CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Aqueous extract from the germinated seeds of sweet *Lupinus albus*

EC Number: **Not allocated**

CAS Number: **Not allocated**

Index Number: **Not allocated**

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1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Aqueous extract from the germinated seeds of sweet *Lupinus albus*
Other names (usual name, trade name, abbreviation)	PROBLAD PLUS
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	Not available
EC name (if available and appropriate)	Not available
CAS number (if available)	Not available
Other identity code (if available)	Not applicable
Molecular formula	Not applicable
Structural formula	Not applicable
SMILES notation (if available)	Not applicable
Molecular weight or molecular weight range	Not applicable
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	The substance is a plant extract.
Degree of purity (%) (if relevant for the entry in Annex VI)	UVCB substance purity is 100% by default. BLAD protein contents is 19.5 – 21.0%w/w.

The one letter code (amino acid sequence) for the lead component BLAD is:

RRQRNPYHFSSQR FQTLYKNRNGKIRVLERFDQ RTNRLENLQNYRIVEFQSKP

NTLILPKHSDADYVLVVLNG RATITIVNPDRRQAYNLEYG DALRIPAGSTSYILNPDDNQ

KLRVVKLAIPINNPGYFYDF YPSSTKDQQSYFSGFSRNTL EATFNTRYEEIQRIILGNED

The substance name was changed during the approval procedure to Aqueous extract from the germinated seeds of sweet *Lupinus albus* . This name is a synonym of Sweet Lupin (seeds), *Lupinus albus* L., germ., ext. and to PROBLAD PLUS.

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
BLAD protein	**19.5 – 21.0%**	**None**	**None**

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
None				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
The substance contains additives, which are claimed confidential. Please refer to the confidential attachment for more information on the substance identity and composition.					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	650-RST-VW-Y	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	-	-	-	-	-	-	-	-	-
Resulting Annex VI entry if agreed by RAC and COM	650-RST-VW-Y	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	-	-	-	-	-	-	-	-	-

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

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	*data conclusive but not sufficient for classification*	Yes
Flammable gases (including chemically unstable gases)	*hazard class not applicable*	No
Oxidising gases	*hazard class not applicable*	No
Gases under pressure	*hazard class not applicable*	No
Flammable liquids	*data conclusive but not sufficient for classification*	Yes
Flammable solids	*hazard class not applicable*	No
Self-reactive substances	*data conclusive but not sufficient for classification*	Yes
Pyrophoric liquids	*data conclusive but not sufficient for classification*	Yes
Pyrophoric solids	*hazard class not applicable*	No
Self-heating substances	*data conclusive but not sufficient for classification*	Yes
Substances which in contact with water emit flammable gases	*hazard class not applicable*	No
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 lacking*	Yes
Acute toxicity via oral route	*data conclusive but not sufficient for classification*	Yes
Acute toxicity via dermal route	*data conclusive but not sufficient for classification*	Yes
Acute toxicity via inhalation route	*data conclusive but not sufficient for classification*	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*	No
Skin sensitisation	*data conclusive but not sufficient for classification*	Yes
Germ cell mutagenicity	*data conclusive but not sufficient for classification*	Yes
Carcinogenicity	*data conclusive but not sufficient for classification*	Yes
Reproductive toxicity	*data conclusive but not sufficient for classification*	Yes
Specific target organ toxicity-single exposure	*data conclusive but not sufficient for classification*	Yes
Specific target organ toxicity-repeated exposure	*data conclusive but not sufficient for classification*	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Aspiration hazard	*data conclusive but not sufficient for classification*	Yes
Hazardous to the aquatic environment	*harmonised classification proposed*	Yes
Hazardous to the ozone layer	*data lacking*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Aqueous extract from the germinated seeds of sweet *Lupinus albus* has not been previously classified by RAC or TC C&L.

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is not registered under REACH.

Aqueous extract from the germinated seeds of sweet *Lupinus albus* has been evaluated within the context of Regulation (EC) No 1107/2009. According to the data presented in the DAR (2019), no classification is proposed for physicalchemical properties, human health or environmental hazards.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is an active substance in the meaning of Regulation (EU) No 1107/2009, therefore, a justification is not required as the classification and labelling has to be discussed anyway.

5 IDENTIFIED USES

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is intended to be used as a fungicide. The intended uses included in the active substance approval dossier are spray application in strawberry (field and greenhouse) and tomato (field and greenhouse).

6 DATA SOURCES

This CLH report was prepared based on the dossier submitted and draft assessment report (DAR) prepared in the context of the approval of this new active substance.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101.3 kPa	Dark brown liquid	Wo, 2012b	
Melting/freezing point	Not determined		The substance is aqueous with an expected freezing point at or below 0°C.
Boiling point	100°C	Wo, 2012a	
Relative density	Relative density at 20°C: 1.255	Wo, 2012b	
Vapour pressure	Not available		The main constituent has a molecular weight of approximately 210 kDa and is expected to be non-volatile.
Surface tension	Surface tension at 1 g/L 29.3 mN/m at 20.2°C	Lien, 2013	
Water solubility	No data available		
Partition coefficient n-octanol/water	4.5 (BLAD protein) Extract: no data (not applicable)		QSAR estimation
Flash point	>100°C. The test item is	Wo, 2012b	

Property	Value	Reference	Comment (e.g. measured or estimated)
	not flammable		
Flammability	Not applicable to liquids.		
Explosive properties	Not explosive	Cage, 2013	
Self-ignition temperature	Not determined		Based on the composition of the extract, the substance is not expected to be self-heating or self-reactive.
Oxidising properties	Not oxidising	Cage, 2013	
Granulometry	Not applicable		
Stability in organic solvents and identity of relevant degradation products	Not determined		Stability in organic solvents is not a data requirement under Regulation 1107/2009/EC.
Dissociation constant	Not applicable		
Viscosity	Viscosity (kinematic) At 20°C 765.932 mm^2s^{-1} At 40°C 230.181 mm^2s^{-1}	Wo, 2012b	

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A14	Not explosive	The test method used is obsolete, but based on theoretical considerations the substance is not expected to be explosive.	Cage, 2013

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

The test method EEC A14 is formally obsolete as it does not allow a conclusion on the explosive properties as required by Regulation 1272/2008/EC. The test involves an investigation on the effects of heat (flame) and shock (impact hammer). The outcome of the test is that the substance is not explosive.

According to the theoretical considerations outlined in Appendix 6 (screening procedures) of the UN Recommendations on the transport of dangerous goods, manual for tests and criteria rev. 6, if a substance or mixture does not contain functional groups related to explosive properties, it does not need to be further tested. The full composition of the extract is not known, however. As the water content is high and it is assumed all but unsaturated hydrocarbons can be excluded to be present in the substance, explosive properties are not expected. If any unsaturated alkyl moieties are present, they are expected to be present in fatty acids, which are known not to be explosive.

8.1.2 Comparison with the CLP criteria

Considering the test was negative, the CLP criteria for classification are not met.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified.

8.2 Flammable gases (including chemically unstable gases)

Not applicable as the substance is not a gas.

8.3 Oxidising gases

Not applicable as the substance is not a gas.

8.4 Gases under pressure

Not applicable as the substance is not a gas.

8.5 Flammable liquids

Table 10: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EEC A9 / OPPTS 830.700 (closed cup)	>100°C		Wo, 2012b

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

The test result is valid. The flashpoint of >100°C can be used to address the flammability of the substance.

8.5.2 Comparison with the CLP criteria

The flashpoint was determined using an adequate method and does not meet the classification threshold of 60°C or lower.

8.5.3 Conclusion on classification and labelling for flammable liquids

Not a flammable liquid.

8.6 Flammable solids

Not applicable as the substance is not a solid.

8.7 Self-reactive substances

No studies were submitted.

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

The substance is claimed not to be flammable or self-reactive based on it being an aqueous solution. This statement does not fully address the criteria of the CLP regulation, because being an aqueous

solution does not exclude the possibility of components within the substance being self-reactive or auto-flammable. However, the data in the dossier shows the substance is stable when heated to its boiling point and it is not flammable. It is therefore not expected to be self-reactive.

8.7.2 Comparison with the CLP criteria

Although the CLP criteria for testing and the screening procedures (appendix 6) of the UN Recommendations on the transport of dangerous goods, manual for tests and criteria rev. 6 were not fully addressed, it is reasonable to assume the substance will not be self-reactive, based on circumstancial evidence within the dossier (composition, flammability, boiling point).

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified.

8.8 Pyrophoric liquids

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Pyrophoric behaviour was not specifically addressed in the substance dossier. However, the substance is stable when heated to its boiling point and it is not flammable. It is therefore not expected to be pyrophoric.

8.8.2 Comparison with the CLP criteria

Although the CLP criteria for testing and the screening procedures of appendix 6 of the UN Recommendations on the transport of dangerous goods, manual for tests and criteria rev. 6 were not fully addressed, it is reasonable to assume the substance will not be pyrophoric, based on circumstancial evidence within the dossier (composition, flammability, boiling point).

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Not classified.

8.9 Pyrophoric solids

Not applicable as the substance is not a solid.

8.10 Self-heating substances

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

The substance is claimed not to be flammable or self-heating based on it being an aqueous solution. This statement does not fully address the criteria of the CLP regulation. However, the substance is stable when heated to its boiling point and it is not flammable. It is therefore not expected to be self-heating either.

8.10.2 Comparison with the CLP criteria

Although the CLP criteria for testing and the screening procedures of appendix 6 of the UN Recommendations on the transport of dangerous goods, manual for tests and criteria rev. 6 were not

fully addressed, it is reasonable to assume the substance will not be self-heating, based on circumstancial evidence within the dossier (composition, flammability, boiling point).

8.10.3 Conclusion on classification and labelling for self-heating substances

Not classified.

8.11 Substances which in contact with water emit flammable gases

Not applicable. The substance is aqueous.

8.12 Oxidising liquids

Table 11: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
US federal register Vol 44 (1979) and OPPTS 830.6314	No oxidising or reducing potential reported except when tested with potassium permanganate where a reaction was observed within two minutes.	Addition justification: Aqueous extract from the germinated seeds of sweet *Lupinus albus* is an aqueous solution containing the lead component BLAD. BLAD is a 210, kDa gylco-oligomer , which is mainly composed by a 20 kDa protein of β-conglutin or characterized as a fragment of the amino acid sequence of β-conglutin. As such it is not expected to be oxidizing. No other components of Aqueous extract from the germinated seeds of sweet *Lupinus albus* contain groups that would imply oxidising properties such as nitrates, metal oxides, hypofluorites, difluoroaminopolynitroaryls, perchlorates, bromates and iodites. Therefore Aqueous extract from the germinated seeds of sweet *Lupinus albus* will not be oxidising.	Wo, 2012b
EEC A21	Not oxidising		Cage, 2013

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

The OPPTS method does not use the same conditions as the UN test method. EEC A21 was originally based on UN method O2, but uses a different ignition source than required by the UN test method. Still, the method is acceptable in this case, taking into account the properties of the test substance.

In the UN Recommendations on the transport of dangerous goods, manual for tests and criteria rev. 6, it is stated that if a substance or mixture contains compounds that contain oxygen, fluorine or chlorine bound only to carbon or hydrogen, oxidising properties do not need to be expected. It should be noted that the substance, being a UVCB, was not fully identified, but as it is of plant

origin, it is not expected any unidentified compounds are present in the substance that may induce oxidising properties.

8.12.2 Comparison with the CLP criteria

Full characterisation of the substance was not possible, therefore, the screening procdures with regard to functional groups related to oxidising potential are not conclusive. Still, the test according to EEC A21 gives sufficient evidence that the substance does not need to be considered for classification.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Not classified.

8.13 Oxidising solids

Not applicable. The substance is a liquid.

8.14 Organic peroxides

Not applicable. The substance does not contain organic peroxides.

8.15 Corrosive to metals

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data based on UN test method C.1 was provided to address the corrosiveness to metals. The pH of the substance is approximately 6 and is not classified with regard to skin and eye irritation. It is therefore not expected to be corrosive, but metal corrosion is a complex process which is difficult to predict.

8.15.2 Comparison with the CLP criteria

Insufficient data is available to compare to the CLP criteria.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Inconclusive.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

No specific studies were submitted or available. As Aqueous extract from the germinated seeds of sweet *Lupinus albus* contains the naturally occurring polypeptide component BLAD (the lead component), the protein will be broken down in the digestive tract, enter the amino acid pool and be consumed by normal catabolic processes. As the substance is susceptible to proteolytic degradation as it is, radiolabelling of the test article is not possible. Therefore, studies on absorption, distribution, metabolism and excretion in mammals have not been undertaken.

The mammalian toxicity studies of Aqueous extract from the germinated seeds of sweet *Lupinus albus* were assessed in the Draft Assessment Report (DAR 2019). Studies considered valid in the DAR (reliability score of 1 or 2) have been included in this report and were considered for classification purposes. The study summaries as presented in the DAR are included in Annex I. All studies were carried out under GLP unless indicated otherwise. All studies were conducted in accordance with OECD guidelines.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 12: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity OECD 425 Deviation from guideline: None	Rat, Sprague-dawley, females 1 animal, followed by 2 animals	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	5000 mg/kg bw	LD_{50} > 5000 mg/kg bw	IIA 5.2.1/01 Doc ID: company report no. 31002

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

One acute oral toxicity study in rat conducted according to the up-and-down procedure is available. An initial limit dose of 5000 mg/kg bw was administered to one female rat by gavage. Due to the absence of mortality in this animal, two additional female rats were administered with the same dose. No clinical signs, effect on body weight or pathological findings were observed. The oral LD_{50} is considered to be higher than 5000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008, a substance should be classified for acute toxicity if the acute oral LD_{50} is below 2000 mg/kg bw. In the available study the LD_{50} was higher than 5000 mg/kg bw and thus classification is not required.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the acute oral LD_{50} value of > 5000 mg/kg bw found, classification for acute oral toxicity is not proposed.

10.2 Acute toxicity - dermal route

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity OECD 402	Rat, Sprague-dawley 5 animals/sex	Aqueous extract from the germinated seeds of sweet *Lupinus*	2000 mg/kg bw	LD_{50} > 2000 mg/kg bw	IIA 5.2.2/01 Doc ID: company report no. 31003

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Deviations from guideline: None		*albus*			

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

One acute dermal toxicity study in rat was conducted according to OECD 402. No clinical signs, effect on body weight or pathological findings were observed. The dermal LD_{50} is considered to be higher than 2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008, a substance should be classified for acute dermal toxicity if the LD_{50} is below 2000 mg/kg bw. In the available study the LD_{50} was higher than 2000 mg/kg bw and thus classification is not required.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the acute dermal LD_{50} value of > 2000 mg/kg bw found, classification for acute dermal toxicity is not proposed.

10.3 Acute toxicity - inhalation route

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity OECD 403 Deviations from guideline: None	Rat, Sprague-dawley 5 animals/sex	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	5.34 mg/L (nose-only)	LC_{50} > 5.34 mg/L	IIA 5.2.3/01 Doc ID: company report no. 30998

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

One acute inhalation toxicity study in rat was conducted according to OECD 403. No clinical signs, effect on body weight or pathological findings were observed. The inhalation LC_{50} is considered to be higher than 5.34 mg/L.

10.3.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008, a substance should be classified for acute inhalation toxicity if the LC_{50} is below 5 mg/L. In the available study the LC_{50} was higher than 5.34 mg/L and thus classification is not required.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the acute inhalation LC_{50} value of > 5.34 mg/L found, classification for acute inhalation toxicity is not proposed.

10.4 Skin corrosion/irritation

Table 15: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Skin irritation *in vivo* OECD 404 Deviations from guideline: None	Rabbit, New-Zealand white 3 males	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	0.5 mL, semi-occluded	Mean values erythema: 0.7 after 24 hours 0 after 48 and 72 hours Mean values of oedema: 0 after 24, 48 and 72 hours	IIA 5.2.4/01 Doc ID: company report no. 31000

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

Skin corrosion/irritation was tested in one *in vivo* study with rabbits conducted according to OECD 404. Slight erythema was observed after 24 hours, whereas no erythema was observed after 48 hours or later time points. No oedema was observed in any of the animals after 24, 48 or 72 hours.

Table 10.4.1-1: Individual and mean skin irritation scores according to the Draize scheme

Animal no	Erythema				Oedema				Severity of irritation[1]
	1♂	2♂	3♂	Mean	1♂	2♂	3♂	Mean	
after 0.5 – 1h	1	2	2	**1.7**	0	1	1	**0.7**	2.4
after 24 hr	0	1	1	**0.7**	0	0	0	**0**	0.7
after 48 hr	0	0	0	**0**	0	0	0	**0**	0.0
after 72 hr	0	0	0	**0**	0	0	0	**0**	0.0
mean score	**Erythema**				**Oedema**				-
24 – 72h	0	0.3	0.3	-	0	0	0	-	-

[1] Primary Dermal Irritation Index reported in study (sum of erythema + oedema scores)

10.4.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.2.2 a substance should be classified for skin irritation Category 2 in the case where

(1) Mean value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or
(3) In some cases where there is pronounced variability of response among animals, with very

definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Aqueous extract from the germinated seeds of sweet *Lupinus albus* does not fulfil the criteria for skin irritation as the scores for erythema and oedema were below 2.3 in all animals at all time points and no signs of inflammation were observed.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the scores for erythema and oedema found in an *in vivo* study, classification for skin corrosion or irritation is not proposed.

10.5 Serious eye damage/eye irritation

Table 16: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Eye irritation *in vivo* OECD 405 Deviations from guideline: None	Rabbit, New-Zealand white 3 females	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	0.1 mL Single installation in conjunctival sac	Corneal opacity was observed after 1h (scores 1, 1, 1), 24h (scores: 1, 1, 0), and in 1 animal after 48 hours (scores: 1, 0, 0). All animals were free of corneal opacity by 72 hours. Iritis was not observed. Conjunctival redness was observed in all three animals after 1 hour (score: 2, 2, 2), 24 hours (score 2, 2, 2), 48 hours (score 1, 2, 1), and 72 hours (score 1, 1, 1). All three animals were free of conjunctival redness by day 4. Conjunctival chemosis was observed in all three animals after 1 hour (3, 3, 2), 24 hours (1, 1, 1), and in two animals after 48 hours (1,1, 0). All animals were free of chemosis by 72 hours.	IIA 5.2.5/01 Doc ID: company report no. 30999

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

Eye damage/irritation was tested in one *in vivo* study with rabbits conducted according to OECD 405. Corneal opacity was observed in all three animals at 1h, in 2 animals at 24h and in 1 animal at 48h, thereafter no corneal opacity was observed. No iritis was observed in any of the animals at any time point. Conjunctival redness was observed in all three animals at 1h, 24h, 48h and 72h, but was no longer observed after 4 days. Conjunctival chemosis was observed in all three animals at 1h and 24h and in two animals at 48h; all animals were free of chemosis by 72 hours.

Table 10.5.1-1: Eye irritation scores according to the Draize scheme – unwashed eye

Time / Rabbit No.	Cornea (opacity/area)			Iris (value/area)			Conjunctiva-					
							redness			chemosis / discharge		
	1	2	3	1	2	3	1	2	3	1	2	3
1 hr	1/2	1/2	1/1	0/0	0/0	0/0	2	2	2	3/2	3/3	2/2
24 hrs	1/1	1/1	0/4	0/0	0/0	0/0	2	2	2	1/2	1/2	1/2
48 hrs	1/1	0/4	0/4	0/0	0/0	0/0	1	2	1	1/1	1/1	0/0
72 hrs	0/4	0/4	0/4	0/0	0/0	0/0	1	1	1	0/1	0/1	0/0
4 d	0/4	0/4	0/4	0/0	0/0	0/0	0	0	0	0/0	0/0	0/0
7 d	0/4	0/4	0/4	0/0	0/0	0/0	0	0	0	0/0	0/0	0/0
means scores 24-72 hrs[1]	0.7	0.3	0	0	0	0	1.3	1.7	1.3	0.7	0.7	0.3

[1]. Area not taken into account as it was assumed that readings were taken from the densest area

10.5.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008 Section 3.3.2 a substance should be classified in Category 1 (serious eye damage) if

a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

b) in at least 2 of 3 tested animals, a positive response of

i) corneal opacity ≥ 3 and/or

ii) iritis > 1.5

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

In the study, all findings were reversible in all animals, no corneal opacity score of 3 or higher was observed and no iritis was observed in any of the animals. Therefore, Cat.1 classification is not required.

According to Regulation (EC) No 1272/2008 Section 3.3.2, a substance should be classified in Cat. 2 (eye irritation) 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 installation of the test material, and which fully reverses within an observation period of 21 days.

In the study, corneal opacity mean score over the time period 24-72 h were 0.7, 0.3 and 0 for the three animals, and thus below the classification threshold of 1. Iritis was not observed in any of the animals. Conjunctival redness scores over the time period 24 h-72 h were 1.3, 1.7, 1.3 for the three animals and thus below the classification threshold of 2. Conjunctival chemosis scores over the time period 24 h-72 h were 0.7, 0.7 and 0.3 for the three animals and thus below the classification threshold of 2. Overall, none of the criteria for classification in cat. 2 (eye irritation) are met, therefore, classification is not required.

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

Based on the scores for corneal opacity, iritis, conjunctival redness and chemosis found in an *in vivo* study, classification for eye damage or irritation is not proposed.

10.6 Respiratory sensitisation

No data available.

10.7 Skin sensitisation

Table 17: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Buehler assay OECD 406 Deviations from guideline: None	Guinea pig, Hartley albino 4 males (pre-test) 20 males (main test) 10 males (control)	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	0.4 ml; 25, 50, 75 and 100% Dermal, occlusive	Very faint erythema (0.5-10 was noted for all test sites during the induction phase. Very faint erythema (0.5) was noted for 12 of the 20 test sites at 24 hours after challenge. Irritation cleared by 48 hours. No evidence of skin sensitisation in this Buehler assay	IIA 5.2.6/01 Doc ID: company report no. 31004

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

The sensitising potential of Aqueous extract from the germinated seeds of sweet *Lupinus albus* was investigated in a Buehler assay in accordance with OECD 406. A preliminary irritation test was conducted at doses of 25% and 50% (w/w) in which no skin reactions were observed; at doses of 75 and 100% 2 out of 4 animals exhibited very faint erythema (score of 0.5). From these results, the highest non irritation concentration was 100%, this was used for both the induction and challenge phase.

In the Buehler assay, very faint to faint erythema (score 0.5-1) was noted for all test sites during the induction phase. Very faint erythema (0.5) was noted for 12 of the 20 test sites at 24h after challenge. Irritation cleared from all affected sites by 48h. Under the conditions of the study performed, the test substance did not show evidence of skin sensitisation.

In addition, a position paper was submitted (R. Boavida Ferreria (2011)) discussing potential allergenicity of lupine seeds with special emphasis on BLAD. In a (draft) scientific opinion of EFSA on the evaluation of allergenic foods and food ingredients for labelling purposes, it is mentioned that clinical reactions to lupin were triggered in peanut allergic individuals at lupin doses varying from 50 mg to 1.6 gram. Considering the (overestimated) calculated amounts of BLAD on fruit surfaces (grape, strawberry, tomato/apple) and the amounts of these fruits eaten (extracted from PRIMO v.2.0), the values of BLAD ingested based on the amounts calculated to be on the fruits is between 0.16 and 4.7 mg. These values are below the trigger dose for clinical reactions of 0.05-1.6 gram lupin mentioned in the EFSA scientific opinion.

BLAD (the lead component of Aqueous extract from the germinated seeds of sweet *Lupinus albus*) was assessed according to the criteria set by Codex Alimentarius (2003) and FAO/WHO (2001). According to the data provided for these criteria there are no allergenicity-related concerns from the use of Aqueous extract from the germinated seeds of sweet *Lupinus albus* as a biological fungicide in agriculture.

Furthermore, the applicant provided a clinical study (Anonymous, 2013) in which serum from patients allergic to either lupine, peanut (or in some situations both sensitizations) and other nuts and legumina. No reaction to BLAD was found, therefore, BLAD is considered not a potential allergen.

Lastly, the applicant provided a position paper on the dermal penetration of BLAD (Gledhill, A., 2019). by reference to proteins of pharmaceutical interest, it can be demonstrated that polypeptides such an insulin are not absorbable by the dermal route without significant measures to disrupt or bypass the stratum corneum. Insulin is a *ca.* 6 kDa polypeptide consisting of 51 amino acid residues, so is a smaller molecule than BLAD

and is thus considered to provide a worse case assessment with respect to dermal absorption. Therefore, by analogy with a polypeptide such as insulin, it is reasonable to conclude that under normal use conditions BLAD will not reach the epidermis or dermis layers within the skin.

10.7.2 Comparison with the CLP criteria

According to Reg (EC) No 1272/2008, Section 3.4.2.2.1:

Substances shall be classified as skin sensitisers (Cat.1) where data are not sufficient for sub-categorisation in accordance with the following criteria:

a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or

b) if there are positive results from an appropriate animal test

Regarding the Buehler assay, animal test results for the two sub-categories are:

Cat. 1A: ≥ 15% responding at ≤ 0.2% topical induction dose; or ≥ 60% responding at > 0.2% to ≤ 20% topical induction dose

Cat. 1B: ≥ 15% to < 60% responding at > 0.2% to ≤ 20% topical induction dose; or ≥ 15% responding at > 20% topical induction dose.

In the study conducted with Aqueous extract from the germinated seeds of sweet *Lupinus albus*, only very faint erythema (score 0.5) was observed at 24h which was resolved in all animals at 48h. This small effect was regarded as indicative of irritation and not of skin sensitisation. Therefore, classification for skin sensitisation is not proposed.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the study results found in the Beuhler assay, no classification for skin sensitisation is proposed.

10.8 Germ cell mutagenicity

Table 18: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Ames assay OECD 471 Deviations from guideline: None	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	Test strains: TA98, TA100, TA1535, TA1537, WP2 uvrA Concentrations tested: Plate 1: 0, 16, 50, 160, 500, 1600, 5000 µg/plate Plate 2: 0, 51.2, 128, 320, 800, 2000, 5000 µg/plate Positive controls: 2-nitrofluorene, 4-nitroquinoline-1-oxide, N-methyl-N'-nitro-N-nitroguanidine, ICR-191, 2-aminoanthracene	Test substance did not increase revertant colony numbers either without or with S9.	IIA 5.4.1.1/01 Doc ID: company report no. 8325399

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Mammalian cell gene mutation OECD 476 Deviations from guideline: None	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	Cells: L5178Y *tk*$^{+/-}$ mouse lymphoma cells Test concentrations: Preliminary cytotoxicity assay was conducted. Test concentrations main assay: 0-5000 µg/ml –S9 and 0-2500 µg/ml +S9. Positive controls: methyl methane sulphonate and Benzo[a]pyrene.	Without S9, the test substance did not induce mutation at the tk locus after 3 hour treatment when tested up to either a precipitating or toxic concentration. With S9, the test substance induced mutation at the *tk* locus after 3 hour treatment when tested up to toxic concentrations.	IIA 5.4.1.2/01 Doc ID: company report no. 8325403
Micronucleus assay OECD 487 Deviations from guideline: None	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	Cells: Human peripheral blood lymphocytes Test concentrations: Preliminary cytotoxicity assay was conducted. Main assay: 0-2000 µg/ml Positive controls: mitomycin C and vinblastine	The test substance did not induce micronuclei in cultured human peripheral blood lymphocytes following treatment for 3 [+21h recovery] and 24 hours [+24h recovery] in the absence of S9. The test substance did not induce micronuclei following treatment for 3 hours [+21 h recovery] in the presence of S9.	IIA 5.4.1.3/01 Doc ID: company report no. 8325400

Table 19: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Comet assay OECD 489 Deviations from guideline: None	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	Test species: Rat, Han Wistar Doses: Range finding: 3 rats/sex, receiving 2 doses at 2000 mg/kg bw separated by 21h Comet assay: 5 male rats/dose. Doses: 0, 500, 1000, 2000 mg/kg bw, two doses separated by 21 hours Dosing by gavage Positive control: ethyl methane suphonate	No clinical signs, effect on body weight or other test-article related toxicity was observed. The test substance Aqueous extract from the germinated seeds of sweet *Lupinus albus* did not induce DNA damage in the stomach of male rats following dosing at 0 and 21 hours with harvesting of the stomach tissue 3 hours later. The positive control induced an acceptable increase in %tail intensity in the stomach, thereby demonstrating sensitivity of the assay.	IIA 5.4.2.1/01 Doc ID: company report no. 8325402

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

In vitro

Ames test:

Aqueous extract from the germinated seeds of sweet *Lupinus albus* was found to be negative for mutagenicity in an Ames test performed according to OECD 471 (Doc ID 8325399).

In vitro mammalian cell gene mutation:

Aqueous extract from the germinated seeds of sweet *Lupinus albus* was found to be negative in a mammalian cell gene mutation assay without S9, induction of mutations at the *tk* locus was found in this assay with S9 (Doc ID 8325403).

Table 10.8.1-1: Mouse lymphoma toxicity and mutant frequency data from experiment 1

Conc. (µg/mL)	-S9		Conc. (µg/mL)	+S9		
	%RTG	MF		%RTG	MF	Proportion of small colony mutants
0	100	53.57	0	100	57.67	0.28
500	105	51.74	150	109	59.47	-
1000	103	50.34	300	119	61.14	--
1500	113	57.34	600	64	155.66	-
2000PP	78	62.39	900	14	449.12***	0.78
MMS 15	68	262.04	B[a]P 2	89	338.13	0.54
MMS 20	44	432.59	B[a]P 3	53	738.42	0.62

+ve controls: MMS - methyl methanesulphonate; B[a]P - benzo[a]pyrene
PP: precipitate observed at the end of treatment
***Sum of the vehicle control mutant frequency (MF) + GEF (126) being exceeded, with accompanying test for linear trend (one-sided) significant at $p \leq 0.001$

Table 10.8.1-2: Mouse lymphoma toxicity and mutant frequency data from experiment 2

Conc. (µg/mL)	-S9		Conc. (µg/mL)	+S9		
	%RTG	MF		%RTG	MF	Proportion of small colony mutants
0	100	59.13	0	100	46.88	0.34
600	76	77.47	300	74	79.30	-
900	108	74.01	400	57	104.33	--
1200	106	73.66	500	49	98.40	-
1500	104	95.85	600	39	127.74	-
1750	89	80.88	700	48	126.07	-
2000	58	111.31	800	42	141.91	-
2250	66	72.07	850	38	129.20	-
2500	16	118.07	900	33	168.13	-
MMS 15	59	439.63	950	28	157.88	-
MMS 20	45	729.04	1000	30	203.14***	0.61
			1100	24	218.12***	0.55
			1200	19	230***	0.62
			B[a]P 2	65	342.06	0.52
			B[a]P 3	58	443.95	0.54

+ve controls: MMS - methyl methanesulphonate; B[a]P - benzo[a]pyrene

Conc. (µg/mL)	-S9		Conc. (µg/mL)	+S9		
	%RTG	MF		%RTG	MF	Proportion of small colony mutants

PP: precipitate observed at the end of treatment
***Sum of the vehicle control mutant frequency (MF) + GEF (126) being exceeded, with accompanying test for linear trend (one-sided) significant at $p \leq 0.001$

In vitro micronucleus:

Aqueous extract from the germinated seeds of sweet *Lupinus albus* was found to be negative for numerical and structural chromosome aberrations in an *in vitro* micronucleus assay conducted according to OECD 487 (Doc ID: 8325400).

Table 10.8.1-3: Human lymphocyte micronucleus data

3 h (+21 h recovery) –S9			3 h (+21 h recovery) +S9			24 h (+24 h recovery) -S9		
Conc (µg/mL)	Cyto (%)[a]	MNBN freq.[b]	Conc (µg/mL)	Cyto (%)[a]	MNBN freq.[b]	Conc (µg/mL)	Cyto (%)[a]	MNBN freq.[b]
0	-	0.55	0	-	0.35	0	-	0.50
800	8	0.60 NS	800	1	0.45 NS	200	0	0.70 NS
1600	22	0.65 NS	1600	9	0.35 NS	400	18	0.65 NS
2000	19	0.95 NS	2000	11	0.35 NS	1200	36	0.75 NS
MMC 0.2	22	3.55***	CPA 2.0	57	1.60 ***	1600	58	0.95 **
						VIN 0.04	59	3.60 ***

** $p \leq 0.05$; *** $p \leq 0.001$; NS Not significant
a cytotoxicity based on replication index
b mean micronucleated binucleate frequency (%)
+ve controls: MMC: Mitomycin C; CPA: Cyclophosphamide; VIN: vinblastine

In vivo

The positive result in the *in vitro* mammalian cell gene mutation assay (with S9 only) was followed up by an *in vivo* Comet assay, an assay which is capable of detecting gene mutations and/or clastogenicity. Furthermore, considering the test substance main component is a protein which will be broken down and consumed by normal catabolic processes, the Comet assay which can investigate DNA damage at the site of contact (stomach) is considered suitable. As systemic exposure could not be demonstrated examination of liver would be deemed redundant. Therefore, it is considered acceptable to address the positive *in vitro* finding with an *in vivo* Comet assay at the stomach (first site of contact, exposure demonstrated).

In the Comet assay, no induction of DNA damage in the stomach of male rats was observed.

Table 10.8.1-4: Group mean stomach data

Dose level (mg/kg bw/d)	No. of animals	No .of cells scored	Mean tail intensity[a] (% ±SD)	SEM	Mean tail moment[a] (% ±SD)	SEM	Mean % hedgehogs
0	6	900	0.95 ±1.20	0.49	0.12 ±0.14	0.06	11.85
500	6	900	1.03 ±0.96	0.39	0.13 ±0.13	0.05	9.84
1000	6	750[b]	0.31 ±0.12	0.05	0.04 ±0.01	0.01	8.79
2000	6	900	0.47 ±0.33	0.13	0.05 ±0.04	0.02	13.57
EMS, 200	3	450	9.91 ±0.98	0.56	1.21 ±0.08	0.05	24.34

Historical control data ranges for rat stomach comet Data generated from 13 studies dosed between January 2010 to July 2014					
Vehicle	116	Mean:	2.75 ±1.63	0.29 ±0.20	11.98 ±3.94
		Median:	2.44	0.25	12.00
95% reference range:			0.66-6.01	0.09-0.66	4.43-17.50
Positive	103	Mean:	26.61 ±7.22	3.78 ±1.38	17.14 ±5.19
		Median:	25.66	3.52	17.50

95% reference range:	16.92-39.73	2.06-6.49	9.00-25.00

+ve control: EMS – ethyl methylsulphonate
SEM =- standard error mean
a median values of each slide calculated. The mean of the slide medians were calculated to give the individual mean animal value. The individual mean animal values were averaged to provide group mean
b no cells present on the slides examined, however data available from 5 animals within the group

10.8.2 Comparison with the CLP criteria

According to Reg (EC) No 1272/2008 Table 3.5.1, classification in Category 2 mutagen is based on:

- positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

- somatic cell mutagenicity tests in vivo, in mammals; or

- other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Aqueous extract from the germinated seeds of sweet *Lupinus albus* tested negative in an Ames test, negative in an *in vitro* micronuecleus assay with human lymphocytes. Aqueous extract from the germinated seeds of sweet *Lupinus albus* tested positive in the *in vitro* mammalian cell gene mutation assay (with S9 only), however, the *in vivo* follow up Comet assay was negative. Overall, it is concluded that the current information does not indicate Aqueous extract from the germinated seeds of sweet *Lupinus albus* fulfils the classification criteria for germ cell mutagenicity.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification for germ cell mutagenicity is proposed for Aqueous extract from the germinated seeds of sweet *Lupinus albus*.

10.9 Carcinogenicity

No long term toxicity and carcinogenicity studies have been conducted and none are considered necessary. Aqueous extract from the germinated seeds of sweet *Lupinus albus* contains 20% BLAD (the lead component). BLAD is a naturally occurring polypeptide formed during day four to twelve of the germination process of sweet lupines (*Lupinus albus*). BLAD is used in human and animal nutrition, as a food and feed item, it has a non-toxic mode of action which is specific to fungi only (BLAD binds to chitin and chitosan which weakens the cell wall structure. It has been found to be a very effective fungicide against powdery mildew and other diseases and is rapidly biodegradable. It is known to be susceptible to proteolytic degradation and the protein will be broken down, enter the amino acid pool and be consumed by normal catabolic processes. A complete genotoxicity test battery confirmed a lack of genotoxic potential. Furthermore, the 90-day study was completely negative and did not give any indication of non-genotoxic carcinogenicity. There is no evidence in the public domain to suggest that proteins similar to BLAD, which contains a segment of β-conglutin (which shares strong homology to other members of the vicilin family (globulin storage protein associated with leguminous seeds such as peas and lentils)) are associated with an increased incidence of cancer. Based on these it can be concluded that the lead component is unlikely to be considered a carcinogen.

10.9.1 Comparison with the CLP criteria

There is no information suggesting a carcinogenic potential of the Aqueous extract from the germinated seeds of sweet *Lupinus albus.* Therefore a comparison with the criteria is not necessary.

10.9.1 Conclusion on classification and labelling for carcinogenicity

Classification for carcinogenicity is not proposed.

10.10 Reproductive toxicity

No reproductive studies (including developmental studies) have been conducted and none are considered necessary. Aqueous extract from the germinated seeds of sweet *Lupinus albus* contains 20% BLAD (the lead component). BLAD, a naturally occurring polypeptide, is formed during day four to twelve of the germination process of sweet lupines (*Lupinus albus*). BLAD is used in human and animal nutrition, as a food and feed item, it has a non-toxic mode of action which is specific to fungi only (BLAD binds to chitin and chitosan which weakens the cell wall structure and so has been found to be a very effective fungicide against powdery mildew and other diseases) and it is rapidly biodegradable. It is known to be susceptible to proteolytic degradation and the protein will be broken down, enter the amino acid pool and be consumed by normal catabolic processes. There is no evidence in the public domain to suggest that proteins similar to BLAD, which contains a segment of β-conglutin (which shares strong homology to other members of the vicilin family (globulin storage protein associated with leguminous seeds such as peas and lentils)) are toxic either in a reproductive or developmental capacity. Based on these it can be concluded that the lead component is unlikely to be considered a reproductive or developmental toxin.

10.10.1 Comparison with the CLP criteria

There is no information suggesting a reprotoxic potential of the Aqueous extract from the germinated seeds of sweet *Lupinus albus.* Therefore a comparison with the criteria is not necessary.

10.10.2 Conclusion on classification and labelling for reproductive toxicity

Classification for reproductive toxicity is not proposed.

10.11 Specific target organ toxicity-single exposure

The only studies available to assess STOT SE are the acute toxicity studies via the oral, dermal and inhalation route.

Table 20: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference

Acute oral toxicity OECD 425 Deviation from guideline: None Rat, Sprague-dawley, females 1 animal, followed by 2 animals	Aqueous extract from the germinated seeds of sweet *Lupinus albus* Gavage, 5000 mg/kg bw, single dose	LD_{50} > 5000 mg/kg bw. No clinical signs, adverse pharmacologic effects or abnormal behaviour were observed. No effect on body weight or body weight gain was observed during the 14-day observation period following the single dosing at 5000 mg/kg bw. No treatment-related pathological findings were observed.	IIA 5.2.1/01 Doc ID: company report no. 31002
Acute dermal toxicity OECD 402 Deviations from guideline: None Rat, Sprague-dawley 5 animals/sex	Aqueous extract from the germinated seeds of sweet *Lupinus albus* Dermal application, 2000 mg/kg bw, single	LD_{50} > 2000 mg/kg bw. Clinical signs of toxicity were limited to red nasal discharge on day 2 in 3/5 males and 1/5 females. No signs of dermal irritation were observed. No effect on body weight or body weight gain was observed during the 14-day observation period following the single dosing at 2000 mg/kg bw No treatment-related pathological findings were observed.	IIA 5.2.2/01 Doc ID: company report no. 31003
Acute inhalation toxicity OECD 403 Deviations from guideline: None Rat, Sprague-dawley 5 animals/sex	Aqueous extract from the germinated seeds of sweet *Lupinus albus* 4h inhalation, nose-only, 5.34 mg/L	LC_{50} > 5.34 mg/L. Clinical signs were limited to one animal of each sex displaying signs of hypoactivity and 7 animals (3M / 4F) exhibiting abnormal respiration. All affected animals recovered by day 8 and appeared normal for the remainder of the observation period. No effect on body weight or body weight gain was observed during the 14-day observation period and no pathological findings were seen.	IIA 5.2.3/01 Doc ID: company report no. 30998

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

In the acute oral toxicity study (Doc ID 31002), no clinical signs, effects on body weight (gain) or pathological findings were observed following a single dosing (by gavage) at 5000 mg/kg bw.

In the acute dermal toxicity study (Doc ID 31003), red nasal discharge was observed in 3/5 males and 1/5 females on day 2. No effects on body weight (gain) or pathology were observed.

In the acute inhalation toxicity study (Doc ID 30998), one male and one female had signs of hypoactivity and 7 animals (3 males and 4 females) had abnormal respiration. All these effects recovered by day 8 and remained normal for the remainder of the observation period. No effects on body weight (gain) or pathology were observed.

Table 10.11.1-1: Individual cage side observations acute inhalation study (Doc ID 30998)

Males			Females		
Animal number	**Findings**	**Day of occurrence**	**Animal number**	**Findings**	**Day of occurrence**
3301	Active and healthy Hypoactivity Irregular respiration	CR, 3-14 0 (1h) 1-2	3306	Active and healthy Hypoactivity	CR, 1-14 0 (1h)
3302	Irregular respiration Active and healthy	CR-2 3-14	3307	Rales (moist) Irregular respiration Active and healthy	CR-2 CR-7 8-14
3303, 3304	Active and healthy	0-14	3308	Irregular respiration Active and healthy	CR-3 4-14

3305	Rales (moist) Irregular respiration Scab* on head Active and healthy	CR-0(1h) CR-4 CR-13 5-14	3309, 3310	Irregular respiration Active and healthy	CR-1 2-14

CR – removal from the exposure tube
* scab was present at pre-exposure observations.

10.11.2 Comparison with the CLP criteria

The only effect observed was clinical signs in the acute inhalation study following dosing at 5.34 mg/L. This value is higher than the values given in the CLP guidance regarding classification in cat. 1 ($C \leq 1$ mg/L) or cat. 2 ($5.0 \geq C > 1.0$ mg/L). Therefore, classification in cat. 1 or cat. 2 is not required.

Category 3 classification only covers respiratory tract irritation and narcotic effects. Regarding respiratory irritation:

The criteria for classifying substances as Category 3 for respiratory tract irritation are:
(a) respiratory irritant effects (characterized by localized redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data.
(b) subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids).
(c) he symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports simply of "irritation" shall be excluded as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation, and dryness, which are outside the scope of classification for respiratory irritation.
(d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation.
(e) *this special classification would occur only when more severe organ effects including in the respiratory system are not observed.*

No human data is available that would indicate respiratory tract irritation.

In the acute inhalation toxicity study in rat, abnormal respiration (irregular respiration) was observed in 3/5 males and 4/5 females. In males this clinical sign was observed on day 1-2 in 1 animal, and in the other two after removal of the exposure tube until day 2 to 4. In females, this clinical sign of irregular respiration was observed following removal of the exposure tube until day 1 for two animals, until day 3 for one animal and until day 7 for one animal. No histopathological findings were observed in this study.

In the CLP Regulation the following is indicated:
It is clearly indicated in the CLP that there are currently no validated animal tests that deal specifically with RTI, but that animal studies can be used as a part of weight of evidence evaluation (CLP Annex I, 3.8.2.2.1.2(d)). However when there are no data in human and animal data suggesting RTI effects, expert judgement is needed to estimate the severity of the effects observed in animals, the conditions of the test, the physical-chemical properties of the substance and whether those considerations alone might be sufficient for a classification in Category 3 for RTI.

Based on the compound type of this substance, volatilisation to air is not anticipated. The substance is a protein based aqueous solution; it is a large molecule (>200 kDa) and vapour pressure is expected to be low. Furthermore, the clinical sign observed in the acute inhalation study followed directly after exposure tube

removal, therefore, it could be related to the stress the animals had during and following 4 h nose-only exposure.

Overall, the effect is not considered severe enough for classification for RTI and based on the characteristics of the compound volatisation to air is not anticipated. Therefore, classification for respiratory tract irritation is not required.

10.11.3 Conclusion on classification and labelling for STOT SE

No classification for STOT SE is proposed for Aqueous extract from the germinated seeds of sweet *Lupinus albus*.

10.12 Specific target organ toxicity-repeated exposure

Table 21: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
90-day oral study in the rat OECD 408 Deviations from guideline: No genotoxicity was assessed (no bone marrow exposure can be determined as no analytical method is available)	Aqueous extract from the germinated seeds of sweet *Lupinus albus* Oral, gavage 0, 250, 500, 1000 mg/kg bw/day Rat, Crl:WI(Han), 10/sex/dose	No mortality or treatment-related clinical signs were observed. No effect on body weight or body weight gain was observed. No effects on food consumption, haematology or clinical chemistry were observed. Functional observation battery (FOB) did not reveal any treatment-related effects. There were no treatment-related effects on organ weights or gross pathology. Histopathology revealed a single female in the high dose with spinal and brain lesions, which was minimal to slight bilateral symmetrical neurophil vacuolation in the grey matter. This effect was not observed in the other animals of this high dose group or the other dose groups. This was considered an incidental finding as no other neurological findings were observed in the FOB. However, during the expert meetin (PREV25) the majority of experts considered the NOAEL should be set at 500 mg/kg bw/day based on vacuolation in the brain and spinal cord seen in 1 female at the high dose. It was noted that this was a precautionary approach.	IIA 5.3.2/01 Doc ID: company report no. 8325453

22-day dermal study in rat OECD 410 Deviations from guideline: None	Aqueous extract from the germinated seeds of sweet *Lupinus albus* Dermal, non-occlusive 0, 100, 300 and 1000 mg/kg bw/day Rat, Crl:WI(Han), 5/sex/dose	No treatment-related effect on body weight or body weight gain, food consumption, haematology or clinical chemistry was observed. Kidney, liver and testis/epididymides weight were not affected; the increase in adrenal weight in top dose males was not significant and was not accompanied by histopathological findings. Increase in hyaline droplets in the kidneys of the high dose males is not considered relevant for humans, as they generally represent accumulation of α2u-globulin (a naturally occurring male rat protein). Following the expet meeting (PREV25), the majority of experts considered the NOAEL should be set at 300 mg/kg bw/day bsed on increased adrenal weight when adjusted for terminal body weight.	IIA 5.3.3/01 Doc ID: company report no. 8297704

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

Oral route of administration:

In a 90-day oral toxicity study in the rat (Doc ID 8325453), doses up to 1000 mg/kg bw/ day were tested. No mortality, clinical signs, effects on body weight or body weight gain, food consumption, haematology or clinical chemistry were observed. Functional observation battery (FOB) did not reveal any treatment-related effects. There were no effects on organ weights or gross pathology. One female in the high dose group had minimal to slight bilateral symmetrical neutrophil vacuolation in the grey matter. As this effect was not observed in any of the other animals and no neurological findings were observed in the FOB, this was considered an incidental finding. Based on a precautionary approach, the NOAEL was set at 500 mg/kg bw/day based on the vacuolation in brain and spinal cord observed in one female at the high dose.

Dermal route of administration:

In a 22-day dermal toxicity study in rat (Doc ID8297704), doses up to 1000 mg/kg bw/day were tested. No mortality, clinical signs, effects on body weight or body weight gain, food consumption, haematology or clinical chemistry were observed. The only effect on organ weight obsed was an increased adrenal weight in males. The adrenal weights (adjusted for terminal body weight) increased by 16%, 18% and 38% for doses of 100, 300 and 1000 mg/kg bw/day, respectively. No histopathological effects in the adrenals were observed. Minimal to slight hyperkeratosis of the treated skin was observed in males and females of the high dose group of 1000 mg/kg bw/day, characterised by a minor increase in thickness of the epidermis with increased keratohylaine granules. In the kidney hyaline droplets were increased in males at 1000 mg/kg bw/day, characterised by eosinophilic cytoplasmic inclusions in the proximal tubular epithelial cells. This effect was not observed in females. Hyaline droplets appear as eosinophilic cytoplasmic inclusions in the proximal tubular epithelial cells and are a common background finding in the kidney of male rats. They generally represent accumulations of α2u-globulin, a naturally occurring male rat protein. This is a common response of the male rat to xenobiotics and is of little relevance to risk assessment in humans.

Table 10.12.1-1: Male Adrenal weights

Parameter	Dose (mg/kg bw/d)			
	0	100	300	1000
Ter. body weight (g)	328.5	305.5	301.8	300.4
Adrenal unadjusted abs. organ weight (g)	0.063	0.063	0.063	0.073
Historical control data[a]:	Mean: 0.058 (n= 195) Range: 0.035 – 0.080 (Sept 2007 – Mar 2011)			
Adrenal wt : ter. body	0.0191	0.0208	0.0208	0.0244

Parameter	Dose (mg/kg bw/d)			
	0	100	300	1000
wt (%)				
Historical control data[a]:	Mean: 0.171 (n= 195) Range: 0.093 – 0.0248 (Sept 2007 – Mar 2011)			
Adjusted for ter. body wt (g) [%increase][b]	0.056	0.065 [+16%]	0.066 [+18%]	0.077* [+38%]

* $p \leq 0.05$
abs absolute
ter terminal

a Laboratory historical control range derived from Han Wistar rats aged 14-26 weeks of age at necropsy. Range: min – max (95% reference range). Refer to Attachments, Historical control data

b As group means are estimated after controlling the effect of terminal body weight, no historical control data are available

Table 10.12.1-2: Selected histopathology findings

Parameter	♂ (mg/kg bw/d)		♀ (mg/kg bw/d)	
	0	1000	0	1000
Hyperkeratosis	5; 5,0,0,0	5; 0,5,0,0	5; 5,0,0,0	5; 1,4,0,0
Kidney, hyaline droplets	5; 0,2,2,1	5; 0,1,1,3	5: 0,0,0,0	5: 0,0,0,0

no. examined;
no finding, minimal, slight, moderate

It was considered that the increase in hyaline droplets in kidneys in high dose males is not relevant for humans and the increase in adrenal weight in top dose males was not significant, within historical control data and not accompanied by histopathological findings.The NOAEL was set at 300 mg/kg bw/day bsed on increased adrenal weight when adjusted for terminal body weight.

10.12.2 Comparison with the CLP criteria

According to Reg (EC) No 1272/2008, Section 3.9.2.1:
Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement (see 1.1.1), on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s), (see 3.9.2.9), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed (Table 3.9.1).

Guidance values for Cat. 1 classification are:
Oral (rat) → $C \leq 10$ mg/kg bw/day for a 90-day study
Dermal (rat/rabbit) → $C \leq 60$ mg/kg bw/day for a 28-day study
Guidance values for Cat. 2 classification are:
Oral (rat) → $10 < C \leq 100$ mg/kg bw/day for a 90-day study
Dermal (rat/rabbit) → $60 < C \leq 600$ mg/kg bw/day for a 28-day study

In the 90-day oral toxicity study with Aqueous extract from the germinated seeds of sweet *Lupinus albus* the only possible treatment related observed effect was vacuolation in the brain and spinal cord in one female at the high dose. As a precautionary approach, this finding was taken into account for NOAEL setting. This possivle effect was only seen at the high dose (1000 mg/kg bw/day), which is above the threshold for classification. Therefore, classification into either Cat. 1 or Cat. 2 is not required.

In the 22-day dermal toxicity study with Aqueous extract from the germinated seeds of sweet *Lupinus albus* the only effect observed was increased adrenal weight at the top dose without any histopathological finding. As this increase in weight was observed at 1000 mg/kg bw/day, which is below the trigger for classification, classification into either Cat. 1 or Cat. 2 is not required.

10.12.3 Conclusion on classification and labelling for STOT RE

No classification for STOT RE is proposed for Aqueous extract from the germinated seeds of sweet *Lupinus albus*.

10.13 Aspiration hazard

Table 22: Summary table of evidence for aspiration hazard

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD 114	Aqueous extract from the germinated seeds of sweet *Lupinus albus*	GLP compliant Capillary viscometer	Viscosity (kinematic) At 20 °C 765.932 mm^2/s At 40 °C 230.181 mm^2/s	Wo, 2012b

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

Data is adequate to assess whether the criterion of 20.5 mm^2/s (measured at 40 °C) is met.

10.13.2 Comparison with the CLP criteria

The substance has a kinematic viscosity higher than the trigger value of 20.5 mm^2/s and does not contain any kwown compounds which are classified as an aspiration hazard.

10.13.3 Conclusion on classification and labelling for aspiration hazard

Not classified

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 23: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD 301 B GLP	BLAD protein (the major component of the substance Aqueous extract from the germinated seeds of sweet *Lupinus albus*) is readily biodegradable.	No remarks.	A. Brunswik-Titze, 2015, CEV S.A, Unpublished report No.: 1035
OECD 301 D	More than 97% of the initial concentration of BLAD protein (the major component of the substance Aqueous extract from the germinated seeds of sweet *Lupinus albus*) was degraded after 14 days of incubation.	A specific analytical technique for detection of the BLAD protein was used; the study therefore describes degradation rather than mineralisation.	A. Carreira, 2014 CEV-ABB-0914

Method	Results	Remarks	Reference
OECD 301 D	Aqueous extract from the germinated seeds of sweet *Lupinus albus* is readily biodegradable.	No remarks.	D. Dengler, 2010, CEV S.A. report NO.: S1002624

11.1.1 Ready biodegradability

Three studies on ready biodegradability of BLAD and Aqueous extract from the germinated seeds of sweet *Lupinus albus* show that Aqueous extract from the germinated seeds of sweet *Lupinus albus* and its major constituent are readily biodegradable.

The biodedegradabilty was tested in an OECD 301 B CO_2-evolution study (Brunswik-Titze, 2015). The mean degradation of BLAD protein (i.e., the major component of the substance Aqueous extract from the germinated seeds of sweet *Lupinus albus*) reached 64.7 after 7 days and therefore the criteria of 60% degradation within a 14 day window is met. The substance is therefore considered as readily biodegradable under the conditions of this CO_2-evolution test. The reference compound sodium benzoate reached the pass levels for ready biodegradability within 4 days. The difference between the replicates at the end of the 10 day window was < 20%. The degradation of the toxicity control was >25% (77.3%) within 14 days. Toxic effects of BLAD can be excluded. The criteria for the OECD 301 B test are met in this study. The test can therefore be considered valid.

Two biodegradability tests using OECD 301 D guidelines are available. In one test (Carreira, 2014) degradation of BLAD protein (the major component of the substance Aqueous extract from the germinated seeds of sweet *Lupinus albus*) was determined using a specific analytical technique for detection of the BLAD protein. As a result, the study describes degradation rather than mineralisation of BLAD protein. The investigation was conducted with two different inoculants from two separate waste treatment plants. The study also looked at two different concentrations of BLAD protein (100 and 200 mg/L) and also two concentration of inoculum (0.5 mL/L and 5 mL/L). Suitable controls were also used to show the stability of the BLAD protein in mineral medium over the duration of the study without the presence of the inoculum and to show that the inoculum did not interfere with the analytical technique. The samples were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The equivalent of 25 µg of BLAD from each of the test systems was sampled and the protein precipitated with cold acetone. The pellet was dissolved in buffer and then boiled before being applied to the gels for the SDS-PAGE analysis. After electrophoresis the gels were stained in order to locate the polypeptides in the gels. Quantification was achieved with a densitometer and imaging software in order to integrate the areas of the gels corresponding to the BLAD protein. The results for the 7 and 14 day samples were expressed relative to the equivalent day 0 sample.
The results show that degradation was rapid with very little or no BLAD remaining after 14 days. With the lower concentration of BLAD (100 mg/L) greater than 90% was degraded in 7 days. Degradation may have been a little slower with higher concentration of BLAD (200 mg/L) but complete degradation was achieved after 14 days (>98%) in all test except the test with lower concentration of inoculum 1, where 3% was remaining after 14 days. With an average of less than 90% of the BLAD protein remaining after 7 days it is clear that BLAD would be rapidly degraded in the environment and suggests a likely DT_{50} < 2 days.

In the second biodegradability test using OECD 301 D guidelines (Dengler, 2010) Aqueous extract from the germinated seeds of sweet *Lupinus albus* (nomimal concentration 2 mg/L) was tested in a closed bottle study. The degradation of Aqueous extract from the germinated seeds of sweet *Lupinus albus* reached 81.4% within a 14 day window and can therefore be considered as readily biodegradable under the conditions of this closed bottle test. Mean biodegradation of the reference substance, sodium benzoate, was 85.4% on Day 14 and 84.6% at the end of the test. The difference of extremes of replicate values of the removal of the test item at the end of the 14 day window was < 20% (9.09%). These data show that the inoculum was viable and exerting normal degradative activity. The degradation of the toxicity control was >25% (71.6%) after 14 days. Therefore, toxic effects of Aqueous extract from the germinated seeds of sweet *Lupinus albus* can be excluded. Therefore, the test can be considered valid.

11.1.2 BOD_5/COD

Information on oxygen demand is not available.

11.1.3 Hydrolysis

No hydrolysis study is available.

11.1.4 Other convincing scientific evidence

No other scientific evidence is available.

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

No field studies or monitoring data are available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No inherent or enhanced ready biodegradability tests are available.

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

No water, water-sediment and soil degradation data are available.

11.1.4.4 Photochemical degradation

No photochemical degradation data are available.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.3 Environmental fate and other relevant information

No other data available which is relevant for the classification.

11.4 Bioaccumulation

Table 24: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
No data available in the DAR. Please see argumentation below.	Not bioaccumulative	The main component of Aqueous extract from the germinated seeds of sweet Lupinus albus is a protein that will be broken down in the digestive track of animals, entering the amino acid pool and consumed into normal catabolic processes and is unlikely to bioaccumulate.	

11.4.1 Estimated bioaccumulation

Bioaccumulation in fishes and other organisms is not expected. According to the Guidance on the Application of the CLP Criteria Version 5.0 (2017), the bioaccumulation potential should be evaluated for substances with a log $K_{ow} \geq 4$. Sweet lupin seeds extract is a complex mixture and consists of numerous components, none of which are isolated during the preparation of the product. It is therefore not possible to

derive a Log K_{ow} for Aqueous extract from the germinated seeds of sweet Lupinus albus as a whole. Furthermore, estimates for many of these components would be very difficult as they are broad classes of compounds and defining their structure would be problematic. Therefore the assessment on the potential for bioaccumulation is focused on the known constituents only (see confidential annex).

The lead component, BLAD is a naturally occurring seed storage protein in germinated sweet lupines. It is a 210 kDa gylco-oligomer which is mainly composed by a 20 kDa protein of β-conglutin, or characterised as a fragment of the amino acid sequence of β-conglutin, therefore, there is no specific molecular or structural formula. As such a Log K_{ow} cannot be estimated using QSAR modelling. A theoretical Log K_{ow} value was calculated (confidential part CLH report) indicating a value of 4.5 for the lead component (BLAD). Similarly for a component that constitutes 0.04% of Aqueous extract from the germinated seeds of sweet *Lupinus albus* a Log $K_{ow} > 4$ was reported. For other two components, theoretical Log K_{ow} values of less than four were calculated (confidential part CLH repport). The main component of Aqueous extract from the germinated seeds of sweet *Lupinus albus* is a protein that will be broken down in the digestive track of animals, entering the amino acid pool and consumed into normal catabolic processes and is unlikely to bioaccumulate. The other constituent present in the product with a Log $K_{ow} > 4$ has a weight concentration of 0.04%. As this percentage is $\leq$0.1%, it is considered as a marginal quantity for the product to have a potential to bioaccumulate.

11.5 Acute aquatic hazard

Table 25: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Reference
OECD 203	*Oncorhynchus mykiss*	Aqueous extract from the germinated seeds of sweet *Lupinus albus*, active substance content 20.0% w/w, Batch no. 201009	[1] No mortality was observed in any of the test groups, and so the LC50 (96 h) was determined to be >50 mg/L (nominal)	Anonymous (2011) Assessment of Toxic Effects of PROBLAD on Rainbow Trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae).Unpublished report No.: S10-02621
OECD 202	*Daphnia magna*	Aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD content 20.0% w/w, Batch no. 201009	[1] Based on nominal concentrations, the 48 hour EC50 of Aqueous extract from the germinated seeds of sweet *Lupinus albus* to *Daphnia magna* was determined to be 79.66 mg/L.	K. Weber (2011) Assessment of Toxic Effects of PROBLAD on Daphnia magna using the 48 h Acute Immobilisation Test. Unpublished report No.: S10-02622
OECD 202 (2006)	*Daphnia magna*	Aqueous extract from the germinated seeds of sweet *Lupinus albus*, Active substance content 100% w/w, Batch no. F0-1218	EC_{50} 48 hr > 75 mg/L (geomean measured concentrations)	Gerke, A.K. and Schneider, S.Z. (2019) PROBLAD PLUS: A 48-hour static-renewal acute toxicity test with the cladoceran (*Daphnia magna*). Unpublised report No.: 896A-101
OECD 201	*Desmodesmus subspicatus*	Aqueous extract from the	[1] The 72 hour EyC50 (yield) was	S. Falk (2011) PROBLAD: Testing of Effects of the Single Cell Green Alga

		germinated seeds of sweet *Lupinus albus* , BLAD content 20.0% w/w, Batch no. 201009	determined to be 14.35 mg /L, this is a nominal value corrected for degradation. The 72 hour EC50 value for growth rate could not be calculated due to statistical reasons.	Desmodesmus subspicatus in a 72 h Static Test. Unpublished report No.: S10-02623
OECD 201	*Raphidocelis subcapitata*	Aqueous extract from the germinated seeds of sweet *Lupinus albus* (100% w/w), 21.0% w/w BLAD protein Batch no. F0-1218	72 hr E_rC_{50} = 51 mg/L. 72 hr E_yC_{50} = 12 mg/L The endpoints are derived from measured concentrations (geomean)	Arnie, J.R. et al. (2019). PROBLAD PLUS: A 72-hour toxicity test with the freshwater alga (*Raphidocelis subcapitata*). Unpublished report No. 896P-101

[1] analysis of the test concentrations in water were not provided and analytical measurements are required according to OECD guidelines.Taking into account the limitations to perform analytical methods of the test substance during the experiment on aquatic organisms, the Dossier Submitter (DS) agrees with the approach of the notifier regarding corrected endpoints considering possible degradation over the course of the tests.

11.5.1.1 Consideration of possible degradation over the course of the fish, *Daphnia* and algal tests

Regarding the studies on the effect of Aqueous extract from the germinated seeds of sweet *Lupinus albus* on *O. mykiss* (Anonymous 2011), *D. magna* (Weber 2011) and *D. subspicatus* (Falk 2011). Although analytical verification of the test solutions was not conducted as part of the aquatic studies named above, it is considered that the results are relevant and reliable as they followed the corresponding protocols as indicated in the guidelines. Additionally, the laboratories in which they were conducted count with GLP certification. However, due to the lack of analytical verification throughout the tests it is not known exactly what the measured concentrations were at the end of the 72-hour test period for the algal study or at the end of the 24-hour renewal periods for the fish and *Daphnia* studies. To address this uncertainty, results of the available ready biodegradation study were used (Brunswik-Titze, 2015. Laboratory report number 1035). In this study, the degradation of the test material, BLAD (marker component of Aqueous extract from the germinated seeds of sweet *Lupinus albus*), under aqueous conditions was determined. By the end of the test on Day 28 the total extent of degradation of the test material was 91.2%. The mean degradation on Day 7 of the test was >60% therefore the criterion to be classed as readily biodegradable (60% degradation within ten days) was met. The table below summarises the biodegradation results achieved over the course of the study.

Table CA 9.2-2
Biodegradation (% of theoretical CO_2) of Aqueous extract from the germinated seeds of sweet *Lupinus albus* achieved during the study

Test vessel (reactor number)	Day							
	0	4	7	11	14	21	28	28 with IC

								reactor
15	0	47.9	61.3	73.9	70.6	87.4	93.7	96.3
16	0	48.8	66.1	75.3	71.4	86.0	97.7	94.1
17	0	50.8	66.8	69.9	75.6	83.3	88.0	83.1
Mean	0	49.2	64.7	73.0	72.5	85.6	93.1	91.2

The results of the study demonstrate that at the first post-initiation measurement timepoint on Day 4 the total biodegradation ranged between 47.9 - 50.8% in the three test replicates with a mean value of 49.2%. Thus, it can be concluded that the degradation of BLAD, under these conditions, was approximately 50% after a period of 4 days. From this, it could be estimated that the degradation of BLAD, the lead component in Aqueous extract from the germinated seeds of sweet *Lupinus albus*, in the aquatic studies is also likely to be approximately 50% after a 4-day period. This assumption of 50% degradation over 4 days could therefore be used to predict the stability of the test material over the course of each test. Application of the 50% degradation value to the results of each test could allow for an estimation of the likely measured concentrations in the test media after a 4-day period. It should be noted that this is a very conservative assumption because in the fish and Daphnia studies the test media was renewed every 24 hours therefore it is a highly conservative approach to assume that all of this 50% degradation would have occurred in the first 24 hours when, in all likelihood, the actual degradation over 24-hours would have been less. Likewise, in the algal study, the test was only 72-hours in duration and therefore this test period is also covered by the 50% degradation value over 4 days.

For simplicity it is proposed to adjust the current endpoints from the three aquatic studies by a factor of 2 to account for an approximate 50% degradation over time. Again, this is very conservative as it does not account for the fact that nominal concentrations were likely to have been achieved at the start of each test or renewal period for each study, thereby leading to mean concentrations much greater than 50% of the nominal value. This means that the reported endpoints (effect concentrations) as obtained from the aquatic studies are *lower* than the actual a.s. concentrations in the study set-up. Thus, any uncertainty in extrapolating the measured result from the biodegradation study to the fish, Daphnia and algal studies is considered to be covered by this correction factor of 2.

The following table summarises the current aquatic toxicity endpoints for Aqueous extract from the germinated seeds of sweet *Lupinus albus* as well as the corrected endpoints for Anonymous (2011), Weber 2011) and Falk (2011).

Table CA 9.2-3
Summary of existing endpoints for aquatic organisms

Test substance	Organism	Endpoint	Value (mg/L)	Corrected value[b] (mg/L)	Reference
Aqueous extract from the germinated seeds of sweet *Lupinus albus*	Rainbow trout (*Oncorhynchus mykiss)*	96 hr LC_{50}	>100[a] mg/L	>50[a] mg/L	Anonymous, (2011)
	Daphnia magna	48 hr EC_{50}	159.32[a] mg/L	79.66[a] mg/L	Weber, K (2011)
	Daphnia magna	48 hr EC_{50}	> 75[d] mg/L	NA	Gerke, A.K. (2019)
	Daphnia magna	21 days EC_{10}	>2.7[d] mg/L	NA	Gerke, A.K. and Schneider, S.Z. (2019)

Test substance	Organism	Endpoint	Value (mg/L)	Corrected value[b] (mg/L)	Reference
	Algae *(Desmodesmus subspicatus)*	72 hr E_rC_{50}	N/D[c]	N/D[c]	Falk, S (2011)
		72 hr E_yC_{50}	28.7 mg/L	14.35 mg/L	
	Algae (*Raphidocelis subcapitata*)	72 hr E_rC_{50}	51[d] mg/L	NA	Arnie, J.R. et al. (2019).
		72 hr E_yC_{50}	12[d] mg/L	NA	

[a] Based on nominal concentrations

[b] Toxicity endpoint corrected by a factor of 2 to account for possible degradation

[c] An E_rC_{50} (growth rate) could not be determined

[d] Additional studies on aquatic organisms in which the concentrations of the active substance were traced during the experiments.

11.5.2 Acute (short-term) toxicity to fish

Anonymous (2011) Assessment of Toxic Effects of PROBLAD on Rainbow Trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae), Unpublished report No.: S10-02621

Guidelines

OECD 203 (1992)

GLP

Yes (certified laboratory)

Executive Summary

The 96-hour acute toxicity of Aqueous extract from the germinated seeds of sweet *Lupinus albus* to *Oncorhynchus mykiss* was determined in a semi-static system, exposed to nominal test concentrations between 0.1 and 100 mg Aqueous extract from the germinated seeds of sweet *Lupinus albus* /L. Based on nominal concentrations the 96 hour LC_{50} was >100 mg/L and the NOEC was >100 mg Aqueous extract from the germinated seeds of sweet *Lupinus albus* /L. No lethal or sublethal effects were observed in any test item concentration.

Materials and Methods

Test Material

Aqueous extract from the germinated seeds of sweet *Lupinus albus*, active substance content 20.0% w/w, Batch no. 201009

Test Design

Fish were exposed over a period of 96 hours to Aqueous extract from the germinated seeds of sweet *Lupinus albus* at nominal concentrations corresponding to 0.01, 0.1, 1, 10 and 100 mg Aqueous extract from the germinated seeds of sweet *Lupinus albus* /L. One test vessel per treatment level and for the control was prepared. The test was initiated when 7 rainbow trout were impartially selected and distributed to each test vessel. All test vessels were examined at 0, 3, 6, 24, 48, 72 and 96 hours of exposure and mortality was recorded. Effects for this study were based on mortality. No analysis of the test media was conducted and results are based on nominal concentrations.

The fish used in this study were obtained from Fischzucht Wagenhausen Germany. The body length of the fish was 4- 6 cm. Fish were held in the laboratory under conditions comparable to those of the test for >12 days. Feeding stopped during the 24-hour period prior to test initiation and during exposure period. The water temperature was 15– 17 °C. Dissolved oxygen was > 60% throughout the study and the pH of the test media was 6.0 to 8.5.

Since no mortality was observed up to the highest test item concentration of 100 mg Aqueous extract from the germinated seeds of sweet *Lupinus albus* /L, the evaluation of the data did not require statistical analysis.

Results and Discussion

Analysis of the test media was not conducted and effects were based on nominal concentrations. No mortality was observed in any of the test groups, and so the LC_{50} (96 h) was determined to be >100 mg/L

Conclusion

The 96-hour LC_{50} of Aqueous extract from the germinated seeds of sweet *Lupinus albus* to *Oncorhynchus mykiss* in a semi- static system, based on nominal concentrations was >100 mg Aqueous extract from the germinated seeds of sweet *Lupinus albus* /L and the NOEC was >100 mg Aqueous extract from the germinated seeds of sweet *Lupinus albus* /L. No lethal or sub-lethal effects were observed at any test item concentration.

Based on the study results and the test conditions, the validity criteria of OECD 203 were fulfilled. No effects on fish were observed during the duration of this test. The report does not contain any method of analysis which is required in order to confirm the exposure of the organisms to the test substance. However, taking into consideration the wide availability of reports in the scientific literature on effects of sweet lupin on fishes and that vertebrate experiments must be avoided, corrected endpoints were used taking into account possible degradation over the course of the aquatic organisms tests. The corrected endpoint is NOEC >50 mg/L.

11.5.3 Acute (short-term) toxicity to aquatic invertebrates

K. Weber (2011) Assessment of Toxic Effects of PROBLAD on *Daphnia magna* using the 48 h Acute Immobilisation Test. Unpublished report No.: S10-02622

Guidelines

OECD 202 (2004)

GLP

Yes (certified laboratory)

Executive Summary

The 48-hour acute toxicity of Aqueous extract from the germinated seeds of sweet *Lupinus albus* (content of BLAD: 20.0%) to *Daphnia magna* was determined in a semi static system, with groups of 20 daphnids per treatment, spanning nominal concentrations between 7.81 and 250 mg/L The 24- and 48-hour immobilisation EC_{50} was >250 and 159.32 mg/L respectively and the 48-hour NOEC was 31.3 mg/L.

Materials and methods

Test Material

Aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD content 20.0% w/w, Batch no. 201009

Test Design

Daphnids were exposed over a period of 48 hours to Aqueous extract from the germinated seeds of sweet *Lupinus albus* at nominal concentrations corresponding to 0 (dilution water control), 7.81, 15.6, 31.3, 62.5, 125 and 250 mg/L. Two concentrations of the reference item, potassium dichromate (1.0 mg/L, 2.0 mg/L) were also tested. Four tests vessels per treatment level and for the control were prepared. Daphnids < 24 hours old were selected and distributed until each vessel contained 20 daphnids. The test was initiated when 5 daphnids were introduced to each replicate exposure vessel. The daphnids were not fed during the 48 hour exposure period. The number of immobilized daphnids observed in each replicate test vessel was recorded at test initiation and after 24 and 48 hours of exposure.

Measurements of temperature, pH and dissolved oxygen concentrations were made at 0, 24 and 48 hours in one replicate of each treatment level and the control. The water temperature was 20.7°C. Dissolved oxygen was above 60% saturation and the total hardness was between 140 – 268 mg/L (as CaCO3). The pH of the test media was 8.16.

Results and Discussion

Details are provided in Table 8.2.4.1. Following 24 hours of exposure, no immobilisation was observed in the control and up to 62.5 mg/L. At 125 mg/L 10% immobilisation was observed. At the highest test item concentration of 250 mg/L 15% immobilisation was observed. After 48 hours no immobilisation was observed in the control and up to 15.6 mg/L. At 31.3 mg/L one immobile daphnid was observed, 15% immobilisation was observed at 62.5 mg/L and 20% immobilisation at 125 mg/L. At the highest test item concentration of 250 mg/L 75% of the daphnids were immobile.

The oxygen concentration at the highest test item concentration of 250 mg/L decreased at t= 24 hours aged to 27% and at t=48 hours aged to 29%. This deviation from the Study Plan was due to the influence of the test item and it is considered to have no impact on the study since the control is valid. The oxygen content in the control test vessels and each of the test item vessels with the exception of the highest test concentration of 250 mg/L remained within the required criteria of > 3 mg/L (approximately 33 %) for the duration of the study. The control group consistently remained above 95 % oxygen saturation and so indicated the test design was valid. For the highest concentration of 250 mg/L in the 24 h and 48 h aged samples only the oxygen saturation dropped marginally below the required criteria of 3 mg/L or equivalent of 33 % dissolved oxygen (27 and 29 % respectively). The fresh media at the renewals were shown to have high oxygen saturation of 98 and 91 % and the mean saturation was 61 % (approximately 6 mg/L) which exceeds the required oxygen concentration. The drop in oxygen in the one test item group is not considered to have affected the study.

Table 8.2.4.1/01-1 Corresponding immobilization and observations made during the 48-hour exposure of daphnids (*D. magna*) to Aqueous extract from the germinated seeds of sweet *Lupinus albus*

Nominal Concentration (mg Aqueous extract from the germinated seeds of sweet *Lupinus albus* /L)	Number of Immobilized Organisms									
	24-Hour					48-Hour				
	1	2	3	4	Total	1	2	3	4	Total
Control	0	0	0	0	0	0	0	0	0	0
7.81	0	0	0	0	0	0	0	0	0	0
15.6	0	0	0	0	0	0	0	0	0	0
31.3	0	0	0	0	0	0	0	0	1	1
62.5	0	0	0	0	0	1	0	1	1	3
125	1	1	0	0	2	1	3	0	0	4
250	1	1	0	1	3	5	5	0	5	15

Conclusion

Based on nominal concentrations, the 48-hour EC_{50} of Aqueous extract from the germinated seeds of sweet *Lupinus albus* to *Daphnia magna* was determined to be 159.32 mg/L. The 48-hour NOEC was determined to be 31.3 mg/L (nominal).

The product seems to have an influence on the oxygen concentration at 250 mg/L. Nevertheless, the testing should have been performed up to a concentration of 100 mg/L as indicated in the EU No. 283/2013. Furtheremore, it is agreed that the drop in dissolved oxygen is not significant and it might not have affected the results of the study. However, an analysis of the test concentrations in water is not provided and analytical measurements are required according to OECD 202. Taking into account the limitations to perform analytical methods of the test substance during the experiments on aquatic organisms, corrected endpoints considering possible degradation over the course of the tests were used. The corrected endpoint is EC_{50} 79.66 mg/L.

In 2019, the applicant provided two additional studies on the effect of Aqueous extract from the germinated seeds of sweet *Lupinus albus* on aquatic invertebrates (48 h and 21 days) and one on algae (72 h).

Gerke, A.K. and Schneider, S.Z. (2019) PROBLAD PLUS: A 48-hour static-renewal acute toxicity test with the cladoceran (*Daphnia magna*). Unpublised report No.: 896A-101

Guidelines

OECD 202 (2004)

GLP

Yes (certified laboratory)

Executive Summary

This study was conducted to determine the effects of Aqueous extract from the germinated seeds of sweet *Lupinus albus* exposure on the survival, growth, and reproduction of the cladoceran *Daphnia magna* during a 48-hour static-renewal exposure.

Materials and methods

Test Material

Aqueous extract from the germinated seeds of sweet *Lupinus albus*, Active substance content 100% w/w, Batch no. F0-1218

Test Design

Twenty daphnids in four replicate groups of five were exposed to each of the measured test concentrations, 2.2, 5.0, 12, 31, and 75 mg/L, along with a dilution water control. Observations of immobility and other signs of toxicity were made approximately 3, 24 and 48 hours after test initiation. Daphnids were exposed to a geometric series of five test concentrations and a negative control (dilution water) for 48 hours under static-renewal conditions. The daphnids were transferred to newly-prepared test solutions at approximately 24 hours. Four replicate test chambers were maintained in each treatment and control group, with five daphnids in each test chamber, for a total of 20 daphnids per concentration. Nominal test concentrations selected were 7.5, 15, 30, 60 and 120 mg test item/L. Test concentrations were measured in samples of test water collected from each treatment and control group at the beginning of the test, before and after renewal at approximately 24 hours, and at the end of the test. Measured concentrations of the samples collected from the newly prepared test solutions at 0 and 24 hours ranged from 17.0 to 67.9% of nominal. Measured concentrations of the samples collected from the 24-hour old test solutions at 24 and 48 hours ranged from 20.1 to 86.5% of nominal. When measured concentrations of the samples collected during the test were averaged, the mean measured test concentrations for this study were 2.2, 5.0, 12, 31 and 75 mg/L, representing 29, 33, 39, 52 and 63% of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations.

Analytical method

Triplicate water samples were collected from each treatment and control group at the beginning and end of each renewal period during the test to determine concentrations of the test substance. Newly prepared batch solutions were sampled on Day 0 and at 24 hours (±1 hour), and the 24-hour old solutions in two of the four replicate test chambers in each group were sampled at 24 and 48 hours (±1 hour). Samples (20 mL) were collected from mid-depth and placed in polypropylene centrifuge tubes. Two sets of samples collected at each sampling interval were stored frozen (i.e., ≤ -18°C) until shipment for analysis. The other set of samples was stored frozen as back-up samples for possible analysis. Samples were analysed by Enzyme-Linked Immunosorbent Assay (ELISA).

Results and discussion

Analytical results

Measured concentrations of the samples collected from the newly prepared test solutions at 0 and 24 hours ranged from 17.0 to 67.9% of nominal. Measured concentrations of the samples collected from the 24-hour old test solutions at 24 and 48 hours ranged from 24-hour old test solutions at 24 and 48 hours ranged from 20.1 to 86.5% of nominal. When measured concentrations of the samples collected during the test were averaged, the mean measured test concentrations for this study were 2.2, 5.0, 12, 31 and 75 mg/L, representing 29, 33, 39, 52 and 63% of nominal concentrations respectively. The results of the study were based on the mean measured concentrations.

Biological results

At test termination, survival in the negative control and each of the 2.2, 5.0, 12, 31, and 75 mg/L treatment groups was 100%. No statistical analyses were conducted on account of the lack of effect.

The resulting NOEC and LOEC for survival were determined to be 75 and >75 mg/L, respectively. EC_{10}, EC_{20}, and EC_{50} values were empirically estimated to be greater than the highest concentration tested (>75 mg/L).

Table CA 8.2.4.1/02-1
Cumulative immobility of *Daphnia magna* exposed to Aqueous extract from the germinated seeds of sweet *Lupinus albus* **for 48 hours**

Mean measured concentration (mg/L)	Number exposed	Cumulative immobility (%)		
		3 hours	24 hours	48 hours

Negative control	20	0.0	0.0	0.0
2.2	20	0.0	0.0	0.0
5.0	20	0.0	0.0	0.0
12	20	0.0	0.0	0.0
31	20	0.0	0.0	0.0
75	20	0.0	0.0	0.0

Conclusion

Validity criteria according to OECD 202 were met: mortality of daphnids in the control group ≤10% (actual: 0%); dissolved oxygen concentration at the end of the test ≥3 mg/L (actual ≥5.1 mg/L). There were no treatment-related effects on survival concentrations ≤75 mg/L. Consequently, the overall NOEC and LOEC for the study were determined to be 75 and >75 mg/L, respectively. The 48-hour EC_{10}, EC_{20}, and EC_{50} values based on immobility were >75 mg/L. All endpoints are based on mean measured concentrations.

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

S. Falk (2011) PROBLAD: Testing of Effects of the Single Cell Green Alga *Desmodesmus subspicatus* in a 72 h Static Test. Unpublished report No.: S10-02623

Guidelines

OECD 201 (2006)

GLP

Yes (certified laboratory)

Executive Summary

The 72-hour effects of Aqueous extract from the germinated seeds of sweet *Lupinus albus* on biomass and growth rate of *Desmodesmus subspicatus* were determined in the laboratory. Based on nominal concentrations the 72 h E_yC_{50} for yield was 28.7 mg /L and NOEC 11.1 mg/L. Due to statistical reasons it was not possible to calculate the E_rC_{50} for growth rate.

Materials and methods

Test Material

Aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD content 20.0% w/w, Batch no. 201009

Test Design

Algae were exposed over a period of 72 hours to Aqueous extract from the germinated seeds of sweet *Lupinus albus* at nominal concentrations corresponding to 0 (dilution water control), 0.0137, 0.412, 1.23, 3.70, 11.1, 33.3 and 100 mg/L. The culture medium used was a synthetic algal assay growth medium prepared by adding appropriate amounts of nutrient stock solutions to sterile, deionised water. The algal medium used to prepare the exposure solutions was the same as the culture medium. The medium were prepared using deionised water and were equilibrated to test temperature. There were three replicate vessels per treatment and six replicate vessels for the control. The flasks were inoculated with volumes providing the required cell density of 0.5×10^4 cells/mL. Cell numbers were counted using a Neubauer chamber after preparation of a dilution series. Observations of the health of the algal cells were also made at each 24-hour interval.

Analysis of the test substance was not performed, and results were based on nominal concentrations. Measurements of pH were performed at t =0 and t = 3 days and the temperature was measured at day 0, 1, 2 and 3. The pH of the test was 6.71 to 8.91. The temperature was 22.5-24.0 °C throughout the entire study.

The statistical analysis for day 3 was performed for cell number, yield and growth rate using SAS (2002-2008). The data set was transformed via Boxcox transformation and NOEC and LOEC were determined by using the Dunnetts-t-test. A test for normality of the data was performed using Shapiro-Wilks' Test and for homogeneity of variance using the Levene-Test. The E_yC_{50} (for yield) values were determined using Moving average analysis. Due to statistical reasons, values of the concentrations 0.137, 0.412, 1.23 and 3.7 were not taken into account. The EC_{50} of growth rate was not calculable due to statistical reasons.

Results and Discussion

Analytical results

Analysis was not conducted and results were calculated based on the nominal concentrations of 0.137, 0.412, 1.23, 3.70 and 11.1, 33.3 and 100 mg/L.

Biological Results

Details are provided in Table 8.2.6.1/01-1 below.

Table 8.2.6.1/01-1 Cell density of *Desmodesmus subspicatus* after 24, 48 and 72 hours of exposure to Aqueous extract from the germinated seeds of sweet *Lupinus albus*

Nominal concentration (mg/L)	Average cell numbers/mL[a]					
	0 hours	24 hours	48 hours	72 hours	72 hours Inhib. of growth rate (%)	72 hours Inhib. of yield(%)
Control	0.5	1.70	9.98	42.49	0.0	0.0
0.137	0.5	1.43	10.08	45.78	-1.8	-7.8
0.412	0.5	1.48	10.59	49.25	-3.6	-16.1
1.23	0.5	1.29	10.78	47.08	-2.5	-10.9
3.70	0.5	1.55	12.43	55.42	-6.2	-30.8
11.1	0.5	1.42	11.40	55.99	-6.4	-32.8
33.3	0.5	1.54	3.30	4.47	51.4	90.5
100	0.5	2.08	5.21	7.84	40.5	82.5

[a]Algae counts are divided by 10000. At the start, the cell density was adjusted to 0.5 x10^4 cells/mL

Conclusion

The NOEC and LOEC values for growth rate and yield were 11.1 and 33.3 mg /L respectively. The 72 hour E_yC_{50} (yield) was determined to be 28.7 mg /L. The 72 hour EC_{50} value for growth rate could not be calculated due to statistical reasons.

The validity criteria as per OECD 201 were fulfilled although the analysis of the test concentration was not performed which is a requirement of OECD 201. Corrected endpoint values considering degradation of the substance were used due to the limitations to perform analysis of the test substance. The corrected value for this endpoint is 14.35 mg /L.

Arnie, J.R. *et al*. (2019). PROBLAD PLUS: A 72-hour toxicity test with the freshwater alga (*Raphidocelis subcapitata*). Unpublished report No. 896P-101

Guidelines

OECD 201 (2006)

GLP

Yes (certified laboratory)

Executive Summary

This study was conducted in order to assess the toxicity of five concentrations of Aqueous extract from the germinated seeds of sweet *Lupinus albus* to the freshwater alga *Raphidocelis subcapitata* over a 72-hour exposure period. Three replicates were assessed per treatment group, and six for the control.

Effects were evaluated based on cell density, yield, and growth rate using nominal and geometric mean measured concentrations. The 72-hour EC50, EyC50, and ErC50 values were determined to be nominally 27, 27, and 87 mg/L, respectively, corresponding to geometric mean measured concentrations of 12, 12, and 51 mg/L. The 72-hour NOEC was determined to be 15 mg/L (6.6 mg/L based on geometric mean measured concentrations).

Materials and methods

Test Material

Aqueous extract from the germinated seeds of sweet *Lupinus albus*, 100% w/w *Lupinus albus* L. germ. extract, 21.0% w/w BLAD protein Batch no. F0-1218

Test design

This study was conducted in order to assess the toxicity of varying concentrations of Aqueous extract from the germinated seeds of sweet *Lupinus albus* to the freshwater alga *Raphidocelis subcapitata* over a 72-hour exposure period. Test concentrations were selected based on the results of a non-GLP range-finding test.

Algal cells were cultured and tested in freshwater AAP medium. Stock nutrient solutions were prepared by adding reagent-grade chemicals to purified water from an on-site well. The pH of the medium was adjusted to 7.5 with 10% hydrochloric acid. The medium was then sterilised by filtration (0.22 µm) and refrigerated prior to use.

Test vessels were sterile, 250-mL glass Erlenmeyer flasks plugged with sterile foam stoppers, and contained 100 mL test or control medium. These were indiscriminately positioned on a mechanical shaker table in an environmental chamber and shaken continuously at approximately 100 rpm.

A primary stock solution was prepared by mixing 0.1200 g Aqueous extract from the germinated seeds of sweet *Lupinus albus* in 1000 mL AAP medium, to achieve a nominal concentration of 120 mg test item/L. Additional test solutions were prepared at nominal concentrations of 7.5, 15, 30, and 60 mg/L by dilution of aliquots of the 120 mg/L stock solution with AAP medium. Samples of the test solutions were collected to measure concentrations of the test substance at test initiation and at test termination after 72 hours. Geometric mean measured concentrations were 3.3, 6.6, 14, 31, and 76 mg/L, respectively, corresponding to 42.9, 44.0, 46.7, 51.7, and 63.3% of nominal.

Prior to test initiation, the concentrations of algal cells in the stock culture was determined using a haemocytometer to be 4.19 $x10^6$ cells/mL. To each replicate test chamber was added 0.239 mL stock culture in order to achieve the desired initial cell density of 10,000 cells/mL.

Samples were taken at approximately 24-hour intervals during the 72-hour exposure period, and were held in the dark for a maximum of three days under refrigerated conditions to inhibit growth until cell counts could be performed. At the end of the exposure period, samples of the test solution were pooled by treatment or control group and examined microscopically for atypical cell morphology. Cells were also assessed for aggregation or flocculation, and adherence to the test chamber.

Statistical analyses were conducted using SAS 9.4. The NOEC values were determined based on the results of statistical analyses and evaluation of the dose-response. ECx values and corresponding 95% confidence intervals were determined using non-linear regression with replicate data and exposure concentration data.

Analytical method

Samples of the Control and treated media were analysed using the validated analytical method.

Results amd Discussion

Analytical results

The measured concentrations of the test item determined during the test are summarised in the table below:

Table CA 8.2.6.1/02-1

Measured concentrations of Aqueous extract from the germinated seeds of sweet *Lupinus albus* **during the test**

Nominal concentration (mg/L)	Geometric mean measured concentration (mg/L)	Percent of nominal
Negative control	<LOQ	-
7.5	3.3	42.9
15	6.6	44.0
30	14	46.7

60	31	51.7
120	76	63.3

LOQ 0.03 mg Aqueous extract from the germinated seeds of sweet *Lupinus albus* /L

Biological Results

Details are provided in Table 8.2.6.1/02-4 below.

Table CA 8.2.6.1/02-4

Summary of endpoints after 72-hour exposure to Aqueous extract from the germinated seeds of sweet *Lupinus albus*

Endpoint	Cell density		Growth rate		Yield	
	Nom.[1] (mg/L)	Geo. mm[2] (mg/L)	Nom.[1] (mg/L)	Geo. mm[2] (mg/L)	Nom.[1] (mg/L)	Geo. mm[2] (mg/L)
NOEC	15	6.6	15	6.6	15	6.6
EC_{10} (95% CI)	11 (8.0 - 15)	4.2 (<3.3 - 6.2)	18 (14 - 25)	7.5 (5.0 - 11)	11 (8.2 - 15)	4.3 (2.9 - 6.3)
EC_{20} (95% CI)	15 (19 - 19)	6.1 (4.4 - 8.5)	31 (25 - 39)	14 (11 - 19)	15 (12 - 19)	6.2 (4.5 - 8.5)
EC_{50} (95% CI)	27 (23 - 32)	12 (10 - 15)	87 (78 - 97)	51 (44 - 59)	27 (23 - 32)	12 (10 - 15)

[1] Nominal concentration

[2] Geometric mean measured concentration

CI Confidence interval

Conclusion

The study has been conducted in accordance with OECD 201 and follows the recommended test methods and procedures. Validity criteria according to OECD 201 were met: mean cell density in negative control replicated to increase by a factor >16 within three days (actual: 266); the coefficient of variation of the average specific growth rate in the negative control replicated during the test period to not exceed 7% (actual: 1.74%); the mean coefficient of variation for section-by-section specific growth rates in the negative control replicates to not exceed 35% (actual: 16.6%). The 72-hour EC_{50}, E_yC_{50}, and E_rC_{50} values were determined to be nominally 27, 27, and 87 mg/L, respectively, corresponding to geometric mean measured concentrations of 12, 12, and 51 mg/L. the 72-hour NOEC and E_rC_{10} were determined to be 15 mg/L and 18 mg/L respectively (6.6 mg/L and 7.5 mg/L respectively based on geometric mean measured concentrations).

11.6 Long-term aquatic hazard

Table 27: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
OECD 201	*Desmo desmus subspic*	Aqueous extract from the	NOEC: 11.1 mg/L (nominal) **Corrected value:**	The analysis of the substance concentration during the test is a requirement of OECD 201.	S. Falk (2011) PROBLAD: Testing of Effects of the Single

	atus	germinated seeds of sweet *Lupinus albus*, BLAD content 20.0% w/w, Batch no. 201009	**5.5 mg/L**	Corrected values of endpoints are used due to the limitations to perform the required analysis of the test substance. The corrected value for the **NOEC is 5.5 mg/L**	Cell Green Alga *Desmodesmus subspicatus* in a 72 h Static Test. CEV SA, Unpublished report No.: S10-02623
OECD 201	*Raphidocelis subcapitata*	Aqueous extract from the germinated seeds of sweet *Lupinus albus* (100% w/w), 21.0% w/w BLAD protein Batch no. F0-1218	E_rC_{10}: 7.5 mg/L (geomean measured concentration)	Measured concentrations below 80% of nominal. Therefore, the endpoints are based on measured concentrations	Arnie, J.R. et al. (2019). PROBLAD PLUS: A 72-hour toxicity test with the freshwater alga (*Raphidocelis subcapitata*). Unpublished report No. 896P-101
OECD 211	*Daphnia magna*	Aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD content 100% w/w, Batch no. FO-1218	EC_{10}: >2.7 mg/L (geomean measured concentrations)	Measured concentrations below 80% of nominal. Therefore, the endpoints are based on measured concentrations	Gerke & Scheneider (2019). PROBLAD PLUS: A semi-static life-cycle toxicity test with the cladoceran (*Daphnia magna*). Unpublised report No.: 896A-102

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

11.6.2 Chronic toxicity to fish

Information on chronic toxicity to fishes is not available

11.6.3 Chronic toxicity to aquatic invertebrates

Gerke, A.K. and Schneider, S.Z. (2019) PROBLAD PLUS: A semi-static life-cycle toxicity test with the cladoceran (*Daphnia magna*). Unpublised report No.: 896A-102

Guidelines

OECD 211

GLP

Yes (certified laboratory)

Executive Summary

This study was conducted to determine the effects of Aqueous extract from the germinated seeds of sweet *Lupinus albus* exposure on the survival, growth, and reproduction of the cladoceran *Daphnia magna* during a 21-day static-renewal exposure.

Materials and methods

Test Material

Aqueous extract from the germinated seeds of sweet *Lupinus albus*, 100.0% w/w, Batch no. F0-1218

Test Design

Daphnids were impartially assigned to exposure chambers at test initiation. First-generation daphnids were observed daily during the test for immobility, the onset of reproduction, and clinical signs of toxicity. Following the onset of reproduction, the numbers of second-generation daphnids were counted daily until test termination on Day 21. Total body lengths and dry weights of the surviving first-generation daphnids were measured at the end of the exposure period. Observations of the effects of Aqueous extract from the germinated seeds of sweet *Lupinus albus* on survival, reproduction, and growth were used to determine the NOEC and LOEC. $EC_{10,}$ $EC_{20,}$ and EC_{50} values were estimated based on survival and immobility, reproduction, and growth at test termination, where possible.

Analytical method

Freshwater samples of Aqueous extract from the germinated seeds of sweet *Lupinus albus* were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) at Eurofins Agroscience Services Ltd.

Triplicate water samples were collected from each treatment and control group at test initiation, at the beginning and the end of a renewal cycle each week, and at test termination. Samples of “new” solutions were collected from the batch test solutions at test initiation and at the beginning of the renewal cycle. Samples of “old” solutions were collected from two alternating replicates of each treatment and control group at the end of the renewal cycle and at test termination. Samples (20 mL) were collected from mid-depth and placed in polypropylene centrifuge tubes. One set of samples collected at each sampling interval was stored frozen until shipment for analysis. One set of samples was stored frozen until shipment in case re-analysis was needed. The other set was stored frozen as back-up samples for possible analysis.

Results and Discussion

Analytical results

Concentrations of Aqueous extract from the germinated seeds of sweet *Lupinus albus* in the new test solutions prepared and sampled on Days 0,11 and 20 ranged from 5.3 to 48.7% of nominal. Concentrations of Aqueous extract from the germinated seeds of sweet *Lupinus albus* in the 24 hour-old test solutions collected on Days 1, 12 and 21 ranged from <LOQ to 15.0% of nominal. When the measured concentrations of the samples collected during the test were averaged, the mean measured test concentrations were determined to be 0.08, 0.20, 0.38, 1.0 and 2.7 mg/L, representing 10,13,17 and 22% of nominal concentrations respectively. The results of the study were based on the mean measured test concentrations.

Biological Results

Table CA 8.2.5.1/01-1
Summary of survival, reproduction, and growth effects on *Daphnia magna* after 21-day exposure to
Aqueous extract from the germinated seeds of sweet *Lupinus albus*

Mean measured concentration (mg/L)	Adult survival (%)	Production rate of first brood	Mean no. neonates per adult at test initiation ±SD	Mean no. neonates per adult at test termination ±SD	Meal total length ±SD (mm)	Mean dry weight ±SD (mm)
Negative control	95.0	0.1333	244 ± 12.4	244 ± 12.4	4.9 ± 0.077	1.20 ± 0.105
0.08	100	0.1333	238 ± 37.9	238 ± 37.9	5.0 ± 0.15	1.23 ± 0.187
0.20	100	0.1333	265 ± 10.4	265 ± 10.4	5.0 ± 0.074	1.21 ± 0.237
0.38	100	0.1333	262 ± 13.6	262 ± 13.6	5.1 ± 0.11	1.24 ± 0.229
1.0	90.0	0.1333	271 ± 12.6	271 ± 12.6	5.1 ± 0.050	1.36 ± 0.154
2.7	90.0	0.1354	274 ± 29.9	274 ± 29.9	5.1 ± 0.093	1.45 ± 0.0714

SD: Standard Deviation

Table CA 8.2.5.1/01-2
Percent inhibition of endpoints compared to the negative control

Mean measured concentration (mg/L)	Adult survival	Production rate of first brood	Mean no. neonates per adult at test initiation[1]	Mean no. neonates per adult at test termination	Meal total length	Mean dry weight
0.08	-5.3	0.00	2.2	2.2	-0.4	-2.6
0.20	-5.3	0.00	-8.8	-8.8	-1.4	-1.3
0.38	-5.3	0.00	-7.7	-7.7	-3.0	-3.5
1.0	5.3	0.00	-11.3	-11.3	-3.9	-13.4
2.7	5.3	-1.6	-12.7	-12.7	-3.0	-21.5

Negative values indicate percentage increase.
[1] Excluding live neonates produced by first generation daphnids which accidentally and/or inadvertently died during the test.

Conclusion

The resulting NOEC and LOEC for growth were determined to be 2.7 and >2.7 mg/L, respectively. EC_{10}, EC_{20}, and EC_{50} values were empirically estimated to be greater than the highest concentration tested (>2.7 mg/L).

11.6.4 Chronic toxicity to algae or other aquatic plants

Reference: S. Falk (2011) PROBLAD: Testing of Effects of the Single Cell Green Alga Desmodesmus subspicatus in a 72 h Static Test. CEV SA, Unpublished report No.: S10-02623

Guideline: OECD 201

Species tested: *Desmodesmus subspicatus*

Test material: Aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD content 20.0% w/w, Batch no. 201009

Test Design: for details see Section 11.4.4

Results: The analysis of the substance concentration during the test is a requirement of OECD 201. Corrected values of endpoints are used due to the limitations to perform the required analysis of the test substance. The corrected value for the **NOEC is 5.5 mg/L.**

Reference: Arnie, J.R. *et al*. (2019). PROBLAD PLUS: A 72-hour toxicity test with the freshwater alga (*Raphidocelis subcapitata*). Unpublished report No. 896P-101

Guideline: OECD 201

Species tested: *Raphidocelis subcapitata*

Test material: Aqueous extract from the germinated seeds of sweet *Lupinus albus*, 100% w/w Lupinus albus L. germ. extract, 21.0% w/w BLAD protein Batch no. F0-1218

Test Design: for details see Section 11.4.4

Results: The 72-hour EC_{50}, E_yC_{50}, and E_rC_{50} values were determined to be nominally 27, 27, and 87 mg/L, respectively, corresponding to geometric mean measured concentrations of 12, 12, and 51 mg/L. the 72-hour NOEC and E_rC_{10} were determined to be 15 mg/L and 18 mg/L respectively (6.6 mg/L and 7.5 mg/L respectively based on geometric mean measured concentrations).

11.6.5 Chronic toxicity to other aquatic organisms

Further information not available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

According to CLP criteria: *"Acute aquatic toxicity is normally determined using a fish 96 hour LC50, a crustacea species 48 hour EC50 and/or an algal species 72 or 96 hour EC50. These species cover a range of trophic levels and taxa and are considered as surrogate for all aquatic organisms. Data on other species (e.g. Lemna spp.) shall also be considered if the test methodology is suitable. The aquatic plant growth inhibition tests are normally considered as chronic tests but the EC50s are treated as acute values for classification purposes".*

Information on acute toxicity for Aqueous extract from the germinated seeds of sweet *Lupinus albus*: Lowest acute endpoint for aquatic organisms: 51 mg/L (algae: E_rC_{50}). The cut-off value to be considered as an aquatic hazard according to CLP criteria is 1 mg/L. Therefore classification of Aqueous extract from the germinated seeds of sweet *Lupinus albus* as Aquatic Acute 1 is not required.

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

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is rapidly degradable and bioaccumulation is not expected as the main component of Aqueous extract from the germinated seeds of sweet *Lupinus albus* is a protein that will be broken down in the digestive track of animals, entering the amino acid pool and consumed into normal catabolic processes, therefore it is considered unlikely to bioaccumulate. Furthermore, the other components identified have an estimated log Kow lower than 4 or are present in concentrations lower than 0.1%. Therefore Aqueous extract from the germinated seeds of sweet *Lupinus albus* is considered to have a low potential to bioaccumulate.

Experimental chronic toxicity endpoints are only available for algae and crustacean. The lowest chronic endpoint is an EC_{10} of >2.7 mg/L for growth rate of *D. magna*. This value is higher than 1 mg/L (Table 4.1.0 (b) (ii) of the CLP guidance) therefore Sweet lupin seed extract should not be classified as aquatic chronic on the basis of the available data for chronic aquatic toxicity. Classification on the basis of the acute data for fish as surrogate is not required because the substance is considered to be rapidly biodegradable and to have a low potential to bioaccumulate. For this reason also classification as Aquatic chronic 4 is not required.

CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

A classification for aquatic acute or chronic toxicity is not required.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Table 28: Summary table of data concerning hazardous properties of the substance for the ozone layer

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		No data concerning hazardous propertiese of the substance for the ozon layer were submitted.		

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

Since the fact that the substance Aqueous extract from the germinated seeds of sweet *Lupinus albus* is an plant extract of the germinated seeds of sweet lupin and therefore a botanical it might be expected the substance has no hazardous effects on the ozone layer. Therefore, no data concerning hazardous propertiese of the substance for the ozone layer were submitted and required.

12.1.2 Comparison with the CLP criteria

No comparison possible since no data is available.

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

Since there is no data on potential effects of Aqueous extract from the germinated seeds of sweet *Lupinus albus* to the ozone layera comparisonto the CLP criteria is not possible.

13 ADDITIONAL LABELLING

None.

14 REFERENCES

A reference list for the studies from the DAR is included below:

Physical and chemical properties

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 2.1/01	Wo, C.	2012a	PROBLAD PLUS: Physical and Chemical Characteristics: Boiling Point Company Report No. 34852 Eurofins PSL, USA GLP, Unpublished
CA 2.3/01, CA 2.10/01, CA 2.13/01	Wo, C.	2012b	PROBLAD PLUS Physical and Chemical Characteristics: Color, Physical State, Odor, Oxidation/Reduction, Flammability, pH, Viscosity, and Density/Relative Density (amended) Company Report No. 32388 Eurofins PSL, USA GLP, Unpublished
CA 2.11/01, CA 2.13/02	Cage, S.	2013	PROBLAD PLUS: Explosive properties and oxidising properties Company Report No. MIB0036 Huntingdon Life Sciences, Eye, UK GLP, Unpublished Study submitted to meet data requirements
CA 2.12/01	Lien, T.P.	2013	Surface tension of PROBLAD PLUS Company Report No. S13-00831 Eurofins, Germany GLP, Unpublished

Toxicology and metabolism

Data point	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 5.2.1/01	2012a	PROBLAD PLUS: Acute oral toxicity up and down procedure in rats (amended report) Company Report No. 31002 GLP, Unpublished
CA 5.2.2/01	2012b	PROBLAD PLUS: Acute dermal toxicity study in rats – limit test (amended report) Company Report No. 31003 GLP, Unpublished
CA 5.2.3/01	2012c	PROBLAD PLUS: Acute inhalation toxicity study in rats – limit test (amended report) Company Report No. 30998 GLP, Unpublished

CA 5.2.4/01	2012d	PROBLAD PLUS: Primary skin irritation study in rabbits (amended report) Company Report No. 31000 GLP, Unpublished
CA 5.2.5/01	2012e	PROBLAD PLUS: Primary eye irritation study in rabbits (amended report) Company Report No. 30999 GLP, Unpublished
CA 5.2.6/01	2012f	PROBLAD PLUS: Dermal sensitisation study in guinea pigs (Buehler method) (amended report) Company Report No. 31004 GLP, Unpublished
R. Boavida Ferreira	2011	Potential allergenicity of lupine seeds (Lupinus sp.) with special emphasis on BLAD, an intermediate in the breakdown process of the major storage protein during germination of lupine seeds. CEV SA, Unpublished report No.: CEV110820
CA 5.3.2/01	2015	PROBLAD PLUS: 13 week oral (gavage) administration toxicity study in the rat Company Report No. 8325453 GLP, Unpublished
CA 5.3.3/01	2015	PROBLAD PLUS. 21 day dermal administration toxicity study in the rat (OECD 410) Company Report No. 8297704 GLP, Unpublished
CA 5.4.1.1/01	2016	PROBLAD PLUS: Bacterial reverse mutation assay using a treat and plate modification Company Report No. 8325399 GLP, Unpublished
CA 5.4.1.2/01	2015a	PROBLAD PLUS: *In vitro* L5178Y gene mutation assay at the *tk* locus Company Report No. 8325403 GLP, Unpublished
CA 5.4.1.3/01	2015	PROBLAD PLUS: *In vitro* human lymphocyte micronucleus assay Company Report No. 8325400 GLP, Unpublished
CA 5.4.2.1/01	2015b	PROBLAD PLUS: Rat alkaline comet assay Company Report No. 8325402 GLP, Unpublished

Environmental fate and behaviour

Data point	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 7.1.1.1/01 CA 7.1.2.1.1/01 CA 7.2.2.1/01	2015	Biodegradability, CO_2-evolution test according to OECD 301 B (July 1992) Hydrotox .- Labor für, Ökotoxikologie und, Gewässerschutz GmbH Report No. 1035 GLP, Unpublished
CA 7.1.1.1/02 CA 7.1.2.1.1/02 CA 7.2.2.1/02	2014	Assessment of the ready biodegradability of BLAD with the closed bottle test and SDS-PAGE CEV S.A Report No. CEV-ABB-0914 Not GLP, Unpublished
CA 7.1.1.1/03 CA 7.1.2.1.1/03 CA 7.2.2.1/03	2010	PROBLAD PLUS: Assessment of ready biodegradability with the closed bottle test. Company report no. S10-02624 Eurofins Agroscience Services GmbH GLP, unpublished

Ecotoxicology

Data point	Author(s)	Year	Title
CA 8.2.1/01	Anonymous	2011	Assessment of Toxic Effects of PROBLAD on Rainbow Trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae) Company Report No. S10-02621 GLP, Unpublished
CA 8.2.4.1/01	Weber, K.	2011	Assessment of Toxic Effects of PROBLAD on *Daphnia magna* using the 48 h Acute Immobilisation Test Company Report No. S10-02622 Eurofins Agroscience Services GmBH GLP, Unpublished
CA 8.2.4.1/02	Gerke, A.K. and Schneider	2019	PROBLAD PLUS: A 48-hour static-renewal acute toxicity test with the cladoceran (*Daphnia magna*). Company report No. 896A-101 Eurofins EAG Agroscience, USA GLP. Unpublised
CA 8.2.5.1/01	Gerke, A.K. and Schneider	2019	PROBLAD PLUS: A semi-static life-cycle toxicity test with the cladoceran (*Daphnia magna*). Company report No.: 896A-102 Eurofins EAG Agroscience, USA Unpublised
CA 8.2.6.1/01	Falk, S.	2011	PROBLAD: Testing of Effects of the Single Cell Green Alga *Desmodesmus subspicatus* in a 72 h Static Test Company Report No. S10-02623 Eurofins Agroscience Services GmBH GLP, Unpublished
CA 8.2.6.1/02	Arnie, J.R.	2019	PROBLAD PLUS: A 72-hour toxicity test with the freshwater alga (*Raphidocelis subcapitata*). Company Report No. 896P-101 Eurofins EAG Agroscience, USA GLP, Unpublished

15 ANNEXES

The study summaries from the DAR of Aqueous extract from the germinated seeds of sweet *Lupinus albus* have been included in Annex I.