317	

Table A.4 Impurities

Impurities					
Constituent (chemical name)	Typical concentration (%(w/w))	Concentration range (%(w/w))	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	**Remarks / Discussion**
N/A - thermally treated garlic juice is a botanical active substance and an UVCB. The term 'impurity' is not relevant. The purity is 100%.					

Table A.5 Additives

Additives						
Constituent (chemical name)	Function	Typical concentration (%(w/w))	Concentration range (%(w/w))	Current CLH in Annex VI Table 3.1	Current self-classification and labelling (CLP)	**Remarks / Discussion**
N/A – thermally treated garlic juice is a botanical active substance and an UVCB. The purity is 100%. The extract does not contain any additives.						

Table A.6 Concentration of constituents (main constituents, impurities, additives) in batches used for (eco)toxicity studies and proposed specification

Constit-uents	**Specifi-cation supported (yes/no)**	**Proposed Specification [% w/w]**	**Batches used for (eco) toxicity studies [% w/w]**			
			Batch No. Study type (Reference)	Batch No. Study type (Reference)	Batch No. Study type (Reference)	Batch No. Study type (Reference)
Active substance	Yes	Purity: 2.58% (w/w) total polysulfides (as diallyl trisufide equivalents) Actual diallyl trisulfide content: 0.93% (w/w). Batch No. 11004007	Batch No. 11004007 OECD 404: Acute dermal irritation study of CLAIL 0021 in rabbits (Anonymous 2011a)	Batch No. 11004007 OECD 405 Acute Eye Irritation Study of CLAIL 0021 in Rabbits (Anonymous, 2011b)	--	--
	Yes	Purity: 2.58% (w/w) total polysulfides (as diallyl trisufide equivalents) Actual diallyl trisulfide content: 0.93% (w/w). Batch Batch No. 11004007	Batch No. 11004007 OECD 203: Fish, acute toxicity Test (Anonymous, 2012a)	Batch No. 11004007 OECD 201: Alga, Growth Inhibition Test (Anonymous 2012b)		
Active substance	Yes	Batch No: AN15463580 Purity: 2.9% (w/w) active ingredient content (total polysulfides)	Batch No: AN15463580 OECD 429: Skin sensitisation study of CLAIL0021 Nemguard liquid by LLNA in mice (Anonymous, 2016e)*	--	--	--
	Yes	Batch No. AN19867020A Purity: 2.92% (w/w) active ingredient content (total polysulfides)	Batch No. AN19867020A OECD 111: Hydrolysis as a function of pH (Anonymous, 2021b)	Batch No. AN19867020A OECD 203: Fish, Acute toxicity test (Anonymous, 2021d)	Batch No. AN19867020A OECD 202: *Daphnia sp.* Acute immobilisation test. (Anon.	Batch No. AN19867020 A OECD 201: Alga, Growth inhibition test. (Anon.

Constit-uents	Specifi-cation	Proposed Specification [% w/w]	Batches used for (eco) toxicity studies [% w/w]			
					2021e)	2021f)
	Yes	Batch No. AN19915120C Purity: 2.90% (w/w) active ingredient content (total polysulfides)	Batch No. AN19915120C; OECD 301B: Ready biodegradability- CO_2 Evolution test. (Anonymous, 2021c)			
	No	Batch No. L0091, Garlic Juice Concentrate 883 **Purity: not determined**	Batch No. L0091 OECD 202: Daphnia sp. Acute immobilisation test. (Anonymous 2000)			
	No	Batch No. AN18825970 **Purity: not determined**	Degradation of Garlic extract in "Local River water" (Anonymous, 2019a)	Batch No. AN18825970 Degradation of CLAIL0021, study similar to OECD 307 (Anonymous, 2019b)		
	No	Batch No. AN22374890 Purity: 2.81% (w/w)	Batch No. AN22374890 OECD 121: Estimation of Adsorption Coefficient (Anonymous, 2022c)			

*If specification is not supported by a batch used in a study, constituent(s) which give concern are highlighted.

A.1.3. Physical and chemical properties of the active substance

Table A.7 Physical and chemical properties of the active substance

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Aggregate state at 20°C and 101.3 kPA	Free-flowing homogeneous liquid	Visual assessment	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2014a
Physical state (appearance) at 20°C and 101.3 kPA	Free-flowing homogeneous liquid	Visual assessment	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2014a
Colour at 20°C and 101.3 kPA	Opaque brown Colour; Munsell code 5YR 4/6	Visual assessment	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2014a
Odour at 20°C and 101.3 kPA	Strong garlic	Olfactory assessment	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2014a
Melting / freezing point	115°C The liquid was dried to a solid in order to perform the test	EC A1 (capillary method). The test item was dried at 85°C to remove moisture and form a solid.	GLP Batch number 12909L	Anonymous 2002a
pH at 20°C	Neat = 5.50 1% dilution = 5.96	CIPAC MT 75	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2016a
Boiling point at	100.3°C	EU A2	GLP Batch number 12909L	Anonymous 2002a

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Relative density	1.2927 at 20°C	EU A3 (pycnometer)	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2014a
Granulometry	N/A		The study does not need to be conducted because the substance is not a solid.	
Vapour pressure	2.18 kPa at 20°C 3.08 kPa at 25°C	EU A4 (static method)	GLP Batch number 12909L	Anonymous 2002a
Henry's law constant	3.6E-6 $atm.m^3.mol^{-1}$.	Calculation	Calculation, performed using a water solubility value of 1000 g/L and a molecular weight of 162 g/mol, which is an average of five identified diallyl sulphide marker compounds. Note: Volatility of the maker molecule DAS1 (Diallyl monosulfide) has been reported in the literature as 1.3 x 10^{-3} $atm.m^3.mol^{-1}$ (predicted value).	- Meylan 1991
Surface tension	41.5 mN/m at 20°C (neat); the substance is considered surface active	EU A5 (plate method)	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2016a
Water solubility at 20°C	>1000g/L at 20°C	EU A6 (preliminary test only)	GLP Batch number 12909L	Anonymous 2002a
Partition coefficient (n-octanol/water)	Log Pow = -1.49 (1:1 octanol:water),	EU A8 (shake flask)	GLP Batch number 12909L	Anonymous 2002a

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
and its pH dependency at 20°C	-2.13 (2:1 octanol:water), -1.69 (1:2 octanol:water)			
Thermal stability and identity of breakdown products	The results of 2-week 54°C and 2-year ambient storage stability studies show no significant changes between pre-storage and post storage samples.	In-house method	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2014a Anonymous 2016a
Reactivity towards container material	The results of a 2-year ambient storage stability study show no significant changes between pre-storage and 24-month storage samples.	In-house method	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2016a
Dissociation constant	N/A		The study does not need to be conducted because the identified marker compounds do not have an ionic structure.	
Viscosity	Results at variable shear rates: 637.1 – 778.1 mPa.s at 20°C, 220.1 - 354.1 mPa.s at 40°C Non-Newtonian liquid	OECD 114 GLP	GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)	Anonymous 2016a

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Solubility in organic solvents, including effect of temperature on solubility	Not determined		The active substance thermally treated garlic juice is a plant extract and soluble in water. The solubility in other solvents was not tested and is not required. As the active substance contain organo-sulfur polysulfides, which are soluble in organic solvents e.g. ethanol, methanol, acetone, dichloromethane and acetonitrile. The analytical studies (see section 5) carried out, used methanol and acetonitrile to extract polysulfides from thermally treated garlic juice.	
Stability in organic solvents used in biocidal products and identity of relevant degradation products	N/A		The active substance as manufactured is not delivered in an organic solvent.	

A.1.4. Physical hazards and respective characteristics

Table A.8 Physical hazards and respective characteristics

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
Explosives			Not explosive Active substance thermally treated garlic juice contains plant matrix (carbohydrates, proteins etc like any other plant and organo-sulfur molecules. None of the known component has any known explosive properties i.e. the molecules within it have high negative oxygen balance and contains no explosophor grouping therefore does not represent an explosive hazard in the formulation. This active substance contains no molecules/ groups listed as oxidants (Urben 2006). Moreover, more than 80 sulfur containing substances (i.e. Diallyl sulfide, diallyl disulfide, diallyl trisulfide, allicin, E/Z-alliin, ajoene, cyclic sulfies, polysulfides…) and 40 non-sulfur-containing substances have been reported. The	Anonymous 2016a Abe 2019

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			concentration of these components in the UVCB can be regarded as very low, and none of them contain structural alerts such as N-O, N-X, O-X, C-metal, N-metal groups, with exception to C-C unsaturation. In the present UVCB, water, carbohydrates and proteins are the main components and are not known for explosive properties. Water even has phlegmatising properties. Based on these findings, it is highly unlikely that thermally treated garlic juice fulfills the classification criteria as explosive substance.	
Flammable gases			N/A – substance is not a gas	
Flammable aerosols			N/A – substance is not an aerosol	
Oxidising gases			N/A – substance is not a gas	
Gases under pressure			N/A – substance is not a gas under pressure	
Flammable liquids	EC A9; Pensky-Martens closed cup GLP Test substance purity = 999 g/L		No flash point observed before boiling at ca. 100°C.	Anonymous 2016a

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
	garlic concentrate (batch number 13105012C)			
Flammable solids			N/A – substance is not a solid	
Self-reactive substances and mixtures			Not self-reactive Active substance thermally treated garlic juice contains plant matrix (carbohydrates, proteins etc. like any other plant and organo-sulfur molecules. None of the known component has any known self-reacting properties It contains no chemical groups indicating self-reactivity (Urben 2006). Moreover, more than 80 sulfur containing substances (i.e. Diallyl sulfide, diallyl disulfide, diallyl trisulfide, allicin, E/Z-alliin, ajoene, cyclic sulfies, polysulfides…) and 40 non-sulfur-containing substances have been reported. The concentration of these components in the UVCB can be regarded as very low, and none of them contain structural alerts such as N-O, N-X, O-X, C-metal, N-metal	Anonymous 2016a Abe 2019

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			groups, with exception to C-C unsaturation. In the present UVCB, water, carbohydrates and proteins are the main components and are not known for self-reactive properties. Water even has phlegmatising properties. Based on these findings, it is highly unlikely that thermally treated garlic juice fulfills the classification criteria as self-reactive substance.	
Pyrophoric liquids			Not pyrophoric Based on experience in handling and use, the active substance is stable in air at room temperature for prolonged periods of time.	
Pyrophoric solids			N/A – substance is not a solid	
Self-heating substances and mixtures			The phenomenon of self-heating generally applies only to solids or liquids adsorbed on a large surface so this hazard class is not applicable in this case.	
Substances and mixtures which in contact with water emit flammable gases			No flammable gases emitted when substance in contact with water. Based on experience in	

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver		Reference
			handling and use and the fact that water is present in the composition of the technical active substance.		
Oxidising liquids			The molecules within the active substance do not show functional groups associated with oxidising properties. Furthermore, Active substance thermally treated garlic juice contains plant matrix (carbohydrates, proteins etc) and organo-sulfur molecules – in all of the mentioned molecules or group of molecules, there is no fluorine or chlorine atom and oxygen is bound only to carbon or hydrogen. Therefore, none of the known component has any oxidizing properties. This active substance contains no molecules/ groups listed as oxidants (Urben 2006).		Anonymous 2016a
Oxidising solids			N/A – substance is not a solid		
Organic peroxides			N/A – the substance is not an organic peroxide		
Corrosive to metals	UN Test C.1. GLP (Batch number AN22374890)	Test duration: 7 days; test temperature: 55°C±1°C; sample volume (weight): steel: 1.74 L (2.21 kg),	Steel corrosion:	Aluminium corrosion:	Anonymous 2023
			No localised corrosion	No localised corrosion	

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver		Reference
		aluminium: 1.63 L (2.07 kg)	observed	observed	
			Uniform corrosion mass loss: 2.80 % (w/w) (gas phase); 1.65 % (w/w) (plate half immersed); 0.27 % (w/w) (liquid phase) Results to not exceed threshold values for uniform corrosion.	Uniform corrosion mass loss: 0.01 % (w/w) (gas phase); 0.06 % (w/w) (plate half immersed); 0.10 % (w/w) (liquid phase) Results to not exceed threshold values for uniform corrosion.	
			Conclusion: the a.s. is not corrosive to metals and does not fulfil classification criteria of corrosive to metals.		
Auto-ignition temperature (liquids and gases)	EC A15 GLP Test substance purity = 999 g/L garlic concentrate (batch number 13105012C)		No self-ignition up to 388°C		Anonymous 2016a
Relative self-ignition			N/A – substance is not a solid		

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
temperature for solids				
Dust explosion hazard			N/A – the substance is not a solid	

A.1.5. Assessment of physical hazards according to the CLP criteria

A.1.5.1. Assessment of physical hazards

The summary of physical hazards and respective characteristics can be found in Part A, section 1.4. (Table A.8). The active substance is not classified for physical hazards.

A.1.5.2. Explosives

Table A.9 Summary table of studies on explosives

Method	Results	Remarks	Reference
Literature reference. oxygen balance	Not explosive Active substance thermally treated garlic juice contains plant matrix (carbohydrates, proteins etc like any other plant and organo-sulfur molecules. None of the known component has any known explosive properties i.e. the molecules within it have high negative oxygen balance and contains no explosophor grouping therefore does not represent an explosive hazard in the formulation. This active substance contains no molecules/ groups listed as oxidants (Urben 2006). Moreover, more than 80 sulfur containing substances (i.e. Diallyl sulfide, diallyl disulfide, diallyl trisulfide, allicin, E/Z-alliin, ajoene, cyclic sulfies, polysulfides…) and 40 non-sulfur-containing substances have been reported. The concentration of these components in the UVCB can be regarded as very low, and none of them contain structural alerts such as N-O, N-X, O-X, C-metal, N-metal groups, with exception to C-C unsaturation. In the present UVCB, water, carbohydrates and proteins are	-	Anonymous 2016a Abe 2019

Method	Results	Remarks	Reference
	the main components and are not known for explosive properties. Water even has phlegmatising properties. Based on these findings, it is highly unlikely that thermally treated garlic juice fulfills the classification criteria as explosive substance.		

A.1.5.2.1 Short summary and overall relevance of the provided information on explosives

Literature indicates no explosivity.

A.1.5.2.2 Comparison with the CLP criteria

The substance fulfils the criteria of the screening procedure and no further testing is required, see table above.

A.1.5.2.3 Conclusion on classification and labelling for explosives

Not explosive

A.1.5.3. Flammable gases (including chemically unstable gases)

Not applicable for CLH report.

A.1.5.4. Flammable aerosols and aerosols

Not applicable for CLH report.

A.1.5.5. Oxidising gases

Not applicable for CLH report.

A.1.5.6. Gases under pressure

Not applicable for CLH report.

A.1.5.7. Flammable liquids

Table A.10 Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EC A9; Pensky-Martens closed cup	No flash point observed before boiling at ca. 100°C	Measurement carried out up to boiling temperature	Anonymous 2016a

A1.5.7.1 Short summary and overall relevance of the provided information on flammable liquids

Study indicates no flash point before boiling at ca. 100°C.

A1.5.7.2 Comparison with the CLP criteria

The substance fulfils the criteria of the screening procedure and no further testing is required, see table above.

A1.5.7.3 Conclusion on classification and labelling for flammable liquids

Not flammable.

A.1.5.8. Flammable solids

Not applicable for CLH report.

A.1.5.9. Self-reactive substances

There are no chemical groups present in the molecule associated with explosive or self-reactive properties.

A.1.5.10. Pyrophoric liquids

Experience in manufacture or handling shows that the liquid does not ignite spontaneously on coming into contact with air at normal temperatures.

A.1.5.11. Pyrophoric solids

Not applicable for CLH report.

A.1.5.12. Self-heating substances

Not applicable for CLH report.

A.1.5.13. Substances which in contact with water emit flammable gases

No flammable gases emitted when substance in contact with water. Based on experience in handling and use and the fact that water is present in the composition of the technical active substance.

A.1.5.14. Oxidising liquids

Table A.11 Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Oxygen balance	The molecules within the active substance do not show functional groups associated with oxidising properties. Furthermore, Active substance thermally treated garlic juice contains plant matrix (carbohydrates,	--	Anonymous 2016a

Method	Results	Remarks	Reference
	proteins etc) and organo-sulfur molecules – in all of the mentioned molecules or group of molecules, there is no fluorine or chlorine atom and oxygen is bound only to carbon or hydrogen. Therefore, none of the known component has any oxidizing properties. This active substance contains no molecules/ groups listed as oxidants (Urben 2006).		

A1.5.14.1 Short summary and overall relevance of the provided information on oxidising liquids

Calculation of oxygen balance indicates no oxidising properties.

A1.5.14.2 Comparison with the CLP criteria

CLP criteria for oxidising liquids not met.

A1.5.14.3 Conclusion on classification and labelling for flammable liquids

Not oxidising

A.1.5.15. Oxidising solids

Not applicable for CLH report.

A.1.5.16. Organic peroxides

Not applicable for CLH report.

A.1.5.17. Corrosive to metals

Table A.12 Summary table of studies on corrosive to metals

Method	Results		Remarks	Reference
UN Test C.1.	Steel corrosion:	Aluminium corrosion:	--	Anonymous 2023
	No localised corrosion observed	No localised corrosion observed		
	Uniform corrosion mass loss: 2.80 % (w/w) (gas phase); 1.65 % (w/w) (plate half immersed); 0.27 % (w/w) (liquid phase) Results to not exceed threshold values for uniform corrosion.	Uniform corrosion mass loss: 0.01 % (w/w) (gas phase); 0.06 % (w/w) (plate half immersed); 0.10 % (w/w) (liquid phase) Results to not exceed threshold values for uniform corrosion.		
	Conclusion: the a.s. is not corrosive to metals and does not fulfil classification criteria of corrosive to metals			

A1.5.17.1 Short summary and overall relevance of the provided information on corrosive to metals

UN Test C.1 indicates no corrosive properties.

A1.5.17.2 Comparison with the CLP criteria

CLP criteria for corrosive to metals not met.

A1.5.17.3 Conclusion on classification and labelling for corrosive to metals

Not corrosive to metals

A.1.6. Analytical methods for detection and identification

Not applicable for CLH report.

A.2. Effects against target organisms

Not applicable for the CLH report.

A.2.1. Intended uses

Biocidal use as olfactory repellent (Product Type 19), which deters cats of all ages from defecating in treated areas. All seasons outdoor use for non-professional user to protect lawns and flower beds. The biocidal active substance is included in a low percentage ratio (~2%) in a carrier material (granules) which is spread on the area to be protected.

Moreover, thermally treated garlic juice is used as plant protection product.

A.2.2. Summary on efficacy

A.2.2.1. Efficacy

Not applicable for the CLH report.

A.2.2.2. Mode of action

The principal biologically active compound produced by garlic is a group of organo-sulfur compounds (sulfanes) with antimicrobial and repellent properties. Various animals including mammals are sensitive to organo-sulfur compounds. In many cases the nasal sensitivity of animals is very high compared to humans. This produce a repellent effect at very low concentrations.

A.2.2.3. Resistance

Not applicable for the CLH report.

A.2.3. Conclusion on efficacy

Not applicable for the CLH report.

A.3. Assessment of effects on Human Health

A number of studies were submitted on skin and eye irritation and skin sensitisation. Thermally treated garlic juice is a skin sensitiser and is self-classified as Skin Sens. 1B, H317 according to Regulation (EC) No. 1272/2008.

A.3.1. Toxicokinetics (TK)

No toxicokinetic study according to an OECD TG and GLP was submitted.

A.3.1.1. Short summary and overall relevance of the provided toxicokinetic information

The applicant submitted two literature publications: Park et al. (2017) provided limited TK information that confirm (S)-allyl-l-cysteine (SAC), one major bioactive compound in garlic is metabolized to (S)-allyl-l-cysteine sulfoxide, N-acetyl-(S)-allyl-l-cysteine, and N-acetyl-(S)-allyl-l-cysteine sulfoxide after oral administration.
In the second study by Lawson and Hunsaker (2018), different garlic preparations were compared to fresh garlic (0.35, 0.70, 1.4 or 2.8 g corresponding to 0.87 g to 6.94 g allicin content) after oral uptake of a single dose in 13 healthy volunteers. The breath detectable metabolite allyl methyl sulphide (AMS) was measured over a period of maximum 32 hours. T_{max} was reached after 1.4 to 3.5 hours, depending on the administration (sandwich or capsule) of the meal. C_{max} were calculated to range between 47 to 69 ng/L, both parameters also correlate with the protein content of the meal. No differences between men and women were found, but the study group was small.

Allicin and other allyl thiosulfinates as well as allyl polysulfides (that can be transformed from allyl thiosulfinate at ambient temperature) are metabolised by glutathione (in case of an allyl functional group) to allyl mercaptan as an intermediate to allyl methyl sulfide (AMS). For crushed garlic, AMS is formed by 90% of allylthiosulfinates and allyl thiosulfinated and allyl polysulfides produce equimolar amounts of AMS (Lawson and Wang, 2005 as cited in Lawson and Hunsaker, 2018). Allicin-derived diallyl disulfide and diallyl trisulfide are also metabolized mainly to AMS. For garlic processed food also y-glutamyl-S-allylcysteine and S-allylcysteine (SAC) played an important contribution to AMS formation (Lawson and Hunsaker, 2018).

Concerning bioequivalence AUC_{AMS} was compared for raw garlic, kitchen prepared garlic or garlic food (n=9) and garlic preparations or supplements (n=13) in 13 subjects. For the bioavailability and bioequivalence experiments 1.4 g homogenate from 0.88 g raw garlic in capsule served as a control. 23 types of "garlic" were tested in total in 43 assays with 7 to 13 subjects in this investigation (Lawson and Hunsaker, 2018).
Alliinase activity was only detected in raw diced garlic amongst the other kitchen-prepared garlic food (such as roasted 160°C or 215°C, boiled 4 min, boiled 45 min) but not in commercial garlic foods (pickled, acid-minced, oil-chopped or black garlic). All of the alliinase-inhibited foods including boiled and roasted kitchen prepared garlic produced less AMS compared to raw garlic homogenate, however the amounts detected indicate that intrinsic AMS formation from S-allyl compounds still occurred. The intensity and duration of cooking made little difference in allicin bioequivalence (temperature, duration). Roasting yielded two times higher allicin bioequivalence as boiling. (For alliinase-inhibited garlic foods, 5.9 g of roasted garlic and 11 g boiled garlic must be consumed to obtain the same equivalence as 2 g raw garlic). C_{max} at 1 hour for garlic foods were 19 to 31% (% referred to the consumption of the control (raw garlic homogenate) at the standard dose). T_{max} for alliinase-inhibited garlic foods was comparable and significantly longer than the control of

raw garlic. In an additional analysis from the same authors it was reported, that the contents of garlic and garlic preparations such as enteric and normal tablet, special extracts or capsules vary both qualitatively and quantitatively.

The manufacturing process of thermally treated garlic juice covers processes similar to the industrial food manufacture and kitchen preparation of the same raw material raw garlic. Rapid formation of the marker substances: allyl polysulfides from thiosulfinates is reported, if garlic is exposed to hot water e.g. via cooking (Lawson and Hunsaker, 2018).
Based on the similarity of these processes, it is reasonable to anticipate that dietary uptake of industrially manufactured and kitchen prepared garlic food products cover human exposure to allyl polysulfides and other compounds of thermally treated garlic juice used in the biocidal product. Exposure to the biocidal product is expected to be a minor contribution to the potential total uptake of an individual referring to the following assessment.
Therefore, waiving of the data requirement of Annex II 8.8 of Regulation (EU) No. 528/2012 was acceptable.
Literature data indicate rapid uptake and metabolism of garlic compounds after oral uptake. Kitchen prepared garlic including alliinase-inhibited foods e.g. boiling produced less breath detectable metabolite allyl methyl sulphide compared to raw garlic homogenate, however the amounts detected indicate that intrinsic AMS formation from S-allyl compounds still occurred. Thus it can be concluded that allyl polysulfides present in thermally treated garlic juices are metabolized similar than kitchen prepared garlic.

A.3.1.2. Values and conclusions used for the risk assessment

Not applicable for the CLH report.

A.3.2. Acute toxicity / STOT SE

A.3.2.1. Acute oral toxicity

A3.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

No acute oral toxicity (OECD and GLP compliant) study was submitted. Garlic (and oil of garlic) is considered as Generally Recognized As Safe (GRAS) food substance by the U.S. Food and Drug Administration[1]. Accordingly this implies that "there is no evidence in the available information on garlic that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or might reasonably be expected in the future" according to the listing by U.S. FDA.
The applicant provided literature publications for acute oral toxicity. Chutani and Bordia (1981) investigated the beneficial effects on fibrinolytic activity of both raw and fried garlic consumption via oral route at a dose of 0.5 g/kg bw in 20 volunteers with previous diagnosed heart diseases. The paper was not considered relevant and reliable for the endpoint acute toxicity based on poor conduct and reporting of the clinical trial, especially no other acute effects other than fibrinolytic activity were reported according to the risk assessment report for plant protection products (Ireland, 2019). eCA AT shares this conclusion concerning the publication.

A3.2.1.2 Comparison with the CLP criteria

Data lacking.

[1] https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=SCOGS&sort=Sortsubstance&order=ASC&startrow=1&type=basic&search=garlic

A3.2.1.3 Conclusion on classification and labelling for acute oral toxicity

No acute oral toxicity data were submitted; thus, no classification is proposed due to lack of data.

A3.2.1.4 Conclusion on acute oral toxicity related to risk assessment

Not applicable for the CLH report.

A.3.2.2. Acute dermal toxicity

A3.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

No reliable acute dermal toxicity study was submitted. The applicant provided a literature publication: Mikail (2010) investigated single doses of aqueous garlic extract at 300, 600, 1200, 2200, 3200 and 4200 mg/kg bw administered subcutaneously to rabbits. The extract was prepared from pulverized air-dried bulbs soaked in distilled water, filtrated and dried (concentrated). The LD_{50} value was 3034 mg/kg bw. Post-mortem macroscopic examination showed slight liver congestion in moribund animals (Mikail, 2010). The application route is not considered relevant for the evaluation of the biocidal uses of thermally treated garlic juice.

A3.2.2.2 Comparison with the CLP criteria

Data lacking.

A3.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

No acute dermal toxicity data were submitted. In skin irritation or sensitisation studies no mortalities occurred at the tested concentrations (cf. see A.3.3, A.3.5). No classification is proposed due to the lack of data for this endpoint and no indications for acute dermal toxicity from other toxicity studies (skin irritation, skin sensitisation).

A3.2.2.4 Conclusion on acute dermal toxicity related to risk assessment

Not applicable for the CLH report.

A.3.2.3. Acute inhalation toxicity

A3.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

No acute inhalation toxicity (OECD TG and GLP compliant) study was submitted. Based on occupational data from manufacturing sites (liquid and granular formulations) no reports of adverse inhalation effects from their production team were made according to the applicant. However, this statement has not been underpinned with medical report data from workers in manufacturing plants; Therefore, this information could not be verified by eCA AT.
According to WHO (1999) and EMA (2019) allergic reactions e.g. contact dermatitis and asthmatic attacks have been reported after inhalative exposure of powdered garlic preparations. One case report of repeated exposure to garlic dust induced asthma in a 30 year old worker in a garlic processing facility (Lybarger et al., 1982).

A3.2.3.2 Comparison with the CLP criteria

Data lacking.

A3.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No acute inhalation toxicity data were submitted with thermally treated garlic juice; thus, no classification is proposed due to lack of data.

A3.2.3.4 Conclusion on acute inhalation toxicity related to risk assessment

Not applicable for the CLH report.

A.3.2.4. Specific target organ toxicity – single exposure Category 1 and 2 (STOT SE 1 and 2)

No data were submitted.

A3.2.4.1 Short summary and overall relevance of the provided information on STOT SE 1 and 2

No data were submitted. Thermally treated garlic juice is processed from food grade material, therefore this endpoint has not been investigated.

A3.2.4.2 Comparison with the CLP criteria

Data lacking.

A3.2.4.3 Conclusion on classification and labelling for STOT SE 1 and 2

No classification is proposed due to a lack of data.

A.3.2.5. Specific target organ toxicity – single exposure Category 3 (STOT SE 3)

No data were submitted.

A3.2.1 Short summary and overall relevance of the provided information on STOT SE 3

No data were submitted. Thermally treated garlic juice is processed from food grade material, therefore this endpoint has not been investigated.

A3.2.2 Comparison with the CLP criteria

Data lacking.

A3.2.3 Conclusion on classification and labelling for STOT SE 3

No classification is proposed due to a lack of data.

A3.2.4 Overall conclusion on acute toxicity related to risk assessment

Not applicable for the CLH report.

A.3.3. Skin corrosion and irritation

Table A.13 Summary table of in vitro studies on skin corrosion/irritation

<table>
<tr><th colspan="4">Summary table of in vitro studies on skin corrosion/irritation</th></tr>
<tr><th>Method, Guideline, GLP status, Reliability, Key/supportive study</th><th>Test substance (including purity), Vehicle, Doses</th><th>Relevant information about the study</th><th>Results and remarks</th><th>Ref.</th></tr>
<tr><td>Acute dermal irritation study
OECD 404 (version 2002)
GLP
Klimisch 1
Key study</td><td>CLAIL 0021 (2.58% w/w total poly-sulfides)
0.5 mL applied undiluted
4 h (semi-occlusive)

Rabbit (New Zealand White), 3 male</td><td>Skin reactions recorded at 1, 24, 48 and 72 h and on day 7 post patch removal.</td><td>Animal 1:
Erythema = 1.67 (mean) 24h-72h, 2.00 (max.)
Oedema = 0.67 (mean) 24h-72h, 1.00 (max.)

Animal 2:
Erythema = 2.00 (mean) 24h-72h, 2.00 (max.)
Oedema = 0.33 (mean) 24h-72h, 1.00 (max.)

Animal 3:
Erythema = 2.00 (mean) 24h-72h, 2.00 (max.)
Oedema = 0.00 (mean) 24h-72h, 0.00 (max.)

Summary: scores represent values averaged over days 1, 2, and 3:
<table><tr><th>Animal No.</th><th>Erythema score</th><th>Oedema score</th></tr><tr><td>1</td><td>1.67</td><td>0.67</td></tr><tr><td>2</td><td>2</td><td>0.33</td></tr><tr><td>3</td><td>2</td><td>0</td></tr></table>At day 7 all scores were 0.
All observed effects were reversible.</td><td>Anonymous 2011a</td></tr>
</table>

A3.3.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

One GLP compliant study, conducted to acceptable regulatory guidelines, provide data regarding the potential for skin irritation for the active substance thermally treated garlic juice.

In the skin irritation study (OECD 404) 0.5 mL undiluted CLAIL0021 was applied evenly to one clipped sites (6 cm^2) of each rabbit and 0.5 mL distilled water was applied to another clipped site of three male, young adult New Zealand White rabbits. The treated and the control sites were covered with gauze patches (semi-occlusive). At the end of the 4 hour exposure period, the residual test item was removed.

Skin reactions were observed at 1, 24, 48 and 72 hours and on day 7 post patch removal. The site of application was visually assessed and scored for erythema and oedema. The mean erythema and oedema scores (average 24/48/72 hours) were 1.67 to 2.00 and 0 to 0.67, respectively, for the three animals. The observed skin lesions recovered completely within 7 days (Anonymous, 2011a).

A3.3.2 Comparison with CLP criteria

According to Table 3.2.2 of Regulation (EC) No. 1272/2008 a classification for skin irritation category 2 applies if:

(1) Mean score 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.

In a reliable GLP study according to OECD 404 the mean erythema and oedema scores in three New Zealand rabbits were 1.67 to 2.00 and 0 to 0.67, respectively after 24 to 72 hours post exposure. The observed skin lesions recovered completely within 7 days (Anonymous, 2011a).

A3.3.3 Conclusion on classification and labelling for skin corrosion/irritation

Thermally treated garlic juice is not classifiable as irritant to skin according to Regulation (EC) No. 1272/2008.

A3.3.4 Overall conclusion on skin irritation and corrosivity related to risk assessment

Not applicable for the CLH report.

A.3.4. Serious eye damage and Eye irritation

Table A.14 Summary table of in vitro studies on serious eye damage and eye irritation

Summary table of in vitro studies on serious eye damage and eye irritation				
Method, Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/group Test substance**	**Dose Duration of exposure**	**Results Average score for corneal opacity, iritis, conjunctiva (24, 48, 72 h) per animal, observations and time reversibility**	**Reference**
Primary eye irritation OECD 405 (version 2002) GLP Klimisch 1 Key study	Rabbit (New Zealand White), 3 female CLAIL 0021 (2.58% w/w total polysulfides)	0.1 mL test item and control saline applied in the conjunctival sac Ocular reaction after 1, 24, 48 and 72 h recorded	*Animal 1:* Opacity = 0.00 (mean and max.) Iritis = 0.00 (mean and max.) Conjunctiva redness = 0.67 (mean 24-72 h) 1 (max.) Chemosis = 0.00 (mean and max.) *Animal 2:* Opacity = 0.00 (mean and max.) Iritis = 0.00 (mean and max.) Conjunctiva redness = 0.33 (mean 24-72 h), 1.00 (max.) Chemosis = 0.00 *Animal 3:* Opacity = 0.00 (mean and max.) Iritis = 0.00 (mean and max.) Conjunctiva redness = 0.33 (mean 24-72 h), 1.00 (max.) Chemosis = 0.00 (mean and max.) All effects were fully reversible within 72 hours	Anonymous 2011b

A3.4.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

One GLP compliant study, conducted according to an acceptable regulatory guideline provide experimental evidence regarding the potential for eye irritation for the active substance.
In a primary eye irritation study according to OECD TG 405 0.1 mL of undiluted CLAIL 0021 was instilled into the conjunctival sac of one eye of three New Zealand White female rabbits. The contralateral eye served as the control and was treated with 0.1 mL 0.9% saline. At 24 hour post instillation, the eyes of all the rabbits were gently washed. Animals were observed for 3 days. Irritation was scored according to OECD TG 405.
Mean eye irritation scores (following assessment at 24, 48 and 72 hour post instillation) of corneal opacity (0.00), iritis (0.00), conjunctival redness (0.33 to 0.67) and chemosis (0.00) were determined (Anonymous, 2011b).

A3.4.2 Comparison with the CLP criteria

According to Table 3.3.2 of Regulation (EC) No. 1272/2008 a classification for eye irritation category 2 applies if:

Substances that produce in at least 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 instillation of the test material, and which fully reverses within an observation period of normally 21 days.

In a reliable GLP study according to OECD 405 the mean eye irritation scores (following assessment at 24, 48 and 72 h post instillation) of corneal opacity (0.00), iritis (0.00), conjunctival redness (0.33 to 0.67, fully reversible after 72 hours) and chemosis (0.00) were determined (Anonymous, 2011b).

A3.4.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Thermally treated garlic juice is not classifiable as eye irritant according to Regulation (EC) No. 1272/2008.

A3.4.4 Overall conclusion on eye irritation and corrosivity related to risk assessment

Not applicable for the CLH report.

A.3.5. Skin sensitisation

Table A.15 Summary table of animal studies on skin sensitisation

Summary table of animal studies on skin sensitisation				
Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/group**	**Test substance, Vehicle, Dose levels, Duration of exposure**	**Results (e.g. EC3-value or amount of sensitised animals at induction dose)**	**Reference**
LLNA Topical application OECD 429 GLP Klimisch 1 Key study	Mouse CBA/J 5 female/dose	CLAIL 0021 (100% thermally treated garlic juice) 1% Pluronic L92 1%, 10% and 25% (v/v)	Positive at 25% test item solution: EC3 value 11.18% (2795 µg/cm²) SI for the 1%, 10% and 25% (v/v) treated groups were 2.16, 2.21 and 12.23, respectively. Positive control: SI of 25% HCA was 9.72	Anonymous 2016e

A3.5.1 Short summary and overall relevance of the provided information on skin sensitisation

A LLNA study according to OECD TG 429 and GLP with the active substance thermally treated garlic juice in mouse was submitted.
In a preliminary assay, ear thickness was measured and was >25% at concentrations of 50%, 75% and 100% CLAIL0021 while a solution of 25% provided <25% ear thickness. Skin erythema were observed at 75% and 100% as well as localized alopecia. The preliminary study served for setting the test concentrations in the main study.

Three groups of 5 female mice were treated with CLAIL0021 at concentrations of 1%, 10% and 25% (v/v) in 1% L92 for three consecutive days (days 0, 1 and 2) on the dorsum of both ears (25 mL per ear). In addition, one group served as vehicle control and was treated with 1% L92, the other group served as a positive control treated with HCA (alpha-hexylcinnamaldehyde) at a concentration of 25% (v/v) in 1% L92.
There were no indications of skin irritation at the treatment site or systemic toxicity in CLAIL0021 treated animals.
On day 5, the uptake of intravenously injected 3H-methyl thymidine into the auricular lymph nodes draining at the site of chemical application was measured (5 hours post-administration) to assess the lymph node proliferative response. Stimulation indices (SI) for the 1%, 10% and 25% (v/v) in 1% L92 treated groups were 2.16, 2.21 and 12.23, respectively. A positive response for HCA (SI = 9.72) confirmed the reliability of the test procedure. The SI obtained for CLAIL0021 at 25% showed a greater than threefold increase over the control value with an EC3 value of 11.18% (Anonymous, 2016e).

A3.5.2 Comparison with the CLP criteria

Hazard categories and sub-categories for skin sensitisers according to Table 3.4.2 and 3.4.4 of Regulation (EC) No. 1272/2008 are as followed:

Subcategory 1A:
- Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.
- For LLNA: EC3 value ≤2%

Subcategory 1B:
- Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.
- For LLNA: EC3 value >2%

With the EC3 value for thermally treated garlic juice being 11.18% (at 25% v/v), classification for skin sensitisation with Skin Sens. 1B, H317 is appropriate.

A3.5.3 Conclusion on classification and labelling for skin sensitisation

The active substance meets classification criteria for Skin Sens. 1B, H317.

A3.5.4 Overall conclusion on skin sensitisation related to risk assessment

Not applicable for the CLH report.

A.3.6. Respiratory sensitisation

A3.6.1. Short summary and overall relevance of the provided information on respiratory sensitisation

Literature data were submitted on respiratory sensitisation.
While EMA (2019) reported garlic to be traditional used against asthma in several regions of the world, literature and case reports indicate occupational asthma from garlic powder or dust (WHO, 1999, Lybarger et al. 1982, Falleroni et al., 1981). In a review paper by Borelli and co-workers respiratory adverse effects like asthma, dyspnoea, cough, rhinitis or rhinoconjunctivitis from occupational exposure (dermal and inhalation) to garlic and garlic dust/powder are described (Borelli et al., 2007).
The literature reported cases of occupational asthma from inhalation of garlic powder (which is chemically different to active substance thermally treated garlic juice), but also dermal contact of different forms of garlic cannot be ruled out as a cause.
Chemical characterisation of plant extracts is a critical factor when relating the published studies to adverse effects. Dried and fresh extract formulations of garlic contain different chemistry compared to thermally treated garlic juice supported under the BPR. However, thermally treated garlic juice also contains dially disulfides, the compounds tested positive in eliciting allergic reactions in humans (Borelli et al., 2007, Papageorgiou et al., 1983).
Occupational data from manufacturing sites (liquid and granular formulations) did not report adverse inhalation effects from their production team on this issue according to the applicant. However, no medical reports were submitted to verify this statement.
The evaluation of thermally treated garlic juice under the plant protection legislation noted the case reports on allergic reactions in humans to garlic or garlic preparations but concluded that sensitisation can be addressed at product level with exposure mitigation measures (Ireland, 2019). EFSA (2020) stated that garlic has the potential to cause asthma under occupational exposure by inhalation.

A3.6.2 Comparison with the CLP criteria

Substances shall be classified as respiratory sensitisers in accordance with the criteria in Table 3.4.1 of Regulation (EC) No. 1272/2008:

Category 1: where data are not sufficient for sub-categorisation in accordance with the following criteria:
a) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity; and/or.
b) if there are positive results from an appropriate animal test.

Sub- category 1A: Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests (1). Severity of reaction may also be considered.

Sub- category 1B: Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests (1). Severity of Severity of reaction may also be considered.

For human evidence Regulation (EC) No. 1272/2008 also notes that it is necessary for a decision on classification to take into account, in addition to the evidence from the cases the size of the population exposed and the extent of exposure.

Occupational exposure to garlic or garlic dust/powder may induce respiratory sensitisation in susceptible persons. Case reports in literature on the active substance thermally treated garlic juice supported under the BPR were not reported in the submitted data package (including literature). Dietary exposure in many regions of the world for a long time indicate that the respiratory sensitisation potential to the general public is low compared to the widespread exposure.

A3.6.3 Conclusion on classification and labelling for respiratory sensitisation

The data were not sufficient to propose classification for respiratory sensitisation to thermally treated garlic juice.

A3.6.4 Overall conclusion on respiratory sensitisation related to risk assessment

Not applicable for the CLH report.

A.3.7. Repeated dose toxicity/STOT RE

A.3.7.1. Short term repeated dose toxicity

A3.7.1.1 Short-term oral toxicity

No short-term oral toxicity data were submitted.

Data waiving	
Information requirement	Short-term oral toxicity
Justification	Ireland (2019) concluded that the nature of the substance as food grade material and a human food source with a global consumption volume of approximately 26 million tons annually (2016 data) makes systemic toxicity testing scientifically unnecessary. Garlic is listed as GRAS by U.S. FDA. Dietary exposure is expected to exceed the systemic exposure by dermal (or inhalation) route. Please see chapter A3.1 for further toxicokinetic and exposure related justifications. Therefore, waiving of the data requirement of Annex II 8.9 of Regulation (EU) No. 528/2012 was acceptable.

A3.7.1.2 Short-term dermal toxicity

No short-term dermal toxicity data were submitted.

Data waiving	
Information requirement	Short-term oral toxicity
Justification	Waiving of the data requirement of Annex II 8.9 of Regulation (EU) No. 528/2012 was acceptable. The proposed conditions for testing by the dermal route are not met. Dermal exposure is very limited/negligible based on the formulation of the carrier-based biocidal product and instructions for use.

A3.7.1.3 Short-term inhalation toxicity

No short-term inhalation toxicity data were submitted.

Data waiving	
Information requirement	Short-term inhalation toxicity
Justification	Waiving of the data requirement of Annex II 8.9 of Regulation (EU) No. 528/2012 was acceptable. The proposed conditions for testing by the inhalation route are not met. Inhalation exposure is negligible based on the formulation of the carrier-based biocidal product and outdoor use.

A3.7.1.4 Overall conclusion on short-term repeated dose toxicity related risk assessment

Not applicable for the CLH report.

A.3.7.2. Sub-chronic repeated dose toxicity

A3.7.2.1 Sub-chronic oral toxicity

No sub-chronic oral toxicity study according to GLP and a regulatory accepted guideline was submitted. The applicant provided a literature paper that investigated garlic enriched diet (dried garlic falkes) administered to horses at a dose of 32 mg/kg bw/d over a course of 83 days. The sample size was limited to 6 horses (6 horses served as a control). While the study results were hampered by the small sample size and horses do not normally represent an accepted animal model for human health, all treated horses showed reduction in haemoglobin and red blood cell counts after the treatment period. No conclusion concerning the benefical effect of improvement of respiratory health could be drawn based on minimal effects and small sample size (Saastamoinen, 2019).

Data waiving	
Information requirement	Sub-chronic oral toxicity
Justification	Please see justification A3.7.1.1.

A3.7.2.2 Sub-chronic dermal toxicity

No sub-chronic dermal toxicity data were submitted.

Data waiving	
Information requirement	Sub-chronic dermal toxicity
Justification	Please see justification A3.7.1.2.

A3.7.2.3 Sub-chronic inhalation toxicity

No sub-chronic inhalation toxicity data were submitted.

Data waiving	
Information requirement	Sub-chronic inhalation toxicity
Justification	Please see justification A3.7.1.3.

A3.7.2.4 Overall conclusion on sub-chronic repeated dose toxicity related risk assessment

Not applicable for the CLH report.

A.3.7.3. Long-term repeated dose toxicity

A3.7.3.1 Long-term oral toxicity

No long-term oral toxicity data were submitted.

Data waiving	
Information requirement	Long-term oral toxicity
Justification	Please see justification under A3.7.1.1

A3.7.3.2 Long-term dermal toxicity

No long-term dermal toxicity data are submitted.

Data waiving	
Information requirement	Long-term dermal toxicity
Justification	Please see justification under A3.7.1.2

A3.7.3.3 Long-term inhalation toxicity

No long-term inhalation toxicity data were submitted.

Data waiving	
Information requirement	Long-term inhalation toxicity
Justification	Please see justification under A3.7.1.3

A3.7.3.4 Overall conclusion on long-term repeated dose toxicity related risk assessment

Not applicable for the CLH report.

A.3.7.4. Specific target organ toxicity – repeated exposure (STOT RE)

A3.7.4.1 Short summary and overall relevance of the provided information on STOT RE

No data were submitted. Thermally treated garlic juice is processed from food grade material, therefore this endpoint has not been investigated.

A3.7.4.2 Comparison with the CLP criteria

Data lacking.

A3.7.4.3 Conclusion on classification and labelling for STOT RE

No repeated dose toxicity studies were submitted; thus, no classification for STOT RE is proposed due to lack of data.

A.3.8. Genotoxicity / Germ cell mutagenicity

A.3.8.1. In vitro

No *in vitro* genotoxicity data were submitted.

Data waiving	
Information requirement	Genotoxicity *in vitro*
Justification	Ireland (2019) concluded that the nature of the substance as food grade material and a human food source with a global consumption volume of approximately 26 million tons (2016 data) makes systemic toxicity testing scientifically unnecessary. Please see chapter A.3.1 for further toxicokinetic and exposure related justifications. Garlic is listed as GRAS by U.S. FDA. Thermally treated garlic juice is processed from food grade material. Dietary exposure is expected to exceed the systemic exposure by the dermal (or inhalation) route. Therefore, waiving of the data requirement of Annex II 8.5 of Regulation (EU) No. 528/2012 was acceptable. Nevertheless, EMA (2019) compiled literature studies which indicate that garlic powder and thermally treated garlic juices (fresh garlic, alcoholic extracts or the components diallyl sulphide and diallyl disulphide) can induce chromosome aberrations *in vitro* and *in vivo* in various models. However, no regulatory accepted test guideline was used and characterisation on the test item and extracts have not been revealed. The active substance under approval is a UVCB substance and contains polysulfides which are chemically different from fresh garlic which contain allicin amongst others. Also in the peer review of the active substance under the plant protection regime, EFSA (2020) identified no critical areas of toxicological concern.

A.3.8.2. In vivo

No *in vivo* genotoxicity data are submitted.

Data waiving	
Information requirement	Genotoxicity *in vivo*
Justification	Based on accepted waiving of information requirement for *in vitro* genotoxicity, no *in vivo* follow-up studies are necessary. Please see also justification under A.3.8.1.

A3.8.2.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

No data were submitted.

A3.8.2.2 Comparison with the CLP criteria

Data lacking.

A3.8.2.3 Conclusion on classification and labelling for germ cell mutagenicity

No mutagenicity or genotoxicity studies were submitted; thus, no classification is proposed due to lack of data.

A3.8.2.4 Overall conclusion on genotoxicity related to risk assessment

Not applicable for the CLH report.

A.3.9. Carcinogenicity

A3.9.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

No carcinogenicity data were submitted. Please see justification A3.7.1.1. Waiving of the data requirement of Annex II 8.11 of Regulation (EU) No. 528/2012 was acceptable.

A.3.9.2 Comparison with the CLP criteria

Data lacking.

A.3.9.3 Conclusion on classification and labelling for carcinogenicity

No carcinogenicity studies were submitted; thus, no classification is proposed due to lack of data.

A.3.9.4 Overall conclusion on carcinogenicity related to risk assessment

Not applicable for the CLH report.

A.3.10. Reproductive toxicity

A.3.10.1. Sexual function and fertility

A3.10.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

No regulatory accepted guideline study under GLP on reproductive toxicity was submitted. Waiving of the data requirement of Annex II 8.11 of Regulation (EU) No. 528/2012 was acceptable. No literature papers were submitted by the applicant for this endpoint.

EMA (2019) indicated in their conclusion on non-clinical data on *Allium sativum L.*, bulbus that a potential impact on male fertility cannot be excluded.
The conclusion was based on a very old fertility study dated back 1947 (no details available) and other investigations by Dixit and Joshi (1982) and Hammami et al. (2008, 2009).
Dixit and co-worker treated male rats with garlic powder (oral administration, 50 mg garlic powder/160 g body weigh) for 45 days or 70 days by oral route. Testes of treated rats over a time course of 45 days showed degenerative changes such as shrinkage of seminiferous tubule and Leydig cells nuclei. After 70 days, severe testicular lesions were seen. Spermatogenesis was arrested at the primary spermatocyte stage and also Sertoli cells showed degenerative changes. The administered limit dose of approximately 300 mg/kg bw/d was considered as LOEL for garlic powder (Dixit and Joshi, 1982).

Hammami and co-workers treated male Wistar rats with crude garlic mixed into standard rat diet for one month (0, 5, 10, 15 and 30% in diet, 6 animals per dose group). Results from the two highest dose groups should be interpreted with caution due to significant body weight reductions of ~12% to 17% (>MTD, please see Table A.16). Histopathology of the testes showed significant and dose-dependent increase in the percentage of empty seminiferous tubules starting from a dose of 10% at which no body weight depression was seen. Decreased serum and testicular testosterone levels were also dose-dependent at 10%, 15% and 30% statistically significant and were associated with elevated LH levels (Hammami et al., 2008).

Table A.16 Selected results on body weight, hormones and testes (Hammami et al., 2008)

Dose groups	Body weight (g)	Serum testosterone (ng/ml)	Luteinizing hormone (LH) in serum (ng/ml)	Testicular testosterone (ng/g)	Empty seminiferous tubules (%)
Control	287.7±20	2.0±0.3	0.3±0.1	1.5±0.02	11.5±2.9
5%	280.2±19	1.8±0.06	0.4±0.15	1.0±0.02*	11.2±4.9
10%	275.8±15	0.5±0.03**	0.6±0.18*	0.4±0.01*	25.7±6.7**
15%	253.2±13**	0.5±0.02**	0.7±0.07*	0.3±0.02*	34.3±1.7**
30%	238.4±18**	0.2±0.01**	1.1±0.18*	0.2±0.01*	37.8±4.1**

* $p<0.05$
** $p<0.01$

In a further mechanistic investigation (one month of dietary treatment, 6 animals per group, 0, 5, 10 and 15% crude garlic) at the two highest dose levels inhibition of Leydig steroidogenic enzyme expression and Sertoli cell markers were found (Hammami et al., 2009). Similar results were reported by Ezz El Arab at al. (2019) in terms of lower serum testosterone levels and histopathological alterations in testes after repeated oral administration with different garlic preparations or extracts for 1 month.
However, other literature studies not reported in EMA (2019) indicate an absence of adverse

effects on testes in rats or increased testosterone serum levels (e.g. Memudu et al. (2015) oral gavage administration of dried garlic powder in aqueous solution to SD rats at 200 mg/kg bw/d for 4 or 8 weeks) or beneficial mixture effects of diallyl sulfides on rat testes and spermatogenesis when co-administered with lead (Hassan et al., 2019).
However, the presented literature studies are not complete and have drawbacks in terms of standardization, validation, low number of animals per dose group (max. 6 animals) and reporting as well as test item characterisation due to the nature of a published literature article.

A3.10.1.2 Comparison with the CLP criteria

Data lacking.

A3.10.1.3 Overall conclusion on sexual function and fertility related to risk assessment

Not applicable for the CLH report.

A.3.10.2. Developmental toxicity

A3.10.2.1 Short summary and overall relevance of the provided information on adverse effects on development

No developmental toxicity data were submitted.

A3.10.2.2 Comparison with the CLP criteria

Data lacking.

A3.10.2.3 Overall conclusion on effects on development related to risk assessment

No developmental toxicity studies were submitted; thus, no classification is proposed due to lack of data.

A.3.10.3. Effects on or via lactation

A3.10.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

No reproductive toxicity data were submitted.

A3.10.3.2 Comparison with the CLP criteria

Data lacking.

A3.10.3.3 Overall conclusion on effects on or via lactation related to risk assessment

No developmental toxicity studies were submitted; thus, no classification is proposed due to lack of data.

A.3.10.4. Conclusion on classification and labelling for reproductive toxicity

Experimental data for reprotoxicity such as two-generation reproduction and pre-natal developmental toxicity studies with the active substance thermally treated garlic juice (supported under the BPR) were not submitted and are not available. Thus, no classification is proposed due to lack of data.

However, repeated dose toxicity studies from open literature indicate effects on male fertility upon dietary or oral gavage administration of crude garlic or garlic preparations/extracts. As thermally treated garlic juice is a UVCB substance and characterisation of test item in literature reported studies is very limited except description of garlic purchase and some information on protocols of preparation, there is considerable uncertainty amongst others if reported effects are also relevant for the active substance thermally treated garlic juice.

In absence of data with thermally treated garlic juice no classification for reproductive toxicity is proposed.

A.3.10.5. Overall conclusion on reproductive toxicity related to risk assessment

Not applicable for the CLH report.

A.3.11. Aspiration hazard

A3.11.1 Short summary and overall relevance of the provided information on aspiration hazard

No aspiration hazard from thermally treated garlic juice is expected (cf. section A.1.3).

A3.11.2 Comparison with the CLP criteria

According to Table 3.10.1 of Regulation (EC) No. 1272/2008 a classification for aspiration hazard applies:

Substances known to cause human aspiration toxicity hazards or to be regarded as if they cause human aspiration toxicity hazard. A substance is classified in Category 1:

(a) based on reliable and good quality human evidence *or*

(b) if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40°C.

Thermally treated garlic juice does not belong to the chemicals class of hydrocarbons. In addition occupational data from manufacturing sites do not report adverse inhalation effects of their production team according to the submission of the applicant. However, this information has not been underpinned with medical report data from workers in manufacturing plants.

In absence of reported adverse human evidence data and chemical properties thermally treated garlic juice is not classified for aspiration hazard.

A3.11.3 Conclusion on classification and labelling for aspiration hazard

No classification proposed.

A.3.12. Neurotoxicity

A3.12.1 Short summary and overall relevance of the provided information on neurotoxicity

No neurotoxicity data were submitted. The conditions for additional data on neurotoxicity according 8.13.2 of Regulation (EU) No. 528/2012 are not met.

A3.12.2 Comparison with the CLP criteria

Data lacking.

A3.12.3 Conclusion on neurotoxicity related to risk assessment

Not applicable for the CLH report.

A.3.13. Immunotoxicity

A3.13.1 Short summary and overall relevance of the provided information on immunotoxicity

No immunotoxicity data were submitted. The conditions for additional data on immunotoxicity according 8.13.4 of Regulation (EU) No. 528/2012 are not met

A3.13.2 Comparison with the CLP criteria

Data lacking.

A3.13.3 Conclusion on immunotoxicity related to risk assessment

Not applicable for the CLH report.

A.3.14. Endocrine disruption

Not applicable for CLH report.

A.3.15. Further Human data

No further human data were submitted. No relevant health data were available nor were adverse health effects reported for the active substance thermally treated garlic juice from manufacturing plants according to the applicant (cf. IUCLID, section: medical surveillance data on manufacturing plant personnel).

A.3.16. Other data

No other data were submitted.

A.4. Environmental effects assessment

A number of studies were submitted for the environmental effect assessment. Thermally treated garlic juice hydrolyses rapidly and can be classified as readily biodegradable. No chronic data regarding aquatic ecotoxicity were available. Based on the evaluated data no classification is warranted for the environment.

A.4.1. Fate and distribution in the environment

A.4.1.1. Degradation

A4.1.1.1 Abiotic degradation

Hydrolysis

Table A.17 Summary table - Hydrolysis

Summary table - Hydrolysis							
Method, Guideline, GLP status, Reliability, Key/supp ortive study	pH	Temp. [°C]	Initial TS concentration, C0 [µg/mL]	Half-life, DT50 [h]	Coeffici -ent of correl- ation, r^2	Remarks	Reference
OECD Guideline 111 and OPPTS 835.2120, GLP, Reliability 1, Key study	4 7 9	20°C 30°C 50°C	100 µg CLAIL0021 (100% thermally treated garlic juice)/mL	**At 20°C:** pH 4: 1.62 h pH 7: 16.3 h pH 9: 5.11 h **At 30°C:** pH 4: 17.4 h pH 7: 18.4 h pH 9: 12.4 h **At 50°C:** pH 4: 23.1 h pH 7: 7.88 h pH 9: 3.12 h	-	Due to the fact that 90% degradat ion was observed , the DT50 was derived from the DT90/3. 32. For pH4 & 9 (T50) DFOP slow phase and for pH9 (T20) HS slow phase was chosen.	Anonymous 2021b

Study summary (Anonymous 2021b):

The hydrolysis of thermally treated garlic juice (in the study named CLAIL0021) was carried out in a tiered approach as per OECD 111 and OPPTS 835.2120. Active substance thermally treated garlic juice in sterile buffer solutions of pH 4.0, 7.0 and 9.0 was incubated for 5 days at 50 ± 0.5ºC in the preliminary test. Hydrolysis reactions were monitored by analyzing the analyte concentration at set intervals using an in-house developed and validated HPLC method. The preliminary test results revealed that the thermally treated garlic juice is nearly 90% hydrolysed at all pH after incubation at 50 ± 0.5ºC. To monitor the rate of hydrolysis a definitive test was performed in 4.0, 7.0 and 9.0 buffer solutions at three temperatures (20 ± 0.5ºC, 30 ± 0.5ºC and 50 ± 0.5ºC). The decay timings were calculated (DT50 and DT90) using CAKE software. For more study details please see Remarks above and Appendix VII: Study summaries.

Estimated photo-oxidation in air

Table A.18 Summary table – Photo-oxidation in air

Summary table – Photo-oxidation in air					
Model	Light protection (yes/no)	Estimated daily (24 h) OH concentration [OH/cm³]	Overall OH rate constant [cm³/molecule sec]	Half-life [hr]	Reference
Atkinson model (ver. 1.92)	NA	5E5	68.87E-12 to 517.17E-12	0.745 to 5.591	Anonymous 2018

Study summary (Anonymous 2018):
The half-life for the atmospheric gas-phase reaction of the thermally treated garlic juice marker molecules with hydroxyl radicals ranged from 0.745 to 5.591 hours, based on an Atkinson structure-activity relationship analysis performed with the Atmospheric Oxidation Program (assuming a constant hydroxyl concentration of 5E5 radicals/cm3). Hydrogen abstractions and reactions with nitrogen and sulfur were predicted to contribute to the overall atmospheric photochemical degradation pathway, and the overall bimolecular rate constant for the process (kOH) was calculated to be 68.87E-12 to 517.17E-12 cm3/moleculesec.

A4.1.1.2 Biotic degradation

A4.1.1.2.1 Biodegradability (ready/inherent)

Table A.19 Summary table – biodegradation studies (ready/inherent)

Summary table - biodegradation studies (ready/inherent)											
Method, Guideline, GLP status, Reliability, Key/supportive study	Test type	Test parameter	Inoculum			Additional substrate	Test sub-stance conc.	Degradation		Remarks [positive control]	Reference
			Type	Con cen-tration	Ad ap-tatio n			Incubation period	Degree [%]		
Experimental, OECD Guideline 301B, GLP, Reliability 1, Key study	Ready biodegradability	CO_2 evolution	Secondary effluent sourced from a STP receiving domestic sewage	6.0 E7 CFU/L	-	-	50 mg/L (equivalent to 15.1 mg TOC/L)	29 d	100% degradation after 29 d (calculated as 140.36% arithmetic mean degradation)	Positive control (sodium benzoate) attained 72.37% after 10 d and 100.72% degradation after 29 d; Toxicity control attained 35.80% degradation after 7 d	Anonymous 2021c

										and 90.87 % degradation after 29 d	

CFU: Colony Forming Units

Study summary (Anonymous 2021c):
The ready biodegradability of thermally treated garlic juice (in the study named CLAIL0021) was investigated using the CO2 Evolution Test, according to OECD Guideline 301B. The test item was added to two test vessels at the concentration of 50 mg/L (equivalent to 15.1 mg of Total Organic Carbon/L). Two flasks as controls containing only the inoculum, one flask as procedure control containing reference item and one flask as toxicity control containing the test item and the reference item were included in the test. All the treatments were added with equal volume of inoculum which was collected from the secondary effluent treatment plant receiving predominantly domestic sewage and volume made with mineral media. Treatment mixtures were aerated for 29 days with carbon dioxide (CO2) free air. The CO2 released was trapped in a series of bottles containing barium hydroxide, which were connected to the outlet of each test vessel. The residual barium hydroxide was measured on days 2, 5, 7, 10, 12, 15, 17, 19, 23, 27 and 29 after the initiation of the test. The arithmetic mean percent degradation of test item was calculated to be 140.36% (note that this calculated value was >100% due to the nature of the calculation, the actual degradation of the test item was 100%) at the end of test (day 29) while, the percent degradation of reference item was 100.72% and the toxicity control was 90.87% at the end of the test.
Ready biodegradability data are available for thermally treated garlic juice which resulted in 100% degradation (based on CO_2 evolution) after 29 days. The tested substance is a UVCB substance and the 10-day window is not appropriate to be applied. It can be anticipated that a sequential biodegradation of the individual structures is taking place. In this case, a case by case evaluation is recommended (see also OECD Guideline for testing chemicals (2006) section 3 page 8 point 43) Considering all the provided data and the degradation curve, 100% degradation was exceeded on day 17. Due to this we would expect the UVCB substance to be readily biodegradable. For more study details please see Remarks above and Appendix VII: Study summaries.

A4.1.1.3 Rate and route of degradation including identification of metabolites and degradation products

A4.1.1.3.1 Biological sewage treatment

Aerobic biodegradation
Not applicable.

Anaerobic biodegradation
Not available.

STP simulation test
Not available.

A4.1.1.3.2 Biodegradation in freshwater

Aerobic aquatic degradation

Table A.20 Summary table – freshwater aerobic biodegradation

Summary table – freshwater aerobic biodegradation							
Method, Guideline, GLP status, Reliability, Key/supportive study	Test type1	Exposure	Test substance concentration	Incubation period	Degradation (DT50)	Remarks	Reference
No guideline study, Non-GLP, Reliability: 3, supportive study	Degradation of thermally treated garlic juice in "Local River water"	14 days	1200 g CLAIL0021 (100% thermally treated garlic juice) in 24 L river water	14 days	4.8 d	pH dropped from 7.4 to 5.4 suggesting formation of acidic species. No test temperature was reported.	Anonym-ous 2019a
Scientific literature study, Non-GLP, Reliability 3, supportive study	The study did not follow a standardised guideline	Radiolabelled diallyl disulfide (a thermally treated garlic juice marker molecule) was applied to tap water and samples taken for monitoring at periods of 0, 1, 2, 4, 8, 16, 20 and 24 hours.	50 ppm [^{35}S] diallyl disulfide	24 h 26°C	<1 day (16% of diallyl disulfide remained 24 h after application)	The study is not focused on the determination of the fate and behaviour of the test substance in water, but on its persistence combined with the toxicity to the mosquito larvae. Consequently, the study might not be directly relevant to a particular data requirement point but it gives an indication towards short life of these	Anony-mous 1989

						molecules.	

Study summaries:

Anonymous 2019a:
The study was carried using local river water. Thermally treated garlic juice (named in the study CLAIL0021) was analysed prior to study for polysulfide contents. A large plastic tub (approx. dimension 36 x 20 x 24cm) containing 24L river water was placed in open atmosphere under a shed. Approximately 1200g of CLAIL0021 (100% thermally treated garlic juice), batch no AN18825970 (expiry date: Jan 2021) was added into the container and mixed well using stirrer. The container was covered with fine cloth. At each time interval a sample (approx. 10mL) was withdrawn from the container. Only 2 g was used for HPLC analysis while rest was returned to the container. PH was measured at each sampling point. Before sampling container water was mixed using handheld stirrer (50rpm). Sampling was carried at day 1, 5, 7, 11 and 14. Study was carried between 10- 25 Oct 2019. The data obtained from both experiments suggest that polysulfides degrade quickly in the aquatic natural environment.
No additional peaks were observed in the HPLC chromatogram upon degradation of polysulfides which may suggest they breakdown in the presence of microbes to soluble sulfur species e.g sulphates. Water contains sulfur reducing bacteria which can degrade the sulfur to sulfate or hydrogensulfide. PH changes from 7.4 to 5.4 suggest formation of acidic species e.g. a weak ion of HSO4-1. For more study details please see Remarks above and Appendix VII: Study summaries.

Anonymous 1989:
The aim of this study was to examine mortality in two developmental stages, larval and pupal, of mosquito *Culex pipiens quinquifasciatus* exposed to diallyl disulfide at concentration 50 ppm over a time period (24 hr). The amount of this substance taken up by the organisms was measured and related to different sensitivities in the two developmental stages. Additional experiment was carried out in which the decrease in concentration of 50 ppm diallyl disulfide from the tap water with no organisms present was measured. This experiment indicates that dially disulfide degrade quickly in water. For more study details please see Remarks above and Appendix VII: Study summaries.

Water/sediment degradation test

Not available.

A4.1.1.3.3 Biodegradation in seawater

Seawater degradation study
Not available.

Seawater/sediment degradation study
Not available.

A4.1.1.3.4 Higher tier degradation studies in water or sediment

Higher tier degradation studies in water or sediment are not available or considered necessary for thermally treated garlic juice, given that the substance is not applied directly to aquatic systems. According to the degradation studies the substance is readily biodegradable and hydrolyses very fast (<1 day).

A4.1.1.3.5 Biodegradation during manure storage

Not available.

A4.1.1.3.6 Biotic degradation in soil

A4.1.1.3.7 Laboratory soil degradation studies

Aerobic biodegradation

Table A.21 Summary table – aerobic biodegradation of thermally treated garlic juice in soil-laboratory study

Summary table – aerobic biodegradation in soil- laboratory study											
Method, Guideline, GLP status, Reliability, Key/supportive study	**Test type[1]**	**Exposure**	**Test system**				**Test substance concentration**	**Incubation period**	**Degradation DT50 (days)**	**Remarks**	**Reference**
			Soil origin	**Soil type**	**pH**	**OC %**					
No guideline study similar to OECD 307, Non-GLP, Reliability: 2, key study	no	Aerobic, 20°C, dark	Soil I: Norwich, Norfolk	Sandy loam	6.7	3.8	20 g CLAIL0021 NEMguard Liquid (100% thermally treated garlic juice)/kg dry soil	6 days	3.01 ± 0.25	The study was terminated on day 6 as DT50's in all soils had been achieved and polysulfides were below the confident HPLC detection limit.	Anonymous 2019b
			Soil II: Manae, Cambridgeshire	Clay loam	6.2	16.7			4.98 ± 0.08		
			Soil III: Ipswich, Suffolk	Sandy silt loam	7.1	2.4			3.63 ± 0.26		
			Soil IV: Kings Lynn, Norfolk	Loamy sand	7.7	5.1			2.01 ± 0.13		
[1] Test according to OECD criteria											

Study summary (Anonymous 2019b):
A new study was conducted to determine the half-life of active substance thermally treated garlic juice (in the study named CLAIL0021 NEMguard Liquid) in different soil types following a methodology similar to that described in OECD guideline 307. HPLC was used as the analytical technique to observe the degradation pattern of the polysulfides within thermally treated garlic juice. Soil textural analysis and biomass determination (microbial activity) was performed by an external lab. The data obtained in this study clearly indicate that the half-life of the active substance is very short across all soil types. The shortest half-life was observed in sandy loams (2-3 days) and longest in clay loams and organic (3-5 days).

Considering the longest half-life, it can be concluded that thermally treated garlic half-life across all soil types is no more than 5-days with no effect observed on microbial activity. For more study details please see Remarks above and Appendix VII: Study summaries.

Anaerobic biodegradation
Not available.

A4.1.1.3.8 Higher tier degradation studies in soil

Not available.

Field dissipation studies (field studies, two soil types)

Not available.

A4.1.1.3.9 Short summary and overall relevance of the provided information on degradation and conclusion on rapid degradation

Thermally treated garlic juice undergoes rapid hydrolysis (DT_{50} = 16.3 h at 20°C & pH 7; Anonymous 2021b).

The photochemical oxidative degradation half-lives of thermally treated garlic juice marker molecules (diallyl sulfide, diallyl disulfide, diallyl trisulfide and diallyl tetrasulfide) ranged from 0.745 to 5.591 hours via the Atkinson model (version 1.92) (e), with the DT_{50} of 5.591hours considered the worst-case representative value for thermally treated garlic juice.

Thermally treated garlic juice can be classified as readily biodegradable according to the results of a ready biodegradability test (OECD 301B, Anonymous 2021c).

Thermally treated garlic juice is derived from a natural plant material consisting of a complex mixture of naturally occurring compounds (polysulfides and plant matrix) which are expected to degrade quickly in the environment like any other plant debris.

In addition, supporting data on the rapid degradation of the active components of thermally treated garlic juice in the aquatic compartment are available in the scientific literature (Anonymous 1989), which showed that only 16% of the active component diallyl disulfide remained 24 hours after the application, implying a DT50water of <1 day for the surface water compartment. In another degradation study, a DT50 of 4.8 days was determined (Anonymous 2019a). However, the provided studies can only be rated as supportive information due to limited information on the study design. The US EPA stated that "garlic is presumed to be non-persistent since it is material known to rapidly degrade in the environment" (US EPA 1992).

Under the proposed uses, any potential residues reaching wastewater treatment plants will be indistinguishable from other naturally occurring residues of biological origin. Results from the toxicity control of a reliable OECD Guideline 301B ready biodegradability study concluded that thermally treated garlic juice is not inhibitory to sewage microorganisms, with the toxicity control having attained 35.8% degradation after 7 days and 90.9% degradation after 29 days (Anonymous 2021c).

Thermally treated garlic juice is therefore concluded to be rapidly degradable (both in the environment and in wastewater treatment plants).

A.4.1.2. Distribution

A4.1.2.1 Adsorption onto/desorption from soils

It is technically not possible to experimentally determine adsorption coefficient for thermally treated garlic juice as it is a complex mixture of naturally occurring substances. Upon release to the environment each of the components in the mixture will behave independently and will exhibit its own mobility and degradation characteristics. In this case it is considered appropriate to determine the adsorption characteristics for the biologically active polysulfides, although it is noted that in the active substance these molecules may behave differently. An OECD 121 laboratory study was conducted to derive the Koc for three marker compounds.

Table A.22 Summary table – Adsorption/desorption

Summary table – Adsorption/desorption									
Method, Guideline, GLP status, Reliability	Soil	Adsorbed AS [%]	Ka	KaOC	Kd KdOC Ka/Kd	Kf	1/n	Remarks	Reference
OECD 121, GLP, Reliability 1	HPLC Method	DAS 1-4	Not relevant for OECD 121	**DAS 1:** 575.44 **DAS 2:** 1778.28 **DAS 3:** 3981.07	Not relevant for OECD 121	Not relevant for OECD 121	Not relevant for OECD 121	The retention time of DAS4 was outside the calibration range and was therefore not calculated.	Anonymous 2022c

Ka = Adsorption coefficient
KaOC = Adsorption coefficient based on organic carbon content
Kd = Desorption coefficient
KdOC = Desorption coefficient based on organic carbon content
Ka/ Kd = Adsorption / Desorption distribution coefficient

A4.1.2.2 Higher tier soil adsorption studies

No data submitted. Thermally treated garlic juice was tested to be readily biodegradable, therefore higher tier soil adsorption studies are not considered necessary.

A4.1.2.3 Volatilisation

Regarding volatilisation, please see Part A, section 1.3 Physical and chemical properties of the active substance.

A.4.1.3. Bioaccumulation

Not available.

A.4.1.4. Monitoring data

No monitoring data are available.

A.4.2. Effects on environmental organisms

A.4.2.1. Atmosphere

The photochemical oxidative degradation half-life of thermally treated garlic juice marker molecules (diallyl sulfide, diallyl disulfide, diallyl trisulfide and diallyl tetrasulfide) was shown to range from 0.745 to 5.591 hours via the Atkinson model (version 1.92) (assuming a constant hydroxyl concentration of 5E5 radicals/cm^3). Hydrogen abstractions and reactions with nitrogen and sulfur were predicted to contribute to the overall atmospheric photochemical degradation pathway, and the overall bimolecular rate constant for the process (kOH) was calculated to range from 68.87E-12 to 517.17E-12 cm^3/molecule-sec. The photochemical oxidative degradation of garlic in air is therefore concluded to be rapid and consequently, air is not expected to be an environmental compartment of concern.

A.4.2.2. Toxicity to sewage treatment plant (STP) microorganisms

Inhibition of microbial activity (aquatic)

Table A.23 Summary table – Inhibition of microbial activity

Summary table – inhibition of microbial activity									
Method, Guideline, GLP status, Reliability, Key/supportive study	Species/ Inoculum	Endpoint	Exposure		Results			Remarks	Reference
			Design	Duration	NOEC	EC10	EC50		
Experimental, OECD Guideline 301B, GLP, Reliability 1, Key study	sewage, domestic	Biodegradation (CO_2 evolution)	Static	28 d	25 mg/L	-	-	Ready biode-grad-ability study toxicity control	Anonymous 2021c

Study summary (Anonymous 2021c):
Please see study summary for point A4.1.1.2.1- Biodegradability.

A.4.2.3. Aquatic compartment

A4.2.3.1 Freshwater compartment

Acute/short-term toxicity (freshwater)

Table A.24 Summary table – acute/short-term aquatic toxicity

Summary table – acute/short-term aquatic toxicity										
Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results [mg thermally treated garlic juice/L]			Remarks	Reference
				Design	Duration	NOEC	LC/EC10	LC/EC50		
Fish										
Fish, acute toxicity test, OECD Guideline 203, GLP, Reliability 1, Key study	*Cyprinus carpio*	LC_{50} (mortality)	Thermally treated garlic juice[1]	Semi-static	96 h	8.23 (geo-metric mean measured)	-	11.7 (geometric mean measured)	24h-LC100: 15.15 mg/L (geometric mean measured)	Anonymous 2021d
Fish, acute toxicity test, OECD Guideline 203, GLP, Reliability 3, Supporting study	*Cyprinus Carpi*	LC_{50} (mortality)	Thermally treated garlic juice[1]	Semi-static	96 h	9.9 (nominal)	-	19.6 (nominal)	Nominal concentrations, no verification of the test substance concentrations at the end of the test	Anonymous 2012a
Invertebrates										
Daphnia sp. Acute immobilisation test, OECD Guideline 202,	*Daphnia magna*	EC_{50} (immobilisation)	Thermally treated garlic	Semi-static	48 h	2.58 (geometric mean	-	13.7 (geometric mean	-	Anonymous 2021e

GLP, Reliability 1, Key study			juice[1]			measured)		measured)		
Daphnia sp. Acute immobilisation test, OECD Guideline 202, GLP, Reliability 3, Supporting study	*Daphnia magna*	EC_{50} (immobilisation)	Garlic Juice concentrate 883	Static	48 h	1.0 (nominal)	-	9.3 (nominal)	No diallylsulfides or any other marker molecules were measured. No information on the tested substance beside the test substance description as "*Garlic Juice concentrate 883*". It is not possible to decide if the used test item in this study equals thermally treated garlic juice in its characteristics.	Anonymous 2000

Algae (growth inhibition)[1]										
Freshwater algae growth inhibition test, OECD Guideline 201, GLP, Reliability 1, Key study	*Pseudokirchneriella subcapitata*	ErC_{50} (growth rate inhibition)	Thermally treated garlic juice[1]	Static	72 h	2.55 (geometric mean measured)	8.52 (geometric mean measured)	19.2 (geometric mean measured)	No DAS3 was detected after 48 hrs.	Anonymous 2021f
Freshwater algae growth inhibition test, OECD Guideline 201, GLP, Reliability 3, Supporting study	*Pseudokirchneriella subcapitata*	ErC_{50} (growth rate inhibition)	Thermally treated garlic juice[1]	Static	72 h	8.1 (nominal)	-	57.8 (nominal)	Nominal concentrations, no verification of the test substance concentrations at the end of the test	Anonymous 2012b

[1] in the study named CLAIL0021

Study summaries:
For more study details please see Remarks above and Appendix VII: Study summaries.

Anonymous 2021d:
The acute toxicity of thermally treated garlic juice to common carp (*Cyprinus carpio*) was determined in a semi static test condition. Fish were exposed to five nominal concentrations of thermally treated garlic juice (CLAIL0021) of 7.0, 11.9, 20.2, 34.3 and 58.3 mg a.s./L and a RCW control. The maximum concentration of thermally treated garlic juice causing no mortality and the lowest concentration tested causing 100 percent mortality within the 96 h test period were 8.24 and 15.15 mg a.s./L based on the analysed mean measured concentration, respectively. The 96 h LC50 was 11.7 a.s./L based on the analysed mean measured concentration.

Anonymous 2012a:
The acute toxicity of thermally treated garlic juice (CLAIL0021) to common carp (*Cyprinus carpio*) was determined in a semi-static test. Fish were exposed to five nominal concentrations (9.9, 14.8, 22.2, 33.3 and 50.0 mg a.s./L) and a dilution water control. The maximum concentration of the active substance causing no mortality and the lowest concentration tested causing 100% mortality within the 96 hour test period were 9.9 and 50.0 mg CLAIL0021/L, respectively. The 96 hour LC50 was determined to be 19.64 mg a.s.21/L.

Anonymous 2021e:
The acute immobilization effect of the test item thermally treated garlic juice (CLAIL0021) was studied on *Daphnia magna* for 48 hours. There was no immobilization of daphnia in the negative control and at the tested concentration of 6 mg a.i./L at 24 and 48 hours of exposure. The immobilization of daphnia was 0, 15, 40 and 80% at 24 h and 20, 30, 70 and 100% at 48 h exposure at 12, 24, 48 and 96 mg/L, respectively. Where mean measured concentrations were 2.51, 5.16, 10.20, 21.05 and 41.93 mg/L of thermally treated garlic juice, respectively. The maximum concentration causing no immobilization and the lowest concentration tested causing 100 % immobilization within the 48 h test period were 2.51 and 41.93 mg a.s./L based on the analysed mean measured concentration, respectively. The 48-hour EC50, NOEC and LOEC values for the test item were 13.69 (95% fiducial limits: 10.46 to 17.71), 2.58 and 5.21 mg/L (based on geometric mean concentrations), respectively.

Anonymous 2000:
The acute toxicity of "Garlic Juice concentrate 883" (test substance was not further specified) to *Daphnia magna* was assessed under static exposure conditions. The. Groups of twenty, first instar Daphnia <24 hours old, were exposed to the test substance in Elendt M4 medium for 48 hours at nominal concentrations of 0.10, 0.22, 0.46, 1.0, 2.2, 4.6, 10, 22, 46 and 100 mg/L. Numbers of immobilised daphnids were recorded for each test and control group after 24 and 48 hours. The 48 hour EC50 (immobilisation) and NOEC values value for the test item with *Daphnia magna* were determined to be 9.3 mg/L and 1.0 mg/L, respectively.

Anonymous 2021f:
The effect of thermally treated garlic juice (CLAIL0021) was tested on the growth of freshwater unicellular green alga *Pseudokirchneriella subcapitata*. The alga was exposed to the test item at the nominal concentrations of 5.8, 9.3, 14.8, 23.8, 38.0, 60.8 and 97.3 mg/ L (factor of 1.6) (respective geometric mean concentrations were 2.55, 4.41, 6.40, 9.65, 14.72, 24.03 and 36.42 mg test item/L) along with a negative control. The cell growth was measured at 24, 48 and 72 hours after the initiation of the test.
The 72-hour ErC50 and ErC10 values for thermally treated garlic juice were 19.22 (95% fiducial limits: 14.70 to 24.67) and 8.517 (4.072 to 15.95) mg/L (based on geometric mean concentrations), respectively. The 72-hour NOEC and LOEC values (based on growth rate) were 2.55 and 4.41 mg/L (based on geometric mean concentrations), respectively.

Anonymous 2012b:
The toxicity of thermally treated garlic juice (CLAIL0021) to the alga *Pseudokirchneriella subcapitata* was determined. The study was run with nominal concentrations of 8.1, 13.4, 22.0, 36.4 and 60.0 mg a.s. /L, together with a negative control. After 72 hours, the median effective concentration, biomass (EbC50) was 27.4 mg a.s./L and the median effective concentration, growth rate (ErC50) was 57.8 mg a.s./L. The NOEC and LOEC values of thermally treated garlic juice for the growth inhibition of *P. subcapitata* over the entire 72 h exposure period were 8.1 and 13.4 mg/L, respectively.

Chronic/long-term toxicity (freshwater)
Not available.

A4.2.3.2 Sediment compartment

Acute/short-term toxicity (freshwater sediment)
Not available.

Chronic/long-term toxicity (freshwater sediment)
Not available.

A4.2.3.3 Marine compartment

Acute/short-term toxicity (seawater)
Not available.

Chronic/long-term toxicity (seawater)
Not available.

A4.2.3.4 Sea sediment compartment

Acute/short-term toxicity (sea sediment)
Not available.

Chronic/long-term toxicity (sea sediment)
Not available.

A4.2.3.5 Higher tier studies on aquatic organisms

Not available.

A.4.2.4. Terrestrial compartment

Not applicable for CLH report.

A.4.2.5. Groundwater

Not applicable for CLH report.

A.4.2.6. Birds and mammals

Not applicable for CLH report.

A.4.2.7. Primary and secondary poisoning

Not applicable for CLH report.

A.4.3. Endocrine disruption

Not applicable for CLH report.

A.4.4. Derivation of PNECs

Not applicable for CLH report.

A.4.5. Overall summary of acute and chronic aquatic toxicity data and Comparison with the CLP criteria

A.4.5.1. Short-term (acute) aquatic hazard

Table A.24 Summary of key information on acute/short-term aquatic toxicity relevant for acute classification

Method	Species	Test material	Results	Remarks	Reference
Fish					
Fish, acute toxicity test, OECD 203, GLP, Reliability 1, Key study	*Cyprinus carpio*	Thermally treated garlic juice[1]	LC_{50}: 11.7 mg/L (geometric mean measured)	-	Anonymous 2021d
Invertebrates					
Daphnia sp. Acute immobilisation test, OECD 202, GLP, Reliability 1, Key study	*Daphnia magna*	Thermally treated garlic juice[1]	EC_{50}: 13.7 mg/L (geometric mean measured)	-	Anonymous 2021e
Algae					
Freshwater alga and cyanobacteria growth inhibition test, OECD 201, GLP, Reliability 1, Key study	*Pseudokirchneriella subcapitata*	Thermally treated garlic juice[1]	ErC_{50}: 19.2 mg/L (geometric mean measured)	-	Anonymous 2021f

[1] in the study named CLAIL0021

A.4.5.2. Chronic/ long-term aquatic hazard (including information on bioaccumulation and degradation)

No chronic aquatic toxicity data are available. Chronic aquatic hazard classification has been based on available acute aquatic toxicity data.

A.4.5.3. Conclusion on classification and labelling for environmental hazards and comparison with the CLP criteria

According to 4.1.2.9.5. of Annex I of Regulation (EC) No 1272/2008 (CLP Regulation) substances are considered rapidly degradable in the environment if one of the following criteria holds true:
(a) if, in 28-day ready biodegradation studies, at least the following levels of degradation are achieved:
(i) tests based on dissolved organic carbon: 70 %;
(ii) tests based on oxygen depletion or carbon dioxide generation: 60 % of theoretical maximum.
or (b) if, in those cases where only BOD and COD data are available, when the ratio of BOD 5 /COD is ≥ 0,5; or
or (c) if other convincing scientific evidence is available to demonstrate that the substance can be degraded (biotically and/or abiotically) in the aquatic environment to a level > 70 % within a 28-day period.

Ready biodegradability data are available for thermally treated garlic juice which resulted in 100% degradation (based on CO_2 evolution) after 29 days. The tested substance is a UVCB substance and the 10-day window is not appropriate to be applied. It can be anticipated that a sequential biodegradation of the individual structures is taking place. In this case, a case by case evaluation is recommended (see also OECD Guideline for testing chemicals (2006) section 3 page 8 point 43) Considering all the provided data and the degradation curve, 100% degradation was exceeded on day 17. Due to this thermally treated garlic juice is expected to be readily biodegradable.

According to Annex I: Table 4.1.0 "Classification categories for hazardous to the aquatic environment", thermally treated garlic juice is considered not to fulfil classification as short-term (acute) aquatic hazard Category Acute 1 as $L(E)C_{50}$ value being 11.7 mg/L (the 96-hour LC_{50} derived from the acute *Cyprinus carpio* toxicity study)> 1 mg/l.

Chronic aquatic ecotoxicological data are not available. According to Annex I: Table 4.1.0 "Classification categories for hazardous to the aquatic environment", thermally treated garlic juice is not considered to fulfil classification as long-term (chronic) aquatic hazard for any chronic category as a rapidly degradable substance with low potential for bioaccumulation.

A.5. Assessment of additional hazards

A.5.1. Hazardous to the ozone layer

Thermally treated garlic juice contains neither Cl, Br nor F substituents. The photochemical oxidative degradation half-life of thermally treated garlic juice marker molecules (diallyl sulfide, diallyl disulfide, diallyl trisulfide and diallyl tetrasulfide) was shown to range from 0.745 to 5.591 hours via the Atkinson model. It is therefore concluded that thermally treated garlic juice degrades rapidly in air and the atmospheric lifetime is not long enough.

A.5.1.1. Short summary and overall relevance of the provided information on ozone layer hazard

See point A.5.1.

A.5.1.2. Comparison with the CLP criteria

Not classified as hazardous to ozone layer.

A.6. Additional Labelling

Supplemental hazard labelling information is not relevant.

A.7. Assessment of exclusion criteria, substitution criteria and POP

Not applicable for CLH report.

B. Appendices

APPENDIX V: OVERALL REFERENCE LIST (INCLUDING DATA OWNER AND CONFIDENTIALITY CLAIM)

Author	Year	Section No / Reference No	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Y/N)	Owner	Applicability	
						CAR	CLH
Anonymous	2002a	A.1.3	Determination of specified physical chemistry parameters of garlic concentrate CLAIL0021, a soluble concentrate formulation in compliance with good laboratory practice Ecospray Ltd. OA00883 GLP Unpublished	Yes	Ecospray Limited	Yes	**No**
Anonymous	2014a	A.1.3	Eagle Green Care SC - Determination of physical chemical properties before and after an accelerated storage procedure for 14 days at 54 deg C ECOSpray OA02407 GLP Unpublished	Yes	EcoSpray Limited	Yes	**No**
Anonymous	2016a	A.1.3 A.1.4	Eagle Green Care SC Determination of physical chemical properties before and after 2 years storage under ambient conditions Ecospray Ltd. OA02406 GLP Unpublished	Yes	Ecospray Limited	Yes	**Yes**
Anonymous	2021b	A.4.1.1.1	Garlic extract (CLAIL0021): Hydrolysis as a function of pH; GLP; Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	2018	A.4.1.1.1	Garlic extract: Fate and behaviour in air; Ecospray Limited UK; Non-GLP; Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	2021c	A.4.1.1.2.1 and A.4.2.2	Garlic extract (CLAIL0021): Ready biodegradability – CO2 evolution test; GLP; Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	1989	A.4.1.1.3.2	Environmental persistence of diallyl disulfide, an	No	Publicly available	Yes	**Yes**

Author	Year	Section No / Reference No	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Y/N)	Owner	Applicability CAR	Applicability CLH
			insecticidal principle of garlic and its metabolism in mosquito, *Culex pipiens quinquifasciatus* Say. *Chemosphere*, 18, 1525-1529; Peer reviewed scientific literature; Non-GLP; Published		literature		
Anonymous	2021d	A.4.2.3.1	Garlic extract (CLAIL0021): Fish, acute toxicity test with common carp (*Cyprinus carpio*); GLP; Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	2012a	A.4.2.3.1	Acute toxicity study of clail 0021 in common carp, Cyprinus carpio; GLP; Unpublished	Yes	Ecospray Ltd., (Co-Sponsor: Intrachem Bio Italia S.p.A)	Yes	**Yes**
Anonymous	2021e	A.4.2.3.1	Garlic extract (CLAIL0021): Daphnia magna, acute immobilization test; GLP; Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	2021f	A.4.2.3.1	Garlic extract (CLAIL0021): Alga, growth inhibition test with *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*); GLP; Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	2012b	A.4.2.3.1	Alga (*Pseudokirchneriella subcapitata*), growth inhibition test with CLAIL 0021; GLP; Unpublished	Yes	ECOspray Ltd., (Co-Sponsor: Intrachem Bio Italia S.p.A)	Yes	**Yes**
Anonymous	2000	A.4.2.3.1	Garlic juice: Daphnia magna, acute immobilization test; GLP; Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	2019a	A4.1.1.3.2	Degradation of Garlic extract in "Local River water" Non GLP Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	2019b	A4.1.1.3.1 A4.1.1.3.7	Soil degradation study of CLAIL0021 NEMguard Liquid Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**

Author	Year	Section No / Reference No	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Y/N)	Owner	Applicability CAR	CLH
Anonymous	2022c	A.4.1.2	Garlic extract (CLAIL0021): Estimation of Adsorption Coefficient GLP Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	2011a	A.3.3	Acute dermal irritation study of CLAIL 0021 in rabbits GLP Unpublished	Yes	Ecospray Ltd., (Co-Sponsor: Intrachem Bio Italia S.p.A)	Yes	**Yes**
Anonymous	2011b	A.3.4	Acute eye irritation study of CLAIL 0021 in rabbits GLP Unpublished	Yes	Ecospray Ltd., (Co-Sponsor: Intrachem Bio Italia S.p.A)	Yes	**Yes**
Anonymous	2016e	A.3.5	Skin sensitisation study of CLAIL 0021 Nemguard liquid by local lymph node assay in mice GLP Unpublished	Yes	Ecospray Ltd.	Yes	**Yes**
Anonymous	**2023**	**A.1.4.**	**Garlic extract (CLAIL0021) – Determination of Corrosion to Metals GLP unpublished**	**Yes**	**Ecospray Ltd**	**Yes**	**Yes**

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Appendix VII: Study summaries

The study summaries can be found in the IUCLID dossier for the biocidal active substance evaluation, UUID: ecc1693c-939a-48c3-8fd4-729286bf9a16.

Additionally, selected study summaries are extracted from the IUCLID dossier and presented below. Please note that these summaries represent the applicant's version. In case there are deviations from the data listed in the dossier parts above, please refer to the data stated above, as that data represent the evaluation of the competent authority.

Toxicological profile for humans and animals

Skin irritation / corrosion (Anonymous, 2011a):

Irritation

Skin irritation / corrosion

ENDPOINT_STUDY_RECORD: Skin irritation / corrosion [redacted]

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:25.000+03:00
Remarks:

Administrative data

EU: BPR

Endpoint
skin irritation: in vivo

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
true

Used for SDS
true

Study period
Experimental Phase: 01 - 17 Sept 2011

Reliability
1 (reliable without restriction)

Data source

Reference

Acute dermal irritation study of CLAIL 0021 in rabbits / [redacted] study report

Data access
data submitter is data owner

Data protection claimed
yes, but willing to share

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
OECD Guideline 404 (Acute Dermal Irritation / Corrosion)

Version / remarks
April 2002

Deviations
no

GLP compliance
yes (incl. QA statement)

Test material

Test material information
Garlic extract

Specific details on test material used for the study
Batch: 11004007
Purity: 2.58% w/w total polysulfides (as diallyl trisufide equivalents)
Actual diallyl trisulfide content: 0.93% w/w.

Test animals

Species
rabbit
common species

Strain
New Zealand White
rabbit

Details on test animals or test system and environmental conditions
3 males
BW: 1.69 - 1.80 kg
Age: 4 - 5.5 mth

Temp: 19 - 23 C
Humidity: 63-65%
Photoperiod: 12 hr light and 12 hr dark; light hrs beng 06:00 - 18:00
Air changes: Minimum of 15 air changes per hour

Test system

Type of coverage
semiocclusive

Preparation of test site
clipped

Vehicle
unchanged (no vehicle)

Controls
yes, concurrent negative control

Amount / concentration applied
0.5mL undiluted per experimental area

Duration of treatment / exposure
4 h

Observation period
7 days

Number of animals
3 per treatment

Details on study design
In a primary skin irritation study, 0.5 mL undiluted CLAIL0021 was applied evenly to one clipped sites of each rabbit and 0.5 mL distilled water was applied the another clipped site of three male, young adult New Zealand White rabbits.
Approximately 24 hours prior to the treatment, fur from the dorso-lumbar region at two contra-lateral sites of each rabbit was closely clipped using a clipper. Care was taken to ensure that the skin was n ot abraded while clipping. An area of approximately 6 cm2 was clipped at both the sites.

The treated and the control sites were covered with gauze patches of approximately 6 cm2 (gauze rolled) which was not more than 8-ply and that was secured at the margins by non-irritating tape to prevent evaporation of the test item and to ensure that the rabbits did not ingest it. At the end of 4 h exposure period, the residual test item was removed with cotton soaked in distilled water.

Skin reactions were observed at 1, 24, 48 and 72 h and on days 7 post patch removal. The site of application was visually assessed and scored for erythema and oedema on a scale of 1 to 4.

The results were interpreted for evaluation according to the Globally Harmonized System (GHS 2009).

Results and discussion

In vivo

Results

Irritation parameter
erythema score

Basis
animal #1

Time point
24/48/72 h

Score

>= 1.67

Max. score
2

Reversibility
fully reversible within: 7 days

Remarks on result
positive indication of irritation

Irritation parameter
erythema score

Basis
animal #2

Time point
24/48/72 h

Score

>= 2

Max. score
2

Reversibility
fully reversible within: 7 days

Remarks on result
positive indication of irritation

Irritation parameter
erythema score

Basis
animal #3

Time point
24/48/72 h

Score

>= 2

Max. score
2

Reversibility
fully reversible within: 7 days

Remarks on result
positive indication of irritation

Irritation parameter
edema score

Basis
animal #1

Time point
24/48/72 h

Score

>= 0.67

Max. score
1

Reversibility
fully reversible within: 7 days

Remarks on result
positive indication of irritation

Irritation parameter
edema score

Basis
animal #2

Time point
24/48/72 h

Score

>= 0.33

Max. score
1

Reversibility
fully reversible within: 7 days

Remarks on result
positive indication of irritation

Irritation parameter
edema score

Basis
animal #3

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Other effects
Scale formation was observed at the site of test item application in all three rabbits during day 4 and 5. No other clinical sigen of toxicity was observed in all rabbits during the experimental period.

Any other information on results incl. tables

Individual and mean skin irritation scores of CLAIL0021 on a 1-4 scale.

Time	Erythema	Oedema

Animal number	1	2	3	1	2	3
after 1 hour	0	1	1	0	0	0
after 24 hours	1	2	2	0	0	0
after 48 hours	2	2	2	1	1	0
after 72 hours	2	2	2	1	0	0
mean score 24-72 hours	1.67	2	2	0.67	0.33	0
after 7 days	0	0	0	0	0	0

Overall remarks, attachments

Overall remarks

Under the experimental conditions, Garlic extract is not a skin irritant.

Attachments

Type
full study report

Applicant's summary and conclusion

Conclusions
Under the experimental conditions, Garlic extract is not a skin irritant.

Executive summary

In a primary dermal irritation study three healthy, adult male albino New Zealand White rabbits were dermally exposed to 0.5 mL undiluted CLAIL0021 for 4 hours. Skin reactions were observed at 1, 24, 48 and 72 h and on day 7 post patch removal.

Mean dermal irritation scores of erythema (1.67 to 2.00) and oedema (0.00 to 0.67) following 24, 48 and 72 h observations were found to be significant in all the three treated rabbits; the treated skin sites of all rabbits recovered completely within 7 days.

Under the experimental conditions, CLAIL0021/NEMguard SC is a mild skin irritant (Category 3). However, the classification criteria of Reg. (EC) No. 1272/2008 are not met and therefore no classification with respect to dermal irritation in required.

Eye irritation (Anonymous, 2011b):

ENDPOINT_STUDY_RECORD: Eye irritation[redacted]

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:25.000+03:00
Remarks:

Administrative data

EU: BPR

Endpoint
eye irritation: in vivo

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
true

Used for SDS
true

Study period
2011

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Justification for type of information
Data requirement.

Data source

Reference

Acute Eye Irritation Study of CLAIL 0021 in Rabbits /[redacted]/ study report

Data access
data submitter is data owner

Data protection claimed
yes, but willing to share

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
OECD Guideline 405 (Acute Eye Irritation / Corrosion)

Version / remarks
Version of April 2002

Deviations
no

GLP compliance
yes (incl. QA statement)

Test material

Test material information
Garlic extract

Specific details on test material used for the study
Batch:No.: 11004007
Expiry Date: Dec. 2011
Purity: 2.58% w/w total polysulfides (as diallyl trisufide equivalents)
Actual diallyl trisulfide content: 0.93% w/w.

Test animals / tissue source

Species
rabbit

Strain
New Zealand White
rabbit

Details on test animals or tissues and environmental conditions
3 females
BW: 1.546 - 1.753 kg
Age: 2.5 - 3.5 mth
Temperature: 19 to 23°C
Humidity: 63-65 %
Photoperiod: 12 hr artificial light and 12 hr dark, light from 06:00 - 18:00
Air Changes: Minimum 15 air changes per hr

Test system

Vehicle
unchanged (no vehicle)

Controls
yes, concurrent vehicle
Saline

Amount / concentration applied
0.1mL undiluted

Duration of treatment / exposure
24h

Observation period (in vivo)
1, 24, 48 & 72 hr post instillation

Any other information on materials and methods incl. tables

The primary eye irritation potential of CLAIL0021 was investigated according to OECD Guideline 405.

On the day of treatment, 0.1 mL of CLAIL0021 was placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of test item. The contralateral eye of each rabbit served as a control and was instilled with 0.1 mL of 0.9% normal saline. At 24 h post instillation of the test item, both the eyes (control and treated) of all the rabbits were gently washed with 0.9% normal saline.

A single animal was treated first. As neither a corrosive effect nor a severe irritant effect was observed after the 1- and 24 hour examinations, the test was completed using the two remaining animals.

The ocular reaction (i.e. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed according to the numerical scoring system given in OECD Guideline 405 (2002) at approximately 1, 24, 48 and 72 hours after instillation.

Results and discussion

In vivo

Results

Irritation parameter
cornea opacity score

Basis
animal #1

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Irritation parameter
cornea opacity score

Basis
animal #2

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Irritation parameter
cornea opacity score

Basis
animal #3

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Irritation parameter
iris score

Basis
animal #1

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Irritation parameter
iris score

Basis
animal #2

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Irritation parameter
iris score

Basis
animal #3

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Irritation parameter
conjunctivae score

Basis
animal #1

Time point
24/48/72 h

Score

>= 0.67

Max. score
1

Reversibility
fully reversible within: 72 hrs

Remarks on result
positive indication of irritation

Irritation parameter
conjunctivae score

Basis
animal #2

Time point
24/48/72 h

Score

>= 0.33

Max. score
1

Reversibility
fully reversible within: 48 hrs

Remarks on result
positive indication of irritation

Irritation parameter
conjunctivae score

Basis
animal #3

Time point
24/48/72 h

Score

>= 0.33

Max. score
1

Reversibility
fully reversible within: 48 hrs

Remarks on result
positive indication of irritation

Irritation parameter
chemosis score

Basis
animal #1

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Irritation parameter
chemosis score

Basis
animal #2

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Irritation parameter
chemosis score

Basis
animal #3

Time point
24/48/72 h

Score

>= 0

Max. score
0

Remarks on result
no indication of irritation

Other effects
None.

Any other information on results incl. tables

Table: Eye irritation scores of Garlic extract/CLAIL0021 according to guideline OECD 405

Time	Cornea			Iris			Conjunctiva					
							Redness			Chemosis		
Animal number	1	2	3	1	2	3	1	2	3	1	2	3
after 1 hour	0	0	0	0	0	0	0	1	1	0	1	0
after 24 hours	0	0	0	0	0	0	1	1	1	0	0	0
after 48 hours	0	0	0	0	0	0	1	0	0	0	0	0
after 72 hours	0	0	0	0	0	0	0	0	0	0	0	0
mean scores 24-72 hours	0	0	0	0	0	0	0.67	0.33	0.33	0	0	0

Overall remarks, attachments

Overall remarks

Under the experimental conditions, Garlic Extract / CLAIL 0021 does not meet the requirements of Reg. (EC) No. 1272/2008 as an eye irritant.

Attachments

Type
full study report

Applicant's summary and conclusion

Interpretation of results
other: Reg. (EC) 1272/2008

Conclusions
Under the experimental conditions, Garlic Extract / CLAIL 0021 does not meet the requirements of Reg. (EC) No. 1272/2008 as an eye irritant.

Executive summary

In a primary eye irritation study, 0.1 mL of undiluted CLAIL 0021 was instilled into the conjunctival sac of one eye of three healthy adult New Zealand White female rabbits. The contralateral eye served as the control and was instilled with 0.1 mL 0.9% normal saline. At 24 h post instillation, both the eyes of all the rabbits were gently washed with 0.9% normal saline. Animals were observed for 3 days. Irritation was scored according to the grading outlined in OECD guideline 405.

Mean eye irritation scores (following assessment at 24, 48 and 72 h post instillation) of corneal opacity (0.00), iritis (0.00), conjunctival redness (0.33 to 0.67) and chemosis (0.00) were determined.

Under the experimental conditions, Garlic Extract / CLAIL 0021 does not meet the requirements of Reg. (EC) No. 1272/2008 as an eye irritant.

Skin sensitization (Anonymous, 2016e):

Skin sensitisation

ENDPOINT_STUDY_RECORD: Skin sensitisation [redacted]

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:26.000+03:00
Remarks:

Administrative data

[CBI]

EU: BPR

Endpoint
skin sensitisation: in vivo (LLNA)

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
true

Used for SDS
true

Study period
Experimental Phase: 09 February to 09 March 2016

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Justification for type of information
Data requirement

Data source

Reference

Skin sensitisation study of CLAIL0021 Nemguard liquid by local lymph node assay in mice / [redacted] / study report

Data access
data submitter is data owner

Data protection claimed
yes

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay)

Version / remarks
Guideline Version of July 2010

Deviations
no

GLP compliance
yes (incl. QA statement)

Type of study
mouse local lymph node assay (LLNA)

Test material

Test material information
Garlic Extract

Specific details on test material used for the study
Batch No.: AN15463580
Purity: 2.9% total polysulfides (active ingredients)
Expiry Date: April 2017

In vivo test system

Test animals

Species
mouse

Strain
CBA:J
mouse

Sex
female

Details on test animals and environmental conditions
5 female mice per group
BW: 17.4 - 23.1 g
Age at Treatment: 8 - 10 wks

Temp: 20 - 23 C
Humidity: 49 - 58%
Photoperiod: 12 hrs light and 12 hr dark with llight hrs being from 06:00 - 18:00
Air changes: Minimun 15 changes per hr

Study design: in vivo (LLNA)

Vehicle
other: Pluronic L92

Concentration
1%

No. of animals per dose
5

Details on study design
Five groups of mice (each comprising 5 females) were selected for the main experiment. Three groups were treated with CLAIL0021 at concentrations of 1%, 10% and 25% (v/v) in 1% L92 for three consecutive days (days 0, 1 and 2) on the dorsum of both ears (25 #L per ear). In addition, one group served as the vehicle control and was treated with 1% L92 and another group served as the positive control and was treated with HCA (#-hexylcinnamaldehyde) at a concentration of 25% (v/v) in 1% L92
.
Group mean body weights of treated animals were comparable with the control group and there were no indications of skin irritation at the treatment site or systemic toxicity in CLAIL0021 treated animals. On day 5, the uptake of intravenously injected 3H-methyl thymidine into the auricular (local) lymph nodes draining at the site of chemical application was measured (5 hours post-administration) to assess the lymph node proliferative response. Stimulation indices (SI) for the 1%, 10% and 25% (v/v) in 1% L92 treated groups were 2.16, 2.21 and 12.23, respectively.
A positive response for HCA (SI = 9.72) confirmed the reliability of the test procedure. The SI obtained for CLAIL0021 at 25% concentration showed a greater than threefold increase over the con-trol value with an EC3 value found to be 11.18%. Therefore, CLAIL0021 demonstrates weak sensi-tisation potential in the local lymph node assay.

Positive control substance(s)
hexyl cinnamic aldehyde (CAS No 101-86-0)

Statistics
Statisitical analysis was carried out for the assessment of the dose response relationship and pair-wise comparison made between the treatment and the solvent/vehicle control group. All the parameters characterised by continuous data such as bodyweight and radioactice disintegrations per minute (DPM) were subjected to Bartlett's test to meet the homogeneity of variance before conducting ANOVA. To compare vehicel and positive control data, Student's t-test was performed to calculate significance.

Results and discussion

Positive control results
Very slight erythema was observed in the group treated with 25% HCA (during days 1 to 4) in all mice (5/5 mouse).

An SI of 9.72, three-fold higher than the control value indicated a positive response, in agreement with the HCD.

In vivo (LLNA)

Results

Key result
true

Parameter
SI

Value

>= 2.16

Test group / Remarks
1% CLAIL0021 NEMGUARD LIQUID

Key result
true

Parameter
SI

Value

>= 2.21

Test group / Remarks
10% CLAIL0021 NEMGUARD LIQUID

Key result
true

Parameter
SI

Value

>= 12.23

Test group / Remarks
25% CLAIL0021 NEMGUARD LIQUID

Key result
true

Parameter
EC3

Value

>= 11.18

Test group / Remarks
25% CLAIL0021 NEMGUARD LIQUID

Cellular proliferation data / Observations
DPM vlaues of 853.8, 1845.0, 1889.2 & 10441.0 were measured for vehicle control, 1%, 10% and 25% (v/v) CLAIL NEMGUARD LIQUID, repectively.

A DPM value of 8301.0 was measured for the positive control (25% (v/v)) HCA.

Any other information on results incl. tables

Table : Individual and mean skin irritation scores of Garlic extract on a 1 to 5 scale.

Group N°	Dose Concentration (%)	Mouse N°	Erythema scores on Day					
			0	1	2	3	4	5
G1	*1% L92*	*1*	*0*	*0*	*0*	*0*	*0*	*0*
		2	*0*	*0*	*0*	*0*	*0*	*0*
		3	*0*	*0*	*0*	*0*	*0*	*0*
		4	*0*	*0*	*0*	*0*	*0*	*0*
		5	*0*	*0*	*0*	*0*	*0*	*0*
G2	*1% CLAIL0021*	*6*	*0*	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*	*0*
		8	*0*	*0*	*0*	*0*	*0*	*0*
		9	*0*	*0*	*0*	*0*	*0*	*0*
		10	*0*	*0*	*0*	*0*	*0*	*0*
G3	*10% CLAIL0021*	*11*	*0*	*0*	*0*	*0*	*0*	*0*
		12	*0*	*0*	*0*	*0*	*0*	*0*
		13	*0*	*0*	*0*	*0*	*0*	*0*
		14	*0*	*0*	*0*	*0*	*0*	*0*
		15	*0*	*0*	*0*	*0*	*0*	*0*
G4	*25% CLAIL0021*	*16*	*0*	*0*	*0*	*0*	*0*	*0*
		17	*0*	*0*	*0*	*0*	*0*	*0*
		18	*0*	*0*	*0*	*0*	*0*	*0*
		19	*0*	*0*	*0*	*0*	*0*	*0*
		20	*0*	*0*	*0*	*0*	*0*	*0*
G5	*25% HCA*	*21*	*0*	*1*	*1*	*1*	*1*	*0*
		22	*0*	*1*	*1*	*1*	*1*	*0*
		23	*0*	*1*	*1*	*1*	*1*	*0*
		24	*0*	*1*	*1*	*1*	*1*	*0*
		25	*0*	*1*	*1*	*1*	*1*	*0*

Overall remarks, attachments

Overall remarks

CLAIL0021 NEMGUARD LIQUID (at 25% (v/v)) was shown to have an SI of 12.23 and and EC3 value of 11.18%. This is indicative of a positive response and indicates that CLAIL0021 NEMGUARD LIQUID has the potential for skin sensitisation.

Attachments

Type
full study report

Applicant's summary and conclusion

Interpretation of results
Category 1B (indication of skin sensitising potential) based on GHS criteria

Conclusions
Under the experimental conditions, CLAIL0021 NEMGUARD LIQUID is a skin sensitiser (category 1B). Thus, classification is required according to Regulation (EC) No. 1272/2008.

Executive summary

The skin sensitisation potential of CLAIL0021 NEMGUARD LIQUID was investigated *in CBA/J strain mice in compliance with the OECD test guideline 429.*

A preliminary assay was conducted to identify the appropriate test concentrations for the Local Lymph Node Assay main study.

Based on the results of the preliminary assay, five groups of mice (each comprising 5 females) were selected for the main experiment. Three groups were treated with CLAIL0021 NEMGUARD LIQUID at concentrations of 1%, 10% and 25% (v/v) in 1% L92 for three consecutive days (days 0, 1 and 2) on the dorsum of both ears (25mL per ear). In addition, one group served as the vehicle control and was treated with 1% L92 and another group served as the positive control and was treated with HCA (alpha-hexylcinnamaldehyde) at a concentration of 25% (v/v) in 1% L92.

Group mean body weights of treated animals were comparable with the control group and there were no indications of skin irritation at the treatment site or systemic toxicity in CLAIL0021 treated animals.

On day 5, the uptake of intravenously injected 3H-methyl thymidine into the auricular (local) lymph nodes draining at the site of chemical application was measured (5 hours post-administration) to assess the lymph node proliferative response. Stimulation indices (SI) for the 1%, 10% and 25% (v/v) in 1% L92 treated groups were 2.16, 2.21 and 12.23, respectively.

*A positive response for HCA (SI = 9.72) confirmed the reliability of the test procedure.*The SI obtained for*CLAIL0021*at 25% concentration showed a greater than threefold increase over the control value with an EC3value found to be 11.18%. Therefore, *CLAIL0021 NEMGUARD LIQUID* demonstrates weak sensitisation potential in the local lymph node assay.

Under the experimental conditions, CLAIL0021 NEMGUARD LIQUID is a skin sensitiser (category 1B). Thus, classification is required according to Reg. (EC) No. 1272/2008.

Ecotoxicological studies

Hydrolysis (Anonymous, 2021b)

ENDPOINT_STUDY_RECORD: Abiotic: Hydrolysis as a function of pH[redacted]

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:29.000+03:00
Remarks:

Administrative data

Endpoint
hydrolysis

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
false

Used for SDS
false

Study period
19 Feb 2021- 10 Apr 2021

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Justification for type of information
New guideline study

Data source

Reference

GARLIC EXTRACT (CLAIL0021): HYDROLYSIS AS A FUNCTION OF PH[redacted]/ study report

Data access
data submitter is data owner

Data protection claimed
yes

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
OECD Guideline 111 (Hydrolysis as a Function of pH)

Version / remarks
NA

Deviations
no

GLP compliance
yes (incl. QA statement)

Test material

Test material information
Garlic extract

Radiolabelling
no

Study design

Analytical monitoring
yes

Duration of test

Duration

5	d

pH
4

Temp.

20	°C

Duration

5	d

pH
7

Temp.

20	°C

Duration

5 d

pH
9

Temp.

20 °C

Any other information on materials and methods incl. tables

pH	Temperature (°C)	DT50(Hours)	DT90(Hours)
4.0	20 ± 0.5°C	4.46	14.8
	30 ± 0.5°C	5.95	19.8
	50 ± 0.5°C	4.45	14.8
7.0	**20 ± 0.5°C**	**5.04**	**16.7**
	30 ± 0.5°C	6.39	21.2
	50 ± 0.5°C	7.87	26.1
9.0	20 ± 0.5°C	5.09	16.9
	30 ± 0.5°C	5.89	19.6
	50 ± 0.5°C	7.75	25.8

Results and discussion

Transformation products
not specified not detected

Dissipation DT50 of parent compound

Key result
true

pH
4

Temp.

20 °C

DT50

>= 4.46 h

Key result
true

pH
7

Temp.

20	°C	

DT50

>= 5.04		h

Key result
true

pH
9

Temp.

20	°C	

DT50

>= 5.09		h

Details on results
For Tier 1 test, analyte at nominal concentration of about 100 #g/mL in sterile buffer solutions of pH 4.0, 7.0 and 9.0 was incubated for 5 days at 50 ± 0.5ºC in the preliminary test. Hydrolysis reactions were monitored by analyzing the analyte concentration at set intervals using an in-house developed and validated HPLC method.
For tier 2, a definitive test was performed in 4.0, 7.0 and 9.0 buffer solutions at three temperatures (20 ± 0.5ºC, 30 ± 0.5ºC and 50 ± 0.5ºC) as hydrolysis of Garlic extract was more than 10% in pH 4.0, 7.0 and 9.0 buffer solutions in the preliminary test. The decay timings were calculated (DT50 and DT90) using software (CAKE version 3.4 (Release)).

Overall remarks, attachments

Overall remarks

the active substance degrades quicker in the environment at environmentally realistic pH.

Attachments

Type
full study report

Applicant's summary and conclusion

Validity criteria fulfilled
yes

Conclusions
From the study it is concluded that the test item Garlic extract is rapidly hydrolysed in pH 4, 7 and 9 buffer solutions and hydrolysis of Garlic Extract was not dependent either on pH or temperature. This

clearly shows that active substance is not persistent in the environment and quickly degrades in the environment with a very short half-life.

Executive summary

The hydrolysis of Garlic Extract was carried out in a tiered approach as per OECD 111 and OPPTS 835.2120.

Active substance garlic extract in sterile buffer solutions of pH 4.0, 7.0 and 9.0 was incubated for 5 days at 50 ± 0.5ºC in the preliminary test. Hydrolysis reactions were monitored by analyzing the analyte concentration at set intervals using an in-house developed and validated HPLC method.

The preliminary test results revealed that the garlic extract is nearly 90% hydrolysed at all pH after incubation at 50 ± 0.5ºC. To monitor the rate of hydrolysis a definitive test was performed in 4.0, 7.0 and 9.0 buffer solutions at three temperatures (20 ± 0.5ºC, 30 ± 0.5ºC and 50 ± 0.5ºC). The decay timings were calculated (DT50and DT90) using software (CAKE version 3.4 (Release)).The results indicated that active substance garlic extract is unstable and quickly degrades confirming the ***DT50 of 5.04 Hours and DT90 of 16.9 Hours.***

From the study it is concluded that the test item Garlic extract is rapidly hydrolysed in pH 4, 7 and 9 buffer solutions andhydrolysisof Garlic Extract was not dependent either on pH or temperature.

Inhibition of microbial activity (Anonymous, 2021c)

Inhibition of microbial activity

ENDPOINT_SUMMARY: Toxicity to microorganisms

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:30.000+03:00
Remarks:

Administrative data

EU: BPR

Link to relevant study record(s)

Study name / type

OECD / Toxicity to microorganisms / Toxicity to microorganisms [redacted] / Garlic, ext. / Garlic ext / 8008-99-9

Description of key information

The ready biodegradability of Garlic extract (CLAIL0021) was investigated using the CO2 Evolution Test, according to OECD Guideline 301B.The study included one flask as a toxicity control, in which 75 mg garlic extract and 39 mg sodium benzoate (reference item) were mixed with 300 ml of test medium to achieve an initial test concentration of 15.1 mg Carbon/L. All the treatments were added with equal volume of inoculum which was collected from the secondary effluent treatment plant receiving predominantly domestic sewage and volume made with mineral media.Final volume in the test flask was 3000 mL. The initial test item concentration was therefore equivalent to 25 mg/L garlic extract.

The toxicity control attained 35.80% degradation after 7 days and 90.87% degradation after 29 days, indicating that garlic extract was not inhibitory to the inoculum microorganisms. The 28-day NOEC is therefore concluded to be 25 mg/L (i.e. the initial concentration of test item that was used in the toxicity control).

Key value for chemical safety assessment

EC10 or NOEC for microorganisms

25	mg/L

ENDPOINT_STUDY_RECORD: Toxicity to microorganisms [redacted]

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:26.000+03:00
Remarks:

Administrative data

EU: BPR

Endpoint
activated sludge respiration inhibition testing Ready biodegradability study toxicity control

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
false

Used for SDS
false

Study period
28 January to 13 May 2021

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Justification for type of information
The inhibition of microbial activity of garlic extract to sewage treatment plant (STP) microorganisms was assessed in the toxicity control of a reliable OECD guideline 301B ready biodegradability test.

Cross-reference

Reason / purpose for cross-reference
reference to same study

Related information
OECD / Biodegradation in water: screening tests / Biotic -> Ready / inherent biodegradability [redacted] / Garlic, ext. / Garlic ext / 8008-99-9

Data source

Reference

Garlic extract (CLAIL0021): Ready biodegradability – CO2 evolution test / [redacted] study report

Data access
data submitter is data owner

Data protection claimed
yes

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
other: OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test)

Version / remarks
17 July 1992

Deviations
no

Principles of method if other than guideline
The inhibition of microbial activity of garlic extract to sewage treatment plant (STP) microorganisms was assessed in the toxicity control of a reliable OECD guideline 301B ready biodegradability test.

GLP compliance
yes (incl. QA statement)

Test material

Test material information
Garlic Extract

Sampling and analysis

Analytical monitoring
not required

Details on analytical methods
The amount of CO2 produced was calculated from the amount of base remaining in the absorption bottle. The amount of CO2 remaining was assessed by titra ting 0.0125 M Ba(OH)2 with 0.05 M HCl. The weights of CO2 produced from the inoculum alone and from the inoculum plus test item was calculated using the respective titration values; the difference is the weight of CO2 produced from the test item alone.

Test solutions

Vehicle
no

Test organisms

Test organisms (species)
sewage, domestic

Details on inoculum
The inoculum was secondary effluent, sourced from a treatment plant receiving predominantly domestic sewage. This effluent was used as test system as it is recommended in the guideline. A fresh sample of secondary effluent was collected from the treatment plant and was kept aerobic during transport. This effluent was allowed to settle for one hour, decanted and the decanted eff luent was used in the test. The decanted effluent was preconditioned by aerating for 6 days at 22.6 to 23.8°C. The bacterial population in the inoculum was determined as colony forming units (CFU/ mL) by diluting the inoculum to an appropriate dilution and then plating on nutrient agar plates. The ba cterial population in the inoculum was 6.0E+7 CFU/L.

Study design

Test type
static

Water media type
freshwater

Limit test
no

Total exposure duration

28 d

Remarks on exposure duration
The exposure duration recommended in the OECD guideline 301B

Test conditions

Test temperature
22.1 to 23.8°C

pH
At start of test: 7.51 to 7.56;
At end of test: 8.43

Nominal and measured concentrations
75 mg garlic extract and 39 mg sodium benzoate (reference item) were mixed with 300 ml of test medium to achieve an initial test concentration of 15.1 mg Carbon/L (equivalent to 25 mg/L garlic extract). Final volume in the test flask was 3000 mL.

Details on test conditions
To each test vessel (5 L flasks), 2400 mL of mineral medium was added and mixed with 300 mL of the pre-conditioned inoculum. A separate 3000 mL of mineral medium was also prepared in a flask to use it for further dilutions. A sample of the mineral medium was checked for the inorganic carbo n content on start of the test. These flasks were aerated with CO2 free air at 43 to 50 mL/minute, o vernight to purge the system of carbon dioxide.

A quantity of 75 mg test item and 39 mg of the reference item was mixed and made up to 300 mL using mineral medium (previously aerated with CO2-free air) and added to test flask 6 (the toxicity control). Final volume in the test flask was 3000 mL.

The outlet of each test flask was connected to the inlet of a gas absorption bottle containing 100 mL of 0.0125 M barium hydroxide solution in a series of 3 keeping the outlet of the last absorption bot tle open. Before each use, the strength of barium hydroxide was determined by titrating against pota ssium hydrogen phthalate (PHP). For this, 10 mL of 0.025 M PHP was transferred into a conical flask,

2 to 3 drops of phenolpthalein indicator was added and this was titrated against 0.0125 M barium hy droxide solution until the colour of PHP just turned pink.

The test was carried out by bubbling carbon dioxide free air through the suspension at a rate of 43-50 mL/minute and continued for 28 days. Once a week, the flow rate of carbon dioxide free air was checked using the bubble flow meter. On the 28th day, pH of the test suspension was recorded and 1 mL of concentrated hydrochloric acid was added to each flask and the bubbling of carbon dioxide free air was continued. On the 29th day, the bubbling was stopped. All the barium hydroxide absor ption bottles were disconnected and were titrated for the determination of carbon dioxide production. The temperature of the room was maintained at 22.1 to 23.8°C during the treatment period.

Carbon dioxide analysis was made every second-third day and then at least every fourth day until the 29th day. The amount of CO2 produced was calculated from the amount of base remaining in the ab sorption bottle. The amount of CO2 remaining was assessed by titrating 0.0125 M Ba(OH)2 with 0.05 M HCl.

Reference substance (positive control)
not required Not applicable for ready biodegradability toxicity control

Any other information on materials and methods incl. tables

Preparation of test medium stock solutions

Stock Solution	Chemical	Quantity (g)
A	*Potassium dihydrogen orthophosphate, KH2PO4*	*4.2501*
	Dipotassium hydrogen orthophosphate, K2HPO4	*10.8542*
	Disodium hydrogen orthophosphate dihydrate, Na2HPO4.2H2O	*16.7069*
	Ammonium chloride, NH4Cl	*0.2562*
	These four constituents were dissolved in and made up to 500 mL using Milli-Q water. The pH of the solution was 7.58.	
B	*Calcium chloride, dihydrate, CaCl2.2H2O*	*18.2046*
	This was dissolved in and made up to 500 mL using Milli-Q water.	
C	*Magnesium sulphate heptahydrate, MgSO4.7H2O*	*11.2641*
	This was dissolved in and made up to 500 mL using Milli-Q water.	
D	*Iron (III) chloride hexhydrate, FeCl3.6H2O*	*0.1261*
	This was dissolved in and made up to 500 mL using Milli-Q water.	

Results and discussion

Effect concentrations

Key result
false

Duration

28 d

Dose descriptor
NOEC

Effect conc.

25 mg/L

Nominal / measured
nominal

Conc. based on
test mat.

Basis for effect
other: Inhibition of biodegradation activity (CO2 evolution) Ready biodegradability toxicity control

Details on results
The cumulative carbon dioxide produced from the toxicity control was 17.82, 38.94, 59.51, 68.42, 91.19, 113.46, 134.63, 149.92, 152.01, 151.08 and 151.08 mg, on days 2, 5, 7, 10, 12, 15, 17, 19, 23, 27 and 29 after the treatment, respectively. The toxicity control attained 35.80% degradation after 7 days and 90.87% degradation after 29 days, indicating that garlic extract was not inhibitory to the inoculum microorganisms.

Results with reference substance (positive control)
Not applicable

Any other information on results incl. tables

Carbon-dioxide production

Day	CO2 produced (mg)							Cumulative CO2 produced (mg) Test – Blank mean			
	Test Flasks				Blank						
	Flask 1	Flask 2	Flask 5	Flask 6	Flask 3	Flask 4	Mean	Flask 1	Flask 2	Flask 5	Flask 6
2	*34.43*	*33.44*	*43.45*	*27.94*	*10.78*	*9.46*	*10.12*	*24.31*	*23.32*	*33.33*	*17.82*
5	*40.15*	*41.47*	*43.34*	*31.02*	*9.46*	*10.34*	*9.90*	*54.56*	*54.89*	*66.77*	*38.94*
7	*12.76*	*13.64*	*43.12*	*32.34*	*11.66*	*11.88*	*11.77*	*55.55*	*56.76*	*98.12*	*59.51*
10	*16.50*	*22.66*	*44.44*	*30.36*	*22.00*	*20.90*	*21.45*	*50.60*	*57.97*	*121.11*	*68.42*
12	*50.60*	*49.50*	*34.32*	*38.06*	*15.95*	*14.63*	*15.29*	*85.91*	*92.18*	*140.14*	*91.19*
15	*52.25*	*51.26*	*32.45*	*34.21*	*11.44*	*12.43*	*11.94*	*126.22*	*131.50*	*160.65*	*113.46*
17	*48.84*	*47.52*	*19.14*	*31.35*	*9.57*	*10.78*	*10.18*	*164.88*	*168.84*	*169.61*	*134.63*
19	*43.01*	*44.22*	*11.22*	*25.85*	*11.44*	*9.68*	*10.56*	*197.33*	*202.50*	*170.27*	*149.92*
23	*39.27*	*37.18*	*9.46*	*11.44*	*8.25*	*10.45*	*9.35*	*227.25*	*230.33*	*170.38*	*152.01*

27	*14.85*	*12.43*	*7.26*	*8.14*	*9.68*	*8.47*	*9.08*	*233.03*	*233.69*	*168.57*	*151.08*
29	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*233.03*	*233.69*	*168.57*	*151.08*

Total CO2produced in the inoculum blank during the test = 119.635 mg/3L

= 39.88 mg/L

Note:

Flask 1 and 2: Contain test item (Garlic extract (CLAIL0021)) and inoculum in mineral media

Flask 3 and 4: Contain inoculum in mineral media

Flask 5: Contain reference item (Sodium benzoate) and inoculum in mineral media

Flask 6: Contain test item, reference item and inoculum in mineral media (toxicity control)

Degradation of test item and reference item

Day	**Test item**			**Reference item**	**Test item + reference item***
	Flask 1	**Flask 2**	**Mean**		
2	*14.62*	*14.03*	*14.33*	*19.92*	*10.72*
5	*32.820*	*33.02*	*32.92*	*39.90*	*23.42*
7	*33.41*	*34.14*	*33.78*	*58.63*	*35.80*
10	*30.44*	*34.87*	*32.66*	*72.37*	*41.15*
12	*51.67*	*55.45*	*53.56*	*83.74*	*54.85*
15	*75.92*	*79.10*	*77.51*	*96.00*	*68.25*
17	*99.18*	*101.56*	*100.37*	*101.35*	*80.98*
19	*118.69*	*121.80*	*120.25*	*101.74*	*90.18*
23	*136.69*	*138.54*	*137.62*	*101.81*	*91.43*
27	*140.16*	*140.56*	*140.36*	*100.72*	*90.87*
29	*140.16*	*140.56*	*140.36*	*100.72*	*90.87*

**Toxicity control*

Applicant's summary and conclusion

Validity criteria fulfilled
yes All OECD guideline 301B validity criteria were met

Conclusions
The toxicity control attained 35.80% degradation after 7 days and 90.87% degradation after 29 days, indicating that garlic extract was not inhibitory to the inoculum microorganisms. The 28-day NOEC is therefore concluded to be 25 mg/L (i.e. the initial concentration of test item that was used in the toxicity control).

Executive summary

The ready biodegradability of Garlic extract (CLAIL0021) was investigated using the CO2 Evolution Test, according to OECD Guideline 301B. The study included one flask as a toxicity control, in which 75 mg garlic extract and 39 mg sodium benzoate (reference item) were mixed with 300 ml of test medium to achieve an initial test concentration of 15.1 mg Carbon/L. All the treatments were added with equal volume of inoculum which was collected from the secondary effluent treatment plant receiving predominantly domestic sewage and volume made with mineral media.Final volume in the test flask was 3000 mL. The initial test item concentration was thereforeequivalent to 25 mg/L garlic extract.

The toxicity control attained 35.80% degradation after 7 days and 90.87% degradation after 29 days, indicating that garlic extract was not inhibitory to the inoculum microorganisms. The 28-day NOEC is therefore concluded to be 25 mg/L (i.e. the initial concentration of test item that was used in the toxicity control).

Short-term toxicity to fish (Anonymous, 2021d)

Short-term toxicity testing on fish

ENDPOINT_SUMMARY: Short-term toxicity to fish

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:32.000+03:00
Remarks:

Administrative data

EU: BPR

Link to relevant study record(s)

Study name / type

OECD / Short-term toxicity to fish / Short-term toxicity testing on fish[redacted] / Garlic, ext. / Garlic ext / 8008-99-9

Description of key information

The acute toxicity of Garlic extract (CLAIL0021) was tested on the freshwater fish, Cyprinus carpio (Common carp) for 96 hours. In the definitive test, fish fasted at least for 24 h were exposed to Garlic extract (CLAIL0021) at nominal concentrations of 7.0, 11.9, 20.2, 34.3 and 58.3 mg/L (respective geometric mean concentrations were 2.92, 5.15, 8.23, 15.15 and 24.80 mg/L), along with a negative control under semi-static mode with renewal of test medium at 24 h interval.

The LC50 value for Garlic extract (CLAIL0021) at 96 hours was 11.65 mg test item/L based on geometric mean concentrations.

Key value for chemical safety assessment

Fresh water fish

Fresh water fish

Dose descriptor
LC50

Effect concentration

11.65	mg/L

ENDPOINT_STUDY_RECORD: Short-term toxicity testing on fish

UUID:
Dossier UUID:
Author: svc-r4bp
Date: 2022-08-01T19:52:24.000+03:00
Remarks:

Administrative data

EU: BPR

Endpoint
short-term toxicity to fish

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
false

Used for SDS
true

Study period
01 to 08 April 2021.

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Justification for type of information
New key study replacing old study due to lack of analytical measurements

Data source

Reference

Garlic extract (CLAIL0021): Fish acute toxicity test with common carp (Cyprinus carpio) /
R.S. / study report

Data access
data submitter is data owner

Data protection claimed
yes

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
OECD Guideline 203 (Fish, Acute Toxicity Test)

Version / remarks
18 June 2019

Deviations
no

GLP compliance
yes (incl. QA statement)

Test material

Test material information
Garlic extract

Sampling and analysis

Analytical monitoring
yes

Details on sampling
Samples were taken for analysis at 0 hour (overall mean concentration obtained for Accuracy/ Precision test was used as concentration at '0' hour/day) and at 24 hours (samples stored at room temperature). Three composite replicate samples for each dose group (low and high) was drawn and analysed for the test item concentrations.

Details on analytical methods
The GC-MS/MS method for the analysis of test item in test samples was validated by assessment of the specificity, linearity, range, precision, accuracy, LOD (Limit of Detection), LOQ (Limit of Quantification). The stability of working standard and processed sample solution(s) under specified storage conditions was also assessed.
The active ingredient in the test samples was determined by injecting the samples (prepared as above) along with a working standard solution into a GC-MS/MS operated under the following conditions:
Instrument : Gas Chromatograph equipped with mass spectrometer Detector and PC based data system Manufacture: Shimadzu, Model: TQ8040
Column : ZB-35, 30 m long, 0.25mm internal diameter, 0.25 µm film thickness or similar
Split ratio : 1:1
Temperatures
Column oven : 60°C
Injection : 200°C
Column Oven : Initial: 60°C hold for 5 min.
Ramp 1: 10°C/min. to 100°C, hold for 2.0 min.
Ramp 2: 15°C/min. to 150°C, hold for 3.0 min
Ramp 1: 30°C/min. to 230°C, hold for 8.0 min

Test solutions

Vehicle
no

Details on test solutions
Test solutions from each concentration received from ecotoxicology lab were analysed using the method detailed above.

Test organisms

Test organisms (species)
Cyprinus carpio

Details on test organisms
Common carp (Cyprinus carpio) was used as the test system as it is a recommended fish species in the guideline. Fish were held in the laboratory at least for 9 days before using it for test.

Study design

Test type
semi-static

Water media type
freshwater

Limit test
no

Total exposure duration

96	h

Test conditions

Test temperature
22-22.7C

pH
7.62-7.73

Dissolved oxygen
84 to 98% saturation

Conductivity
< 10 µS/cm.

Nominal and measured concentrations
In the definitive test, fish fasted at least for 24 h were exposed to Garlic extract (CLAIL0021) at nominal concentrations of 7.0, 11.9, 20.2, 34.3 and 58.3 mg/L
(respective geometric mean concentrations were 2.92, 5.15, 8.23, 15.15 and 24.80 mg/L), along with a negative control under semi-static mode with renewal of test medium at 24 h interval. Fish were o bserved for toxic signs and mortality for 96 h. Each group had single replicate with ten fish.

Details on test conditions
Fish were exposed to a range of concentrations of test item, Garlic extract (CLAIL0021) in the test medium for a period of 96 h in semi-static mode with renewal of test solution at every 24 h interval during both range finding test and definitive test. Fish were observed for sub-lethal effects and m ortalities at 2 ± 0.5 h, 5 ± 1 h, 24 ± 2, 30 ± 2, 48 ± 2, 54 ± 2, 72 ± 2, 78 ± 2 and 96 ± 2 h
intervals and the median lethal concentration (LC50) was calculated.

Reference substance (positive control)
no

Any other information on materials and methods incl. tables

The acute toxicity of Garlic extract (CLAIL0021) to common carp (*Cyprinus carpio*) was determined in a semi static test condition. Fish were exposed to five nominal concentrations of Garlic extract (CLAIL0021)7.0, 11.9, 20.2, 34.3 and 58.3mg Garlic extract (CLAIL0021)/L and a RCW control.

Results and discussion

Effect concentrations

Key result
true

Duration

96 h

Dose descriptor
LC50

Effect conc.

11.65 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
mortality (fish)

Details on results
The maximum concentration of Garlic extract (CLAIL0021) causing no mortality and the lowest concentration tested causing 100 percent mortality within the 96 h test period were 8.24 and 15.15 mg Garlic extract (CLAIL 0021)/L based on the Analysed mean measured concentration, respe ctively. The 96 h LC50was 11.65 Garlic extract (CLAIL0021)/L based on the Analysed mean measured concentration.

Overall remarks, attachments

Attachments

Type
full study report

Applicant's summary and conclusion

Validity criteria fulfilled
yes

Conclusions
The maximum concentration of Garlic extract (CLAIL0021) causing no mortality and the lowest concentration tested causing 100 percent mortality within the 96 h test period were 8.24 and 15.15

mg Garlic extract (CLAIL 0021)/L based on the Analysed mean measured concentration, respe ctively. The 96 h LC50 was 11.17 Garlic extract (CLAIL0021)/L based on the Analysed mean m easured concentration.

Executive summary

The acute toxicity of Garlic extract (CLAIL0021) to common carp (*Cyprinus carpio*) was determined in a semi static test condition. Fish were exposed to five nominal concentrations of Garlic extract (CLAIL0021)7.0, 11.9, 20.2, 34.3 and 58.3mg Garlic extract (CLAIL0021)/L and a RCW control. The maximum concentration of Garlic extract (CLAIL0021) causing no mortality and the lowest concentration tested causing 100 percent mortality within the 96 h test period were 8.24 and 15.15 mg Garlic extract (CLAIL 0021)/L based on the Analysed mean measured concentration, respectively. The 96 h LC*50* was 11.17 Garlic extract (CLAIL0021)/L based on the Analysed mean measured concentration.

Short-term toxicity to aquatic invertebrates (Anonymous, 2021e)

ENDPOINT_STUDY_RECORD: Short-term toxicity testing on aquatic invertebrates [redacted]

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:27.000+03:00
Remarks:

Administrative data

EU: BPR

Endpoint
short-term toxicity to aquatic invertebrates

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
false

Used for SDS
true

Study period
2021

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Justification for type of information
New key study replacing old study due to lack of analytical measurements

Data source

Reference

Daphnia magna, acute immobilization test, [redacted] study report

Data access
data submitter is data owner

Data protection claimed
yes

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)

Version / remarks
13 April 2004

Deviations
no

GLP compliance
yes

Test material

Test material information
Garlic extract

Sampling and analysis

Analytical monitoring
yes

Details on sampling
All test concentrations and the negative control were analysed for the test item concentration at the beginning and end of test. For analysis, single composite sample was drawn from prepared test concentrations.

Details on analytical methods
The active ingredient in the test samples was determined by injecting the samples (prepared as above) along with a working standard solution into a GC-MS/MS operated under the following conditions:
Instrument : Gas Chromatograph equipped with mass spectrometer Detector and PC based data system Manufacture: Shimadzu, Model: TQ8040
Column : ZB-35, 30 m long, 0.25mm internal diameter, 0.25 µm film thickness or similar
Split ratio : 1:1
Temperatures
Column oven : 60°C
Injection : 200°C
Column Oven : Initial: 60°C hold for 5 min.
Ramp 1: 10°C/min. to 100°C, hold for 2.0 min.
Ramp 2: 15°C/min. to 150°C, hold for 3.0 min
Ramp 1: 30°C/min. to 230°C, hold for 8.0 min
Ion Source : 280°C
Interface : 300°C
Column Flow : 2.40 mL/min

Test solutions

Vehicle
no

Details on test solutions
Test samples were received from Ecotoxicology for analysis. The two replicates for control samples i .e. G1 and three replicates from G2 to G6 groups samples from tested concentrations.

Test organisms

Test organisms (species)
Daphnia magna

Details on test organisms
Daphnia magna was used as test system as it is a recommended species in the guideline. Daphnia were maintained in the test medium. The test medium in which the daphnia were maintained was ch anged at least twice a week and they were fed with the unicellular green alga, Chlorella vulgaris.

Study design

Test type
semi-static

Water media type
freshwater

Limit test
no

Total exposure duration

48	h

Test conditions

Test temperature
19.1-19.5°C

pH
7.62-7.89

Dissolved oxygen
6.9-7.3 mg O2/L

Conductivity
< 10 µS/cm

Nominal and measured concentrations
the test concentrations of 6, 12, 24, 48 and 96 mg/L (respective geometric mean concentrations were 2.58, 5.21, 10.20, 21.05 and 41.93 mg/L)

Details on test conditions
The acute immobilization effect of the test item Garlic extract (CLAIL0021) was studied on Daphnia m agna for 48 hours. There was no immobilization of daphnia in the negative control and at the tested concentration of 6 mg/L at 24 and 48 hours of exposure. The immobilization of daphnia was 0, 15, 40 and 80% at 24 h and 20, 30, 70 and 100% at 48 h exposure at 12, 24, 48 and 96 mg/L, respectively. Where mean measured concentrations were 2.51, 5.16, 10.20, 21.05 and 41.93 mg/L of Garlic extract (CLAIL0021), respectively.

Reference substance (positive control)
yes Potassium dichromate

Results and discussion

Effect concentrations

Key result
true

Duration

48 h

Dose descriptor
EC50

Effect conc.

13.69 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
mobility

Remarks on result
other: 95% fiducial limits: 10.46 to 17.71

Key result
false

Duration

48 h

Dose descriptor
NOEC

Effect conc.

2.58 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
mobility

Key result
false

Duration

48 h

Dose descriptor
LOEC

Effect conc.

5.21 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
mobility

Details on results
The maximum concentration of Garlic extract (CLAIL0021) causing no Immobilization and the lowest concentration tested causing 100 percent Immobilization within the 48h test period were 2.51 and 41.93 mg Garlic extract (CLAIL0021)/L based on the Analysed mean measured concentration, respectively. The 48-hour EC50 value was calculated to be 12.68 mg test item/L based on the Analysed mean measured concentration.

Results with reference substance (positive control)
The EC50 valued for the reference substance (potassium dichromate) at 24 and 48 hours were 0.5761 (95% fiducial limits: 0.44569 to 0.74459) and 0.3212 (95% fiducial limits: 0.23574 to 0.43765) mg/L (based on nominal concentrations).

Overall remarks, attachments

Attachments

Type
full study report

Applicant's summary and conclusion

Validity criteria fulfilled
yes

Conclusions
The 48-hour EC50, NOEC and LOEC values for Garlic extract (CLAIL0021) were 13.69 (95% fiducial limits: 10.46 to 17.71), 2.58 and 5.21 mg/L (based on geometric mean concentrations), respectively.

Executive summary

The acute immobilization effect of the test item Garlic extract (CLAIL0021) was studied on *Daphnia magna* for 48 hours. There was no immobilization of daphnia in the negative control and at the tested concentration of 6 mg/L at 24 and 48 hours of exposure. The immobilization of daphniawas 0, 15, 40 and 80% at 24 h and 20, 30, 70 and 100% at 48 h exposure at 12, 24, 48 and 96 mg/L, respectively. Where mean measured concentrations were 2.51, 5.16, 10.20, 21.05 and 41.93 mg/L of Garlic extract (CLAIL0021), respectively. *The maximum concentration of* Garlic extract (CLAIL0021) *causing no Immobilization and the lowest concentration tested causing 100 percent Immobilization within the 48 h test period were 2.51 and 41.93 mg* Garlic extract (CLAIL0021)/L based on the Analysed mean measured concentration*, respectively.*

The 48-hour EC50, NOEC and LOEC values for Garlic extract (CLAIL0021) were 13.69 (95% fiducial limits: 10.46 to 17.71), 2.58 and 5.21 mg/L (based on geometric mean concentrations), respectively.

Growth inhibition on algae (Anonymous, 2021f)

Growth inhibition study on algae

ENDPOINT_SUMMARY: Toxicity to aquatic algae and cyanobacteria

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:32.000+03:00
Remarks:

Administrative data

EU: BPR

Link to relevant study record(s)

Study name / type

OECD / Toxicity to aquatic algae and cyanobacteria / Growth inhibition study on algae [redacted] / Garlic, ext. / Garlic ext / 8008-99-9

Description of key information

The effect of Garlic extract (CLAIL0021) was tested on the growth of freshwater unicellular green alga *Pseudokirchneriella subcapitata*.The alga was exposed to the test item at the nominal concentrations of 5.8, 9.3, 14.8, 23.8, 38.0, 60.8 and 97.3 mg/ L (factor of 1.6) (respective geometric mean concentrations were 2.55, 4.41, 6.40, 9.65, 14.72, 24.03 and 36.42 mg test item/L along with a negative control. The cell growth was measured at 24, 48 and 72 hours after the initiation of the test.

The 72-hour ErC50 and ErC10 values for Garlic extract (CLAIL0021) were 19.22 (95% fiducial limits: 14.70 to 24.67) and 8.517 (4.072 to 15.95) mg/L (based on geometric mean concentrations), respectively. The 72-hour NOEC and LOEC values (based on growth rate) were 2.55 and 4.41mg/L (based on geometric mean concentrations), respectively.

Key value for chemical safety assessment

EC50 for freshwater algae

19.22 mg/L

EC10 or NOEC for freshwater algae

2.55 mg/L

ENDPOINT_STUDY_RECORD: Growth inhibition study on algae [redacted]

UUID: [redacted]
Dossier UUID: [redacted]
Author: svc-r4bp
Date: 2022-08-01T19:52:27.000+03:00
Remarks:

Administrative data

EU: BPR

Endpoint
toxicity to aquatic algae and cyanobacteria

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
false

Used for SDS
false

Study period
03 – 11 April 2021

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Justification for type of information
New key study replacing old study due to lack of analytical measurements

Data source

Reference

Garlic extract (CLAIL0021): Alga, Growth Inhibition Test with Raphidocelis subcapitata [redacted] study report

Data access
data submitter is data owner

Data protection claimed
yes

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
OECD Guideline 201 (Alga, Growth Inhibition Test)
before 23 March 2006

Version / remarks
23 March 2006

Deviations
no

GLP compliance
yes (incl. QA statement)

Test material

Test material information
Garlic extract

Sampling and analysis

Analytical monitoring
yes

Details on sampling
The test concentration along with the negative control was analysed for the test item concentration at the beginning and at the end of test. For analysis, single composite sample was drawn from prepared test concentrations and from the negative control.

Details on analytical methods
The active ingredient in the test samples was determined by injecting the samples (prepared as above) along with a working standard solution into a GC-MS/MS operated under the following conditions:
Instrument : Gas Chromatograph equipped with mass spectrometer Detector and PC based data sy stem Manufacture: Shimadzu, Model: TQ8040
Column : ZB-35, 30 m long, 0.25mm internal diameter, 0.25 µm film thickness or similar
Split ratio : 1:1
Temperatures
Column oven : 60°C
Injection : 200°C
Column Oven : Initial: 60°C hold for 5 min.
Ramp 1: 10°C/min. to 100°C, hold for 2.0 min.
Ramp 2: 15°C/min. to 150°C, hold for 3.0 min
Ramp 1: 30°C/min. to 230°C, hold for 8.0 min
Ion Source : 280°C
Interface : 300°C
Column Flow : 2.40 mL/min

Test solutions

Vehicle
no

Details on test solutions
Test samples were received from Ecotoxicology for analysis. The two replicates for control samples i .e. G1 and three replicates from G2 to G8 groups test samples.

Test organisms

Test organisms (species)
Raphidocelis subcapitata (previous names: Pseudokirchneriella subcapitata, Selenastrum capricornutum)
green algae

Details on test organisms
Raphidocelis subcapitata (formerly Pseudokirchneriella subcapitata) ATCC® 22662 was used as the test system as it is a recommended algal species in the guideline.

Study design

Test type
static

Water media type
freshwater

Limit test
no

Total exposure duration

72	h

Test conditions

Test temperature
22.2 - 22.9°C

pH
7.68 - 7.85

Nominal and measured concentrations
The alga was exposed to the test item at the nominal concentrations of 5.8, 9.3, 14.8, 23.8, 38.0, 60.8 and 97.3 mg/ L (factor of 1.6) (respective geometric mean concentrations were 2.55, 4.41, 6.40, 9.65, 14.72, 24.03 and 36.42 mg test item/L along with a negative control.

Details on test conditions
Exponentially growing cultures of unicellular green alga, Pseudokirchneriella subcapitata were expos ed to various concentrations of Garlic extract (CLAIL0021) under static mode over several generation s under defined conditions. The inhibition of growth in relation to a negative control culture was d etermined over a period of 72 hours.

Reference substance (positive control)
yes Potassium dichromate

Results and discussion

Effect concentrations

Key result
true

Duration

72 h

Dose descriptor
EC50

Effect conc.

19.22 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
growth rate

Remarks on result
other: 95% fiducial limits: 14.70 to 24.67

Key result
true

Duration

72 h

Dose descriptor
NOEC

Effect conc.

2.55 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
growth rate

Key result
false

Duration

72 h

Dose descriptor
LOEC

Effect conc.

4.41 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
growth rate

Key result
false

Duration

72 h

Dose descriptor
EC50

Effect conc.

8.41 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
yield

Remarks on result
other: 95% fiducial limits: 7.349 to 9.650

Key result
false

Duration

72 h

Dose descriptor
NOEC

Effect conc.

2.55 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
yield

Key result
false

Duration

72 h

Dose descriptor
LOEC

Effect conc.

4.41 mg/L

Nominal / measured
meas. (geom. mean)

Conc. based on
test mat.

Basis for effect
yield

Details on results
After 72 hours, the median effective concentration, biomass (EbC50) was 8.62 mg garlic extract (CLAIL0021)/L and the median effective concentration, growth rate (ErC50) was 18.12 mg garlic extract (CLAIL0021)/L based on the analysed mean measured concentration.

Results with reference substance (positive control)
The 72-hour ErC50, ErC20, ErC10, EyC50, EyC20 and EyC10 values for the reference substance (potassium dichromate) were 0.6450 (95% fiducial limits: 0.2836 – 1.3701), 0.3196 (95% fiducial limits: 0.1405 – 0.6786), 0.2214 (95% fiducial limits: 0.0973 – 0.4700), 0.2130 (95% fiducial limits: 0.1664 – 0.2735), 0.0786 (95% fiducial limits: 0.0613 – 0.1008), and 0.0466 (95% fiducial limits: 0.0364 – 0.0598) mg/L (based on nominal concentrations), respectively.

Overall remarks, attachments

Attachments

Type
full study report

Applicant's summary and conclusion

Validity criteria fulfilled
yes

Conclusions
The 72-hour ErC50 and ErC10 values for Garlic extract (CLAIL0021) were 19.22(95% fiducial limits: 14.70 to 24.67) and 8.517 (4.072 to 15.95) mg/L (based on geometric mean concentrations), respectively. The 72-hour NOEC and LOEC values (based on growth rate) were 2.55 and 4.41 mg/L (based on geometric mean concentrations), respectively.

Executive summary

The effect of Garlic extract (CLAIL0021) was tested on the growth of freshwater unicellular green alga Pseudokirchneriella subcapitata. The alga was exposed to the test item at the nominal concentrations of 5.8, 9.3, 14.8, 23.8, 38.0, 60.8 and 97.3 mg/ L (factor of 1.6) (respective geometric mean concentrations were 2.55, 4.41, 6.40, 9.65, 14.72, 24.03 and 36.42 mg test item/L along with a negative control. The cell growth was measured at 24, 48 and 72 hours after the initiation of the test.

The 72-hour ErC50 and ErC10 values for Garlic extract (CLAIL0021) were 19.22 (95% fiducial limits: 14.70 to 24.67) and 8.517 (4.072 to 15.95) mg/L (based on geometric mean concentrations), respectively. The 72-hour NOEC and LOEC values (based on growth rate) were 2.55 and 4.41 mg/L (based on geometric mean concentrations), respectively.