



# **Draft Assessment Report**

## **Evaluation of Active Substances**

Plant Protection Products

Prepared according to **assimilated Regulation No 1107/2009**  
as it applies in Great Britain

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

**Volume 1**

Great Britain

February 2025

### Version History

When	What
June 2024	Initial DAR
February 2025	Updates made after ECP
February 2025	Updates made after additional information submitted post ECP
	Updates made after public consultation
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	[Updates made after any additional steps not covered by the above]

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## **Level 1**

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



# 1. Statement of Subject Matter and Purpose for Which this Report has been Prepared and Background Information on the Application

## 1.1. Context in which this draft assessment report was prepared

### 1.1.1. Purpose for which the draft assessment report was prepared

This dossier is submitted for first approval of aqueous extract from the germinated seeds of sweet *Lupinus albus* in Great Britain (GB) according to assimilated Regulation No. 1107/2009 with the evaluation performed by the Chemical Regulation Division of the Health and Safety Executive.

The applicant is proposing aqueous extract from the germinated seeds of sweet *Lupinus albus* as a low-risk botanical active substance for use as a fungicide on a variety of crops, in both field and protected situations. The representative product (PROBLAD PLUS) is identical to the active substance. The representative uses are field and protected crops of strawberry and tomato.

As aqueous extract from the germinated seeds of sweet *Lupinus albus* is considered a botanical active substance, the 'lead component' concept has been used throughout the assessment. The 'lead component' concept is explained in SANCO/11470/2012 (guidance document on botanical active substances used in plant protection products).

There may be references to PROBLAD PLUS within the DAR, however the applicant has confirmed that the tradename for the product will be PROBLAD in GB.

### 1.1.2. Regulatory history for use in Plant Protection Products

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is a new active for GB, however it is an approved active substance in the EU. The Rapporteur Member State for the EU evaluation was Netherlands. EFSA conclusion and EU commission implementing regulation are available.

The applicant has provided proposals for both MRL setting and Classification & Labelling:

- Addition of the active substance to Part 4 of the GB MRL Regulation 396/2005 (for all crops). In the EU, aqueous extract from the germinated seeds of sweet *Lupinus albus* has been adopted into Annex IV of Regulation (EC) No 396/2005 under EU 2021/1807 to include all crops

- Non-classified status for the active substance on the GB MCL (Mandatory Classification List), noting the EU CLH report (and other information relating to the ongoing EU CLH assessment) from the EU assessment is available from the ECHA website

It is noted that all of the technical/scientific consideration culminating in EU low-risk approval was conducted prior to the end of the EU-Exit Implementation period (noting UK did not participate in the public consultation/peer review).

### 1.1.3. Evaluations carried out under other regulatory contexts

EU approval was granted as a low-risk active substance on 27 April 2021. ECHA CLH report is available.

## 1.2. Applicant information

### 1.2.1. Name and address of applicant(s) for approval of the active substance

CEV SA

Zona Industrial de Cantanhede/BIOCANT PARK

Lote 120

3060-197 Cantanhede

Portugal

### 1.2.2. Producer or producers of the active substance

Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.

## 1.3. Identity of the active substance

<b>1.3.1. Common name proposed or ISO-accepted and synonyms</b>	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i>
<b>1.3.2. Chemical name (IUPAC and CA nomenclature)</b>	
IUPAC	Not applicable (natural plant protein)
CA	Not applicable (natural plant protein)

<b>1.3.3. Producer's development code number</b>	None
<b>1.3.4. CAS, EEC and CIPAC numbers</b>	
CAS	Not available for aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i>  Lead component BLAD: 1219521-95-5
EEC	701-313-1
CIPAC	Not applicable
<b>1.3.5. Molecular and structural formula, molecular mass</b>	
Molecular formula	Not applicable for aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> which is a UVCB substance (Substance of Unknown or Variable composition, Complex reaction product or of Biological material). Please refer to the Volume 4 (Confidential Information) section of the DAR for more information.
Structural formula	Not applicable for aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> which is a UVCB substance. Please refer to the Volume 4 (Confidential Information) section of the DAR for more information.
Molecular mass	Not applicable for aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> which is a UVCB substance. Please refer to the Volume 4 (Confidential Information) section of the DAR for more information.

<b>1.3.6. Method of manufacture (synthesis pathway) of the active substance</b>	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
<b>1.3.7. Specification of purity of the active substance in g/kg</b>	Lead component BLAD: 20% w/w (nominal), min-max: 18.8% w/w-21.2% w/w
<b>1.3.8. Identity and content of additives (such as stabilisers) and impurities</b>	
<b>1.3.8.1. Additives</b>	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
<b>1.3.8.2. Significant components impurities</b>	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
<b>1.3.8.3. Relevant impurities</b>	<p>Quinolizidine alkaloids (QAs) with a limit of 0.005% w/w.</p> <p>None</p> <p>It is noted that the applicant proposed inclusion of quinolizidine alkaloids at total QAs max. 0.005 % w/w. Quinolizidine alkaloids (QAs) may be present in lupin seeds, which subsequently may then be found in the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> at low levels. If the content of the total QAs in the extract exceeds 0.006 % w/w, which may be indicated by the content of lupanine, they are designated as relevant impurities and a clause may be required to limit their concentration.</p>
<b>1.3.9. Analytical profile of batches</b>	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.

## 1.4. Information on the plant protection product

<b>1.4.1. Applicant</b>	CEV SA
<b>1.4.2. Producer of the plant protection product</b>	CEV SA
<b>1.4.3. Trade name or proposed trade name and producer's development code number of the plant protection product</b>	PROBLAD <b>PLUS</b>
<b>1.4.4. Detailed quantitative and qualitative information on the composition of the plant protection product</b>	
<b>1.4.4.1. Composition of the plant protection product</b>	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
<b>1.4.4.2. Information on the active substances</b>	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> Lead component: BLAD CAS No: 1219521-95-5
<b>1.4.4.3. Information on safeners, synergists and co-formulants</b>	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
<b>1.4.5. Type and code of the plant protection product</b>	Soluble concentrate (SL)
<b>1.4.6. Function</b>	Fungicide

<b>1.4.7. Field of use envisaged</b>	Agricultural/horticultural use in strawberry and tomato
<b>1.4.8. Effects on harmful organisms</b>	Preventative biofungicide against botrytis grey mould and powdery mildew that functions via contact action. Multiple modes of action, that inhibit fungal growth and destroy fungal cells.

## **1.5. Detailed Uses of the plant protection product**

## 1.5.1. Details of representative uses

Crop and/or situation (a)	Member State	Product Name	F G I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc of a.i. g/kg (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	Kg a.i./hl min max (g/hl)	Water l/ha min max	L a.i./ha min max (*) (kg/ha)		
Strawberry	GB	PROBLAD PLUS	F	Foliar fungi BOTRCI SPHRMA	Soluble concentrate (SL)	1000 g/kg (PROBLAD PLUS is a UVCB substance and is considered to be 100% pure with the lead component BLAD at 250g/L)	Foliar overall	BBCH 61-89 Spring to Summer	1-6	8 days	0.251-0.893 kg a.i./hL	450-1000	Min 2.0 (2.51)  Max 3.2 (4.016)	01	Equivalent to maximum 800 g/ha lead component (BLAD)  Note: (kg/ha) is based on a density of 1.255 g/mL.
Strawberry	GB	PROBLAD PLUS	G	Foliar fungi BOTRCI SPHRMA	Soluble concentrate (SL)	1000 g/kg (PROBLAD PLUS is a UVCB substance and is considered to be 100% pure with the lead component)	Foliar overall	BBCH 61-89 All seasons	1-6	8 days	0.251-0.893 kg a.i./hL	450-1000	Min 2.0 (2.51)  Max 3.2 (4.016)	01	Equivalent to maximum 800 g/ha lead component (BLAD)  Note: (kg/ha) is

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						BLAD at 250 g/L)									based on a density of 1.255 g/mL.
Tomatoes	GB	PROBLAD PLUS	F	Foliar fungi BOTRCI OIDINL	Soluble concentrate (SL)	1000 g/kg (PROBLAD PLUS is a UVCB substance and is considered to be 100% pure with the lead component BLAD at 250 g/L)	Foliar overall	BBCH 61-89 Spring to Summer	1-6	8 days	0.251- 2.01 kg a.i./hL	200- 1000	Min 2.0 (2.51)  Max 3.2 (4.016)	Ø1	Equivalent to maximum 800 g/ha lead component (BLAD)  Note: (kg/ha) is based on a density of 1.255 g/mL.
Tomatoes	GB	PROBLAD PLUS	G	Foliar fungi BOTRCI OIDINL	Soluble concentrate (SL)	1000 g/kg (PROBLAD PLUS is a UVCB substance and is considered to be 100% pure with the lead component BLAD at 250 g/L)	Foliar overall	BBCH 61-89 All seasons	1-6	8 days	0.251- 2.01 kg a.i./hL	200- 1000	Min 2.0 (2.51)  Max 3.2 (4.016)	Ø1	Equivalent to maximum 800 g/ha lead component (BLAD)  Note: (kg/ha) is based on a density of 1.255 g/mL.

\* For uses where the column „Remarks“ is marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s).

(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthialdicarb-isopropyl).



- (a) For crops, the EU and Codex classification (both) should be taken into account ; where relevant, the use situation should be described (e.g. fumigation of a structure)
- (b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
- (c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds
- (d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
- (e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989
- (f) All abbreviations used must be explained
- (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
- (h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated
- (j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (k) Indicate the minimum and maximum number of application possible under practical conditions of use
- (l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000g/ha or 12.5 g/ha instead of 0.0125 kg/ha)
- (m) PHI - minimum pre-harvest interval

For a product consideration the applicant should consider GB agronomic practices; tomatoes are grown in fully protected conditions, whilst strawberries are grown in tunnels, glasshouses and outdoors. Proposed uses GB are usually addressed with a single justified dose and not a range. Adjustment to rates depending on disease pressure will need to be justified in the product submission.

### 1.5.2. Further information on representative uses

#### Method of application

Products containing the aqueous extract from the germinated seeds of sweet *Lupinus albus* are applied as a foliar spray and can be applied via spray equipment commonly used for making ground applications as well as sprinkler/irrigation systems commonly used for chemigation. Intended water volumes are 450-1000 L/ha in strawberry and 200-1000 L/ha in tomato.

**Table 1.5.2-1: Number/timing of applications and duration of protection**

<b>Crop</b>	<b>Number of application</b>	<b>Timing of application</b>	<b>Duration of protection (days)</b>
Strawberry	1-6	BBCH 61-89	7-10
Tomato	1-6	BBCH 61-89	7-10

#### **Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops**

No residues are detectable in the treated crop. Waiting periods or limitations for succeeding crops are not required. A full risk assessment on succeeding crops will be conducted at the product authorisation stage.

#### **Proposed instructions for use**

Products containing the aqueous extract from the germinated seeds of sweet *Lupinus albus* are proposed for use in agriculture and horticulture as foliar fungicide sprays in the crops detailed above.

### 1.5.3. Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
Strawberry	UK	PROBLAD	F	Botrytis cinerea (BOTRCI)  Powdery mildew, including Podosphaera aphanis (PODOAP), Oidium sp. (OIDISP), Erysiphe sp. (ERYSSP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 40-94  Spring to Summer	6	8 days	0.4016-0.892	450-1000	4.016	1	Open field, open protective structures (nets and open plastic covers) and walk-in tunnels. Note permanent protected uses assessed in interzonal dRR
Table and Wine Grapes	UK	PROBLAD	F	Botrytis cinerea (BOTRCI)  Erysiphe necator (UNCINE)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 55-89  Spring to Autumn	6	7 days	0.4016-2.008	200-1000	4.016	1	LWA conversions will be proposed and considered in the dRR/BAD

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Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
Stone Fruit (Peach, Plum, Sweet Cherry, Sour Cherry, Apricot, Nectarine)	UK	PROBLAD	F	<p>Monilinia sp. (MONISP), including: M. laxa, (MONILA), M. fructicola (MONIFC) and M. fructigena (MONIFG)</p> <p>Powdery mildew, including:</p> <p>Podosphaera pannosa (SPHRPA), Podosphaera sp. (PODOSP), Podosphaera leucotricha (PODOLE), Sphaerotheca sp. (SPHRSP)</p>	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 71-87  Spring to Autumn	3	7 days	0.2677-0.4016	1000-1500	4.016	1	<p><u>Qualified recommendations for use proposed.</u></p> <p>Extrapolated from SEU stone fruit efficacy data.</p> <p>Note environmental risk assessment for UK specific GAP (which differs from CEU GAP in time of application), is provided in the national addenda B8 and 9</p>

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Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
Walnuts	UK	PROBLAD	F	<p>Monilinia sp. (MONISP), including M. laxa (MONILA), M. fructicola (MONIFC) and M. fructigena (MONIFG)</p> <p>Powdery mildew, including Phyllactinia guttata (PHYLGU), Podosphaera pannosa (SPHRPA), Podosphaera sp. (PODOSP), Podosphaera leucotricha (PODOLE), Sphaerotheca sp. (SPHRSP)</p>	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 71-87  Spring to Autumn	3	7 days	0.2677-0.4016	1000-1500	4.016	1	<p><u>Qualified recommendations for use proposed.</u></p> <p>Extrapolated from SEU stone fruit efficacy data.</p> <p>Note environmental risk assessment for UK specific GAP (which differs from CEU GAP in time of application), is provided in the national addenda B8 and 9</p>

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

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Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
Hazelnuts	UK	PROBLAD	F	<p>Monilinia sp. (MONISP), including M. laxa, (MONILA), M. fructicola (MONIFC) and M. fructigena (MONIFG)</p> <p>Powdery mildew, including Phyllactinia guttata (PHYLGU), Podosphaera pannosa (SPHRPA), Podosphaera sp. (PODOSP), Podosphaera leucotricha (PODOLE), Sphaerotheca sp. (SPHRSP)</p>	Soluble Concentrate	UVCB 100%	Foliar overall	<p>BBCH 71-87</p> <p>Spring to Autumn</p>	3	7 days	0.2677-0.4016	1000-1500	4.016	1	<p><u>Qualified recommendations for use proposed.</u></p> <p>Extrapolated from SEU stone fruit efficacy data. Note environmental risk assessment for UK specific GAP (which differs from CEU GAP in time of application), is provided in the national addenda B8 and 9</p>

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

**Volume 1 – Level 1**

Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
Chestnuts	UK	PROBLAD	F	<p>Monilinia sp. (MONISP), including M. laxa, (MONILA), M. fructicola (MONIFC) and M. fructigena (MONIFG)</p> <p>Powdery mildew, including Erysiphe flexuosa (ERYSFL), Podosphaera pannosa (SPHRPA), Podosphaera sp. (PODOSP), Podosphaera leucotricha (PODOLE), Sphaerotheca sp. (SPHRSP)</p>	Soluble Concentrate	UVCB 100%	Foliar overall	<p>BBCH 71-87</p> <p>Spring to Autumn</p>	3	7 days	0.2677-0.4016	1000-1500	4.016	1	<p><u>Qualified recommendations for use proposed.</u></p> <p>Extrapolated from SEU stone fruit efficacy data.</p> <p>Note environmental risk assessment for UK specific GAP (which differs from CEU GAP in time of application), is provided in the national addenda B8 and 9</p>

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

**Volume 1 – Level 1**

Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
Almonds	UK	PROBLAD	F	Monilinia sp. (MONISP), including M. laxa, (MONILA), M. fructicola (MONIFC) and M. fructigena (MONIFG)  Powdery mildew, including Erysiphe flexuosa (ERYSFL), Podosphaera pannosa (SPHRPA), Podosphaera sp. (PODOSP), Podosphaera leucotricha (PODOLE), Sphaerotheca sp. (SPHRSP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 71-87  Spring to Autumn	3	7 days	0.2677-0.4016	1000-1500	4.016	1	<u>Qualified recommendations for use proposed.</u> Extrapolated from SEU stone fruit efficacy data. Note environmental risk assessment for UK specific GAP (which differs from CEU GAP in time of application), is provided in the national addenda B8 and 9
Strawberry	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew,	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 40-94  Spring to Summer	6	8 days	0.4016-0.892	450-1000	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance)



# Aqueous extract from the germinated seeds of sweet Lupinus albus

## Volume 1 – Level 1

Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
				including Podosphaera aphanis (PODOAP), Oidium sp. (OIDISP), Erysiphe sp. (ERYSSP)											
Tomato	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew, including:  Oidium neolycopersici (OIDINL), Podosphaera sp. (PODOSP), Leveillula taurica (LEVETA), Leveillula sp. (LEVESP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD
Aubergine	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew,	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions

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

**Volume 1 – Level 1**

Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
				Leveillula taurica (LEVETA) and Oidium neolycopersici (OIDINL).											will be proposed and considered in the dRR/BAD
Cucumber	UK	PROBLAD	G	Powdery mildew, including Golovinomyces cichoracearum (ERYSCI), Erysiphe spp. (ERYSSP), Podosphaera fuliginea (SPHRFU) and Sphaerotheca spp. (SPHRSP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD
Courgette and Summer Squash	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew, including:  Golovinomyces cichoracearum (ERYSCI), Erysiphe spp. (ERYSSP),	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD

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

**Volume 1 – Level 1**

Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
				Podosphaera fuliginea (SPHRFU) and Sphaerotheca spp. (SPHRSP)											
Melon	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew, including:  Golovinomyces cichoracearum (ERYSCI), Erysiphe spp. (ERYSSP), Podosphaera fuliginea (SPHRFU) and Sphaerotheca spp. (SPHRSP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD
Watermelon	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew, including:	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed

# Aqueous extract from the germinated seeds of sweet Lupinus albus

## Volume 1 – Level 1

Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
				Golovinomyces cichoracearum (ERYSCI), Erysiphe spp. (ERYSSP), Podosphaera fuliginea (SPHRFU) and Sphaerotheca spp. (SPHRSP)											and considered in the dRR/BAD
Pumpkin and Winter squash	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew, including:  Golovinomyces cichoracearum (ERYSCI), Erysiphe spp. (ERYSSP), Podosphaera fuliginea (SPHRFU) and Sphaerotheca spp. (SPHRSP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD

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

**Volume 1 – Level 1**

Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
Gherkins	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew, including:  Golovinomyces cichoracearum (ERYSCI), Erysiphe spp. (ERYSSP), Podosphaera fuliginea (SPHRFU) and Sphaerotheca spp. (SPHRSP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD
Pepper	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew, including:  Leveillula taurica (LEVETA), Leveillula sp. (LEVESP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD

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

## Volume 1 – Level 1

Crop and/or situation (i)	GB or Country for IT	Product name	F or G or I (ii)	Pests or Group of pests (iii)	Preparation type (iv)	Preparation conc. a.s. (v)	App Method kind (vi)	Range of growth stages & season (vii)	No of apps min-max (viii)	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max (ix)	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max (x)	PHI (days) (xi)	Remark
Chilli Pepper	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew, including:  Leveillula taurica (LEVETA), Leveillula sp. (LEVESP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD
Okra	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Powdery mildew, including:  Golovinomyces cichoracearum (ERYSCI), Erysiphe spp. (ERYSSP), Podosphaera fuliginea (SPHRFU) and Sphaerotheca spp. (SPHRSP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD

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

### Volume 1 – Level 1

Crop and/or situation ( <sup>i</sup> )	GB or Country for IT	Product name	F or G or I ( <sup>ii</sup> )	Pests or Group of pests ( <sup>iii</sup> )	Preparation type ( <sup>iv</sup> )	Preparation conc. a.s. ( <sup>v</sup> )	App Method kind ( <sup>vi</sup> )	Range of growth stages & season ( <sup>vii</sup> )	No of apps min-max ( <sup>viii</sup> )	Interval between apps (min)	App rate per treatment (kg a.s./hL) min-max ( <sup>ix</sup> )	Water per treatment (L/ha) min-max	App rate per treatment (kg a.s./ha) min-max ( <sup>x</sup> )	PHI (days) ( <sup>xi</sup> )	Remark
Sweetcorn	UK	PROBLAD	G	Botrytis cinerea (BOTRCI)  Erysiphe spp. (ERYSSP)	Soluble Concentrate	UVCB 100%	Foliar overall	BBCH 20-89  All seasons	6	7 days	0.2677-2.008	200-1500	4.016	1	Permanent glasshouses and walk-in tunnels (define as per EFSA guidance). LWA conversions will be proposed and considered in the dRR/BAD

<sup>i</sup> For crops, the GB and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)

<sup>ii</sup> State if the use is outdoor, field use (F) or glass house (G) or indoor use (I)

<sup>iii</sup> e.g. biting and sucking insects, soil born insects, foliar fungi, weeds

<sup>iv</sup> e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR). CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide

<sup>v</sup> g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).

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

### Volume 1 – Level 1

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<sup>vi</sup> All abbreviations used must be explained. Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench. Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated

<sup>vii</sup> Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

<sup>viii</sup> Indicate the minimum and maximum number of applications possible under practical conditions of use

<sup>ix</sup> The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha)

<sup>x</sup> The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha)

<sup>xi</sup> PHI - minimum pre-harvest interval



**1.5.4. Overview on authorisations in EU Member States**

EU approval was granted on 27 April 2021 for the following GAP:

Crop and/or situation (a)	Member State	Product Name	F G I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)
					Type (d-f)	Conc of a.i. g/kg (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	a.i./hL min max (g/hL)	Water L/ha min max	kg a.i./ha min max (kg/ha) (l) based on a density of 1.255 g/mL	
Strawberry	SEU/CEU	PROBLAD PLUS	F	Foliar fungi BOTRCI SPHRMA	SL	100%	Foliar overall	BBCH 61-89 Spring to Summer	1-6	8 days	401.6 g/hL	450-1000	4.016	0
Strawberry	EU	PROBLAD PLUS	G	Foliar fungi BOTRCI SPHRMA	SL	100%	Foliar overall	BBCH 61-89 All seasons	1-6	8 days	401.6 g/hL	450-1000	4.016	0
Tomatoes	SEU/CEU	PROBLAD PLUS	F	Foliar fungi BOTRCI OIDINL	SL	100%	Foliar overall	BBCH 61-89 Spring to Summer	1-6	8 days	401.6 g/hL	200-1000	4.016	0

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

### Volume 1 – Level 1

Tomatoes	EU	PROBLAD PLUS	G	Foliar fungi BOTRCI OIDINL	SL	100%	Foliar overall	BBCH 61-89 All seasons	1-6	8 days	401.6 g/hL	200-1000	4.016	0
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- (a) For crops, the EU and Codex classification (both) should be taken into account ; where relevant, the use situation should be described (e.g. fumigation of a structure)
- (b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
- (c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds
- (d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
- (e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989
- (f) All abbreviations used must be explained
- (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
- (h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated
- (i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypry). **In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**
- (j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (k) Indicate the minimum and maximum number of application possible under practical conditions of use
- (l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha)
- (m) PHI - minimum pre-harvest interval

## **Level 2**

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

## 2. Summary of active substance hazard and of product risk assessment

### 2.1. Identity

Acceptable data have been submitted to support the manufacturing site of aqueous extract from the germinated seeds of sweet *Lupinus albus*. The proposed specification detailed in Volume 4 is considered supported by the available data.

The content of the lead component BLAD protein (a Lupin protein of the  $\beta$ -conglutins family) in the aqueous extract from the germinated seeds of sweet *Lupinus albus* is 20% w/w. The quinolizidine alkaloids (QAs) are considered relevant impurities and are limited to a maximum content of 0.005% w/w in the aqueous extract from the germinated seeds of sweet *Lupinus albus*, with lupanine as a marker compound, max. 0.0035% w/w.

Please refer to Volume 4 for more detailed information on the identity of the aqueous extract from the germinated seeds of sweet *Lupinus albus* and PROBLAD PLUS.

### 2.2. Physical and chemical properties

#### 2.2.1. Summary of physical and chemical properties of the active substance

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is an aqueous solution containing the lead component BLAD. The lead component is not separated during the manufacturing process and the proposed representative product PROBLAD PLUS has the same composition as the aqueous extract from the germinated seeds of sweet *Lupinus albus*. It is a viscous, dark brown liquid with a pungent odour and is not classified as explosive, flammable or oxidising. Due to low surface tension, it is a surface active compound.

Solubility in water and organic solvents, as well as partition coefficient were not determined, as the aqueous extract from the germinated seeds of sweet *Lupinus albus* contains significant amount of water in its composition and the determination of these properties was not possible. Freezing point was not determined; it was expected to be close to the freezing point of water. As the active substance is a complex mixture which is an aqueous extract, the vapour pressure and dissociation constant were not determined. It may be possible to provide data for the lead component: BLAD protein. Vapor pressure is likely to be very low due to the high molecular weight of the protein.

The applicant provided UV-Vis spectra of purified BLAD standard. No other analytical techniques were used to characterise the substance. Considering the UVCB nature

of the aqueous extract from the germinated seeds of sweet *Lupinus albus* and the nature of the lead component BLAD (large molecule, protein composition), it is not feasible to conduct any other spectra on the substance; no further data are required.

Sufficient information (including mass spectra present with the method validation data) is available to characterise the relevant impurities (QAs).

### 2.2.2. Summary of physical and chemical properties of the plant protection product

PROBLAD PLUS is an SL formulation (soluble concentrate). The formulation is a dark viscous liquid with a pungent odour. The lowest in use concentration is 0.251% w/v (equal to 0.2% v/v), and the highest in use concentration is 2.0% w/v (equal to 1.6% v/v).

PROBLAD PLUS does not require classification as flammable, oxidising or explosive and it is not classified as aspiration hazard. However, PROBLAD PLUS is considered as surface active product due to surface tension of 22.4 mN/m at 1.6% w/v at 20°C.

The pH of 1% solution was measured before and after storage and it does not change significantly after storage and remains in the range of 5.9-6.4. The pH of the neat formulation after storage was similar (6.2-6.3).

Acceptable physical, chemical and technical data have been provided indicating that the product fulfils the requirements of an SL formulation.

Adverse persistence of foaming data was reported, however this was justified based on the batch tested being from a pilot scale process and that incorrect test concentrations and CIPAC Standard Water were tested. New acceptable data were generated (testing the correct concentrations, correct CIPAC Standard Water used and amount of foam after 1 minute within the acceptable limits) and the proposed product label contains instructions on mixing the product to avoid excessive foaming.

Dilution stability tests showed an unstable suspension was formed when testing a concentration of 0.4% w/v which is lower than the highest possible in-use concentration (2% w/v). Additional evidence that the separated material will not block application equipment has been provided: A test on sprayability was performed at the highest in use concentration (1.6% v/v) on the commercial scale plant protection product, showing that no nozzle blockage occurs when the product is applied at the highest use rate and that a constant amount of the product is sprayed throughout the spray duration.

Accelerated storage stability data generated at 54°C for 2 weeks were provided demonstrating that the formulation retains its properties during storage. The content of lead component BLAD did not change significantly. Low temperature storage stability data indicated that there is no effect on the stability of the formulation. Ambient shelf-life data were submitted supporting a two-year shelf life when the product is stored in HDPE containers. Further information regarding the levels of the

relevant impurities before and after storage is presented in Volume 4, section C.1.3.1. Tank mixing is not anticipated for PROBLAD PLUS therefore tank mix compatibility data are not required.

## **2.3. Data on application and efficacy**

### **2.3.1. Summary of effectiveness**

PROBLAD PLUS is a novel biofungicide. The soluble concentrate (SL) formulation contains 1000 g/kg of the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus* and 250 g/L of the lead component BLAD.

PROBLAD PLUS is intended for control of Botrytis and powdery mildew in strawberry and tomato. The applicant has presented nine efficacy trials, five conducted in Portugal (2007-2013) and 4 in the USA (2011).

The Portuguese trials were conducted according to EPPO standards by GEP accredited organisations. Of these trials, two were preliminary screening trials that applied between 200-700 g BLAD/ha (equivalent to 0.8-2.8 L/ha PROBLAD PLUS formulation) to assess control of powdery mildew on grapes or Botrytis on strawberry. A further three efficacy field trials were conducted that applied between 250-750 g BLAD/ha (equivalent to 1-3 L/ha of PROBLAD PLUS formulation) to assess control of powdery mildew on tomato or Botrytis on tomato and strawberry.

Four efficacy field trials were conducted in the USA that applied between 340-840 g BLAD/ha (equivalent to 1.35-3.37 L/ha of PROBLAD PLUS formulation) to assess control of powdery mildew on grapes or Botrytis on strawberry, tomato and grapes.

The data support the proposed GAP and demonstrate that the intended dose would be 'sufficiently effective'.

Please refer to Volume 3 CP B.3.9.

### **2.3.2. Summary of information on the development of resistance**

PROBLAD PLUS is a fungicide with contact action. BLAD is the lead component of PROBLAD PLUS, it is a naturally occurring polypeptide, present in *Lupinus albus* seedlings. BLAD binds strongly to chitin, a major component of the fungal cell wall, inhibiting fungal growth. In addition, BLAD degrades chitin by catalysing the successive removal of the N-acetyl-D-glucosamine terminal chitin monomers, destroying the fungal cells.

BLAD is classified by FRAC (Fungicide Resistance Action Committee) in Group BM01 (previously Group M12), having multi-site contact activity with multiple effects and is thus considered low risk of developing resistance. It is intended that no more than two sequential applications should occur before alternating to a labelled fungicide with a different mode of action. Further information will be required at product evaluation stage.

### **2.3.3. Summary of adverse effects on treated crops**

Crop safety in strawberry, tomato and grapes has been considered in all efficacy trials conducted in Portugal and USA. PROBLAD PLUS (250 g BLAD/L formulation) has been tested at rates between 1 and 3.37 L/ha. No phytotoxicity to foliage or fruits were observed from any of the PROBLAD PLUS treatments in the nine efficacy trials conducted in Portugal (5) and USA (4). A detailed evaluation of all potential adverse effects on the treated crops, including phytotoxicity, yield quantity and quality, effects on plant parts for propagation and transformation processes, must be conducted at the product authorisation stage.

### **2.3.4. Summary of observations on other undesirable or unintended side-effects**

Across the five efficacy trials conducted in Portugal, PROBLAD PLUS applications were found to have no effect on a variety of insect species. No undesirable or unintended side-effects on succeeding crops or on beneficial organisms are expected from applications of PROBLAD PLUS according to Good Agricultural Practices. A detailed evaluation of all potential undesirable or unintended side-effects, including the impact on succeeding crops, other plants such as adjacent crops, tank cleaning, and beneficial and non-target organisms will be conducted at the product authorisation stage.

### **Candidate for substitution: efficacy activity**

Not relevant.

## **2.4. Further information**

### **2.4.1. Summary of methods and precautions concerning handling, storage, transport or fire**

#### **Handling:**

Use handling procedures that minimize formation of dust.

#### **Warehouse storage**

Store in a cool, dry, well-ventilated place in original container. Keep container closed when not in use. Do not contaminate other pesticides, fertilizers, water, or feed by storage or disposal.

#### **User level storage**

Store in a cool, dry, well-ventilated place in original container. Keep container closed when not in use. Do not contaminate other pesticides, fertilizers, water, or feed by storage or disposal.

#### **Transport**

Land transport:	ADR / RID Class: Not classified as dangerous for transport
Inland waterways transport:	Not classified as dangerous for transport
Air transport:	ICAO/IATA: Not classified as dangerous for transport
Marine transport	IMDG/IMO: Not classified as dangerous for transport
Other information:	None

**Protective clothing and equipment proposed for use in storage, transport or in the event of fire – nature**

For firefighters: In the event of fire, wear self-contained breathing apparatus.

**Protective clothing and equipment proposed for use in storage, transport or in the event of fire – characteristics**

For firefighters: In the event of fire, wear self-contained breathing apparatus.

**Fire Fighting Measures**

For firefighters: In the event of fire, wear self-contained breathing apparatus.

**2.4.2. Summary of procedures for destruction or decontamination**

A neutralisation procedure is not proposed.

Dispose of contents/container via an approved waste disposal plant.

Dike to prevent runoff. Absorb with earth, sand or other non-combustible material and transfer to containers for later disposal.

Clean and neutralize spill area, tools and equipment by washing with bleach water and soap. Absorb rinsate and add to the collected waste. Waste must be classified and labelled prior to recycling or disposal.

**2.4.3. Summary of emergency measures in case of an accident**

**Personal precautions:** Isolate and post spill area. Wear suitable protective clothing, gloves and eye/face protection.

**Environmental precautions:** Keep people and animals away from and upwind of spill/leak. Keep material out of lakes, streams, ponds, and sewer drains.

**Methods for containment:** Dike to prevent runoff. Absorb with earth, sand or other non-combustible material and transfer to containers for later disposal.

**Methods for cleaning up:** Clean and neutralise spill area, tools and equipment by washing with bleach water and soap. Absorb rinsate



and add to the collected waste. Waste must be classified and labelled prior to recycling or disposal.

### **Decontamination of areas, vehicles and buildings**

Ensure compliance with EC, national and local regulations. Do not dispose of wastes in the local sewer or drainage system.

### **Disposal of damaged packaging, adsorbents and other materials**

Containers must be disposed of in accordance with local regulations.

### **Protection of emergency workers and residents, including bystanders**

Use personal protective equipment.

### **First aid measures**

Eye contact:	Hold eyes open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control centre or doctor for further treatment advice.
Upon inhalation:	Move to fresh air. If person is not breathing, call an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control centre or doctor for further treatment advice.
Following skin contact:	Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control centre or doctor for treatment advice.
Upon swallowing:	Call a poison control centre or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by a poison control centre or doctor. Do not give anything by mouth to an unconscious person.

## 2.5. Methods of analysis

### 2.5.1. Methods used for the generation of pre-authorisation data

Acceptable methods have been submitted for the determination of the lead component BLAD. The lead component is not separated during manufacturing process and the proposed representative product PROBLAD PLUS has the same composition as the aqueous extract from the germinated seeds of sweet *Lupinus albus*. Therefore, additional methods to address the determination of BLAD in the technical material and plant protection product are not required; the available data is sufficient.

Acceptable methods have been submitted for the determination of the relevant impurities, the QAs. Similarly to the lead components, the methods provided address the determination of the QAs in the technical material and plant protection product as these are identical.

Acceptable methods have been submitted for the determination of BLAD in various studies in support of the ecotoxicology and toxicology areas of the risk assessment. For the residues risk assessment, the method is not considered acceptably validated but is indicative of the possible levels of BLAD in treated crops.

### 2.5.2. Methods for post control and monitoring purposes

No methods have been submitted and no methods are required as no residue definitions for any matrix have been proposed.

## 2.6. Effects on human and animal health

The active substance, aqueous extract from the germinated seeds of sweet *Lupinus albus*, is a plant extract with fungicidal properties intended for use on food and non-food crops. It is extracted from the germinated seeds of sweet *Lupinus albus* and formulated into the active substance. In general, botanical active substances are complex mixtures comprising of numerous components, therefore, the whole technical grade material is regarded as the active substance which is described as a UVCB substance (Substance of Unknown or Variable composition, Complex reaction product or of Biological material). Within the technical grade plant extract, the lead component has been identified as 'Banda de *Lupinus albus* doce', known as BLAD.

BLAD is a naturally occurring seed storage protein in germinated sweet lupines. It is a 210 kDa glyco-oligomer which is mainly composed of a 20 kDa polypeptide (also termed BLAD), alongside several other polypeptides. The 210 kDa polypeptide is comprised of 173 amino acid residues and is a stable intermediate of the catabolism of  $\beta$ -conglutin, or characterised as a fragment of the amino acid sequence of  $\beta$ -conglutin, therefore, there is no specific molecular or structural formula.

Quinolizidine alkaloids (QAs) are considered toxicologically relevant impurities. A limit of 0.005% w/w has been set.

The other components within the active substance are not expected to present any concerns for human health and are not discussed further in this document. Please refer to Volume 4 of this Assessment Report for further details on these.

The representative product of aqueous extract from the germinated seeds of sweet *Lupinus albus* is referred to as 'PROBLAD PLUS'. PROBLAD PLUS is intended for use as a fungicide in edible crops and appears as the test article name in all the individual study reports.

Where relevant, the methods of analysis for the active substance in the different matrices used in the in vivo toxicological studies are considered fit for regulatory purposes (see Volume 3CA B5).

The classification of aqueous extract from the germinated seeds of sweet *Lupinus albus* for Human Health effects has been addressed in a GB Technical Report,

produced by HSE (HSE, 2023)<sup>1</sup> in which no classification for human health effects has been proposed.

The data requirements of assimilated Regulations 1107/2009 and 283/2013 have been met and HSE concludes that there are no data gaps.

#### **2.6.1. Summary of absorption, distribution and excretion in mammals**

Considering the composition of aqueous extract from the germinated seeds of sweet *Lupinus albus*, the ADME characteristics of the active substance are addressed on the basis of known mammalian metabolic processing of proteins and other biological substances which are expected to arise from plant extracts. Oral absorption is considered to be complete (100%), with the exception of intact BLAD for which oral absorption is negligible since it is degraded to its constituents in the gastro-intestinal tract. Absorption by the inhalation and dermal routes is low. Following oral absorption, the lead substance, BLAD polypeptide, is broken down under enzymatic processes in the gastrointestinal tract. Any amino acids resulting from the proteolysis of BLAD will be absorbed, enter the amino acid pool and subsequently consumed into normal metabolic processes.

Please refer to Volume 4 of this Assessment Report for the summary of other components which have been identified in the technical grade active substance.

#### **2.6.2. Summary of acute toxicity**

The acute toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus* was investigated in vivo, in studies conducted via the oral, dermal and inhalation route of exposure. In vivo studies of skin irritancy, eye irritancy and skin sensitisation were also performed, however experimental investigation of phototoxicity was waived based on physico-chemical characteristics of the active substance. All submitted studies were OECD and GLP-compliant.

These data confirm that aqueous extract from the germinated seeds of sweet *Lupinus albus* is of extremely low oral, dermal and inhalation toxicity and is not a dermal or eye irritant, nor is it a skin sensitiser. The table below provides an overview of the acute toxicity, irritation and skin sensitisation potential of aqueous extract from the germinated seeds of sweet *Lupinus albus*.

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<sup>1</sup> HSE (2023) MCL Technical Report: proposal for mandatory classification and labelling (MCL) of aqueous extract from the germinated seeds of sweet *Lupinus albus*, based on Annex VI, Part 2 of the assimilated CLP Regulation No. 1272/2008 as amended for Great Britain. Date of report: November 2023. Accessed date: 30<sup>th</sup> April 2024. Available at <https://www.hse.gov.uk/>

**Table 2.6.2-1: Summary of acute toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus***

<b>Guideline, reference</b>	<b>Species</b>	<b>Result</b>	<b>Classification</b>
<b>Acute Oral toxicity</b>			
OECD 425 ██████ (2012a)	Rat	LD <sub>50</sub> > 5000 mg/kg bw	<b>None</b>
<b>Acute Dermal toxicity</b>			
OECD 402 ██████ (2012b)	Rat	LD <sub>50</sub> > 2000 mg/kg bw	<b>None</b>
<b>Acute Inhalation toxicity</b>			
OECD 403 ██████ (2012c)	Rat	LC <sub>50</sub> 4h > 5.34 mg/L nose-only	<b>None</b>
<b>Skin Irritation</b>			
OECD 404 ██████ (2012d)	Rabbit	Mild signs of initial irritation which were reversible	<b>None</b>
<b>Eye Irritation</b>			
OECD 405 ██████ (2012e)	Rabbit	Mild signs of initial irritation which were reversible	<b>None</b>
<b>Skin Sensitisation</b>			
OECD 406 ██████ (2012f)	Guinea Pig	Not sensitising (Buehler)	<b>None</b>

### 2.6.3. Summary of short-term toxicity

The short-term repeat dose toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus* was assessed in the rat only, in an oral 13 week (90-day) gavage study and via the dermal route, in a 22-day dermal toxicity study. No studies in the mouse or dog were available. Considering the botanical nature of the active substance, and the known components in it, HSE considers the metabolic fate of the active substance will be well-preserved amongst all mammalian species. Therefore, the absence of experimental data from the mouse and dog is acceptable. Based on pattern of use and physicochemical considerations, the data from the oral route is considered predictive of the systemic toxicity via other routes. The active substance demonstrated very low toxicity by both the oral and dermal routes of exposure, however, some marginal effects were noted in both studies.

In the oral 90-day study, there was slight to minimal vacuolation in the brain and spine in a single female at the top dose of 1000 mg/kg bw/d. There were no other adverse effects noted in the study on any parameter in either sex and although it is plausible that the lesions were an artefact of the histopathology tissue preparation method, a precautionary LOAEL was established at this dose level. Therefore, the resulting NOAEL from this oral 90-day study was set at 500 mg/kg bw/d.

In the dermal 22-day study, no systemic toxicity was observed up to the top dose. Therefore, the systemic NOAEL in this dermal RDT study is established at the limit dose of 1000 mg/kg bw/d.

However, the repeated dermal application of test article led to an increase in local irritation at the exposed sites at the top dose in this study. Histopathological examination of the skin revealed minimal hyperkeratosis and overall, a treatment-related and adverse effect on the skin was identified at 1000 mg/kg bw/d, leading to a local effects NOAEL of 300 mg/kg bw/d.

Classification for STOT-RE is not required (HSE, 2023)<sup>1</sup>.

**Table 2.6.3-1: Summary of repeated-dose toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus***

<b>Study, guideline, reference</b> <b>Acceptability</b>	<b>Species, doses tested</b>	<b>NOAEL mg/kg bw/day</b>	<b>LOAEL mg/kg bw/day</b>	<b>Effects at the LOAEL</b>
90 day, oral (gavage)	Rat	500	1000	Vacuolation in the spine and brain in a single individual.
22 day, dermal	Rat	300	1000	Local irritation (erythema and scabbing) at the site of application, and minimal grade hyperkeratosis.

#### 2.6.4. Summary of genotoxicity

The genotoxic potential of the aqueous extract from the germinated seeds of sweet *Lupinus albus* was tested in an in vitro and in vivo battery of valid and reliable OECD TG-compliant tests. In vitro testing was performed via a bacterial reverse mutation assay (Ames test), an in vitro mammalian cell gene mutation test (mouse lymphoma assay) and an in vitro micronucleus assay in cultured human peripheral lymphocytes. Testing in vivo was performed as a comet assay in the rat (site of contact target tissue - stomach) (Table 2.6.4-1).

The aqueous extract from the germinated seeds of sweet *Lupinus albus* did not induce gene mutations in bacteria, nor did it produce evidence of clastogenicity or aneugenicity (micronuclei) in mammalian primary cells, either with or without metabolic activation. However, in the in vitro mammalian gene mutation assay (MLA) with S9 metabolic activation, the active substance induced a positive response (indicative of gene mutations and clastogenicity); this was seen under acceptable levels of cytotoxicity and in the absence of test substance precipitation. All in vitro tests were conducted up to the maximum limit concentration, or up to levels precluded by test substance precipitation or cytotoxicity, ensuring the studies were reliable. The HSE notes that the positive finding in vitro was only found in the presence of S9 metabolic activation in the mammalian cell gene mutation assay, with no similar finding in the comparable bacterial test system in the presence of S9.

The Applicant chose to follow-up the positive in vitro findings in the mammalian gene mutation assay, with an in vivo comet assay, which is an acceptable test method for the detection of mutagens and/or clastogens. The study was well-performed and reliably negative at concentrations up to the limit dose.

In the in vivo comet assay, the target tissue was the stomach, with no sampling of the liver. As there was some residual uncertainty due to the lack of comet analysis in liver tissues which would have been exposed to metabolic activation of the active substance, the Applicant provided the following statement in support of the site of contact sampling strategy:

“...As PROBLAD PLUS contains the naturally occurring polypeptide component, BLAD, the protein will be broken down, enter the amino acid pool and be consumed into normal catabolic processes. Consequently, no bioanalytical method is available to detect target organ exposure, radiolabelling of the test article is neither possible nor cost effective. To overcome this the in vivo comet assay investigating DNA damage at the site of contact (the stomach following dosing via oral gavage) was deemed to be a valid and appropriate way forward to address concerns regarding target organ exposure following consultation with the Dutch authorities ctgb (College voor de toelating van gewasbeschermingsmiddelen en biociden [Board for the Authorisation of Plant Protection Products and Biocides])”.

Due to the direct gavage dosing of the animals in the in vivo comet assay, exposure to the stomach lining was achieved at the maximum dose recommended in the test guideline, and the negative comet results are acceptable as reliable evidence for a lack of concern regarding the potential of the aqueous extract from the germinated seeds of sweet *Lupinus albus* to act at the initial site of contact.

Considering the following points, the absence of liver tissue comet analysis is not considered to be a critical data gap by HSE:


- 1) expected metabolism of the active substance in the mammalian system
- 2) it is not possible to demonstrate exposure of the active substance or the lead component BLAD, to the liver tissue. BLAD in its entirety will not be systemically available. The protein will be susceptible to proteolytic degradation in the acidic environment of the stomach upon oral dosing, consequently, radiolabelling the test article is neither possible nor cost effective, with a liver comet assay being devoid of any relevance as BLAD will not be assessed as it will have completely been consumed into the nitrogen pool.
- 3) considering the clear negative results from Ames test ( $\pm$ S9) and the MNvit ( $\pm$ S9), it is possible that the positive finding in the mammalian cell gene mutation assay (+S9) as a misleading positive, resulting from exposure to a protein-rich test article. It is recognized that within the in vitro battery required by assimilated Regulation No 283/2013, the mammalian cell gene mutation assay is not considered a core test guideline by the UK CoM due to concerns over low specificity (CoM guidance, 2021).

In conclusion, the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not genotoxic in vivo and the data requirements of assimilated Regulation No



283/2013 have been met. Therefore, classification of the active substance for mutagenicity is not warranted (see also aligned HSE Technical Report, HSE (2023)).

**Table 2.6.4-1: Summary of genotoxicity of the aqueous extract from the germinated seeds of sweet *Lupinus albus***

Type of study	Test system	Dose range tested	Result	Reference
In vitro	Bacterial (5 strain, Ames) gene mutation (treat and plate methodology)	+/-S9: 16 to 5000 <sup>a</sup> µg/plate	±S9: negative	Ballantyne (2016)
In vitro	Mammalian (L5178Y tk <sup>+/+</sup> ) gene mutation	Expt. 1: 3 h –S9: 500 to 2000 <sup>b</sup> µg/mL 3 h +S9: 150 to 900 <sup>c</sup> µg/mL  Expt 2: 3 h –S9: 500 to 2500 <sup>c</sup> µg/mL 3 h +S9: 300 to 1200 <sup>c</sup> µg/mL	-S9: negative +S9: positive	Keig-Shevlin (2015a)
In vitro	Mammalian (cultured human lymphocytes) micronucleus	3 h +/-S9: 0 to 2000 <sup>d</sup> µg/mL  24 h –S9: 100 to 1600 <sup>c</sup> µg/mL	±S9: negative	Lloyd (2005)
In vivo	Rat stomach comet	0, 500, 1000, 2000 <sup>e</sup> mg/kg bw/d	negative	 (2015b)

a maximum recommended concentration according to current regulatory guidelines for the Ames test

b precipitate observed at the end of treatment

c concentration not limited by excessive toxicity; RTG 10-20% moderate cytotoxicity

d maximum recommended concentration for the in vitro micronucleus assay

e maximum recommended dose in accordance with OECD 489

### **2.6.5. Summary of long-term toxicity and carcinogenicity**

No long-term chronic toxicity and carcinogenicity studies have been conducted and none are considered necessary. The lead component in aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD, contributes to approximately 20% w/w of the active substance. BLAD is a naturally occurring polypeptide formed during the early days of the germination process of sweet lupin seeds (*Lupinus albus*) and it is also used in human and animal nutrition, as a food and feed item. The BLAD pesticidal mode of action is specific to fungi only, and it is rapidly biodegradable, being susceptible to proteolytic degradation, adding to the amino acid pool in the body. The other components are those which occur naturally in botanical extracts and there is no evidence that any of these substances give rise to toxicological concern (human health). A complete genotoxicity test battery confirmed a lack of genotoxic potential and, there were no indications of potential non-genotoxic carcinogenicity in the short-term database. There is no evidence in the public domain to suggest that BLAD is associated with an increased incidence of cancer. Based on all these considerations, it can be concluded that neither the lead component, or the active substance as a whole, are likely to be considered a carcinogen.

### **2.6.6. Summary of reproductive toxicity**

No reproductive or fertility studies have been conducted with the active substance, and none are considered necessary. The lead component in aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD, contributes to approximately 20% w/w of the active substance. BLAD is a naturally occurring polypeptide formed during the early days of the germination process of sweet lupin seeds (*Lupinus albus*) and it is also used in human and animal nutrition, as a food and feed item. The BLAD pesticidal mode of action is specific to fungi only, and it is rapidly biodegradable, being susceptible to proteolytic degradation, thereby adding to the amino acid pool in the body. The other components in the active substance are those which occur naturally in botanical extracts and there is no evidence in the public domain that any of these substances give rise to reproductive or developmental toxicity. Based on this, it can be concluded that neither the lead component, or the active substance as a whole, is likely to be considered a reproductive or developmental toxicant.

### **2.6.7. Summary of neurotoxicity**

The requirement for a specific neurotoxicity study in rodents is not triggered; BLAD (the lead component) does not belong to a class of chemicals known to be associated with neurotoxicity and there were no signs of neurotoxicity in acute dose

studies. In the 90-day rat repeat dose toxicity study with aqueous extract from the germinated seeds of sweet *Lupinus albus*, there was slight to minimal vacuolation in the brain and spine in a single female at the top dose of 1000 mg/kg bw/d. However, this was not considered to be robust evidence of neurotoxicity since it was an isolated finding and the functional observational battery and locomotor assessment in this study did not reveal any adverse effects. Refer to Volume 4 for consideration of other components within the UVCB substance.

### 2.6.8. Summary of further toxicological studies on the active substance

No further studies have been performed, and none are considered necessary. The Applicant provided additional information on the following endpoints:

#### Oral allergenicity

The Applicant provided a literature-based reasoned case and a clinical study to support the lack of allergenic potential resulting from exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus*. Unlike certain other legume-derived polypeptides, BLAD (a Lupin protein of the  $\beta$ -conglutins family), the lead component within the active substance, exhibits is predicted to have a very high sensitivity to proteases and it does not contain amino acid sequences with significant antigenic potential. In addition, in a clinical study, there was no evidence of binding of the BLAD protein to serum IgE from 26 individuals allergic to lupine and/or peanut. Overall, there is no concern for oral allergenicity of the aqueous extract from the germinated seeds of sweet *Lupinus albus*. It should be noted that the relevant impurities quinolidizine alkaloids (QAs), of which lupanine is the major component, are not allergenic. It is the Lupin proteins which have allergenic potential.

#### Endocrine Disruption

No specific studies on endocrine disruption have been conducted with the active substance, and none are considered necessary. The lead component in aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD, contributes to approximately 20% w/w of the active substance. BLAD is a naturally occurring polypeptide formed during the early days of the germination process of sweet lupin seeds (*Lupinus albus*) and it is also used in human and animal nutrition, as a food and feed item. The BLAD pesticidal mode of action is specific to fungi only, and it is rapidly biodegradable, being susceptible to proteolytic degradation, thereby adding to the amino acid pool in the body. The other components in the active substance are those which occur naturally in botanical extracts and there is no evidence in the public domain that any of these are endocrine disruptors. Following repeated dose toxicity testing of the active substance, there were no adverse effects in endocrine-sensitive organs. Based on all these considerations, it can be concluded that neither the lead component, or the active substance as a whole, is likely to pose endocrine disrupting properties.

### **2.6.9. Summary of toxicological data on impurities and metabolites**

No studies have been performed, and none are considered necessary.

### **2.6.10. Summary of medical data and information**

The manufacture of the active substance involves a continuous non-stop process occurring at the manufacturing site, with 34 workers present at the site. No incidents of poisoning or allergenic symptoms have been attributed to exposure associated with the handling or manufacturing of either the active substance or exposure to the raw material. No specific therapeutic regime is known for the active substance and first aid measures follow symptomatic treatment.

### **2.6.11. Overview of studies and points of departure relevant to reference value derivation**

Derivation of the reference values is based on the available repeat dose toxicity data that have been generated with the aqueous extract from the germinated seeds of sweet *Lupinus albus*. A 90-day gavage study in the rat is available, and the results relevant to establishing human health-based guidance values are summarised in the following sections.

### **2.6.12. Toxicological end point for assessment of risk following long-term dietary exposure – ADI**

The repeat dose oral toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus* was investigated in a 90-day gavage study in the rat. Although the most suitable studies for the derivation of the ADI are usually chronic studies, HSE considers that due to the chemical nature of the active substance, combined with the lack of relevant findings in the published literature or significant toxicity in the short-term database, an absence of chronic and lifetime substance-specific studies is not of concern. No additional assessment factors are required to account for an incomplete database.

In the 90-day study, a NOAEL of 500 mg/kg bw/d was established, based on vacuolation in the brain and spinal cord in a single individual animal at the limit dose of 1000 mg/kg bw/d. The dose level of 500 mg/kg bw/d is considered to be a suitable starting point for derivation of the ADI. After application of the standard assessment factors of 10 x 10 to account for intra-species and inter-individual differences, this results in an ADI of 5 mg/kg bw/d.

### **2.6.13. Toxicological end point for assessment of risk following acute dietary exposure – ARfD (acute reference dose)**

An acute reference value is not necessary as there is no evidence of acute effects following the ingestion of aqueous extract from the germinated seeds of sweet *Lupinus albus*.

#### 2.6.14. Toxicological end point for assessment of occupational, bystander and residents risks – AOEL

A systemic AOEL can be derived on the same basis and rationale by which the ADI has been established. A correction factor for oral bioavailability is not required, based on the expected ADME properties of aqueous extract from the germinated seeds of sweet *Lupinus albus*. Therefore, the resulting systemic AOEL is 5 mg/kg bw/d.

#### 2.6.15. Toxicological end point for assessment of occupational, bystander and residents risk – AAOEL

An acute AOEL is not necessary as there is no evidence of acute effects following exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus*.

#### 2.6.16. Summary of product exposure and risk assessment

##### Operator Exposure

Estimates of operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to outdoor and protected tomatoes and strawberries have been calculated using the Online EFSA OPEX Model (Version 1.0.2). The results are summarised in the tables below:

**Table 2.6.16-1: Summary of short-term operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to outdoor tomatoes and strawberries**

Application Equipment	PPE Requirements	% of systemic AOEL
Vehicle mounted boom sprayer	Workwear (no PPE)	23.9
Manual handheld (tank and lance) sprayer	Workwear (no PPE)	13.6
Handheld knapsack	Workwear (no PPE)	4.5

**Table 2.6.16-2: Summary of short-term operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to protected tomatoes and strawberries**

Application Equipment	Crop culture	PPE Requirements	% of systemic AOEL
Manual handheld (tank and lance) sprayer	Normal	Workwear, no PPE	14.2

Handheld knapsack	Normal	Workwear, no PPE	14.7
Manual trolley sprayer	Normal	Workwear, no PPE	5.4
Manual handheld (tank and lance) sprayer	Dense	Workwear & gloves during mixing/loading, gloves and protective coveralls during application	66.5
Handheld knapsack	Dense	Workwear and gloves during mixing/loading, gloves and protective coveralls during application	66.5

The predicted short term operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to outdoor and protected tomatoes and strawberries is calculated to be within acceptable limits.

It is considered that application to protected crops via vehicle mounted equipment is likely to be within acceptable limits with the following additional operator protection phases:

- Broadcast air-assisted sprayers must only be used where the operator's normal working position is within a closed cab with a suitable in-cab filtration system\* during application in protected situations.  
\*Closed cabin meeting at least EN 15695 category 3.
- Vehicle-mounted or trailed horizontal or vertical boom sprayers must only be used where the operator's normal working position is within a closed cab with a suitable in-cab filtration system\* or suitable respiratory protective equipment\*\* must be worn during application in protected situations.  
\*Closed cabin meeting at least EN 15695 category 3  
\*\*Disposable filtering facepiece respirator to at least EN149 FFP3 or equivalent.

The product 'PROBLAD PLUS' is not classified for human health effects. Thus, there are no additional PPE requirements.

#### Resident/Bystander Exposure

For the proposed outdoor uses of 'PROBLAD PLUS', estimates of resident exposure using the Online EFSA OPEX Model (Version 1.0.2) predict that short term exposure to a child and adult is within acceptable limits for all exposure pathways, with the sum of the mean for all pathways equivalent to 13.3% of the AOEL of aqueous extract

from the germinated seeds of sweet *Lupinus albus* for a child resident and 6.7% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for an adult resident. The short-term exposure to bystanders is covered by the resident exposure assessment.

For the proposed indoor uses of 'PROBLAD PLUS', estimates of resident exposure using the Online EFSA OPEX Model (Version 1.0.2) predict that short term exposure to a child and adult is within acceptable limits for all exposure pathways, with the sum of the mean for all pathways equivalent to 9.8% of the AOEL of aqueous sweet lupin for a child resident and 5.3% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for an adult resident. The short term exposure to bystanders is covered by the resident exposure assessment.

### Worker Exposure

Estimates of worker exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to outdoor and protected tomatoes and strawberries have been calculated using the Online EFSA OPEX Model (Version 1.0.2) and the UK Decline Calculator. Exposure estimates have been calculated assuming a maximum application rate of 6 x 4.016 kg a.s./ha for both crops for outdoor and protected uses. An additional assessment has been conducted at a reduced application rate to protected strawberries at 6 x 3.5 kg a.s./ha. The results are summarised in the tables below:

**Table 2.6.16-3: Summary of short-term worker exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to outdoor and protected strawberries**

Application rate	Situation	Worker activity	Clothing/ PPE Requirements	% of systemic AOEL	PHI / Re-entry restriction
6 x 4.016 kg a.s./ha	Outdoor	Reaching/picking	Workwear (no PPE)	95.7	0
6 x 4.016 kg a.s./ha	Protected	Searching/Reaching /picking	Workwear (no PPE)	98.1	6
6 x 4.016 kg a.s./ha	Protected	Inspection/irrigation and general maintenance	Workwear (no PPE)	44.8	0
6 x 3.5 kg a.s./ha	Protected	Searching/Reaching /picking	Workwear (no PPE)	96.0	4

**Table 2.6.16-4: Summary of short term worker exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to outdoor and protected tomatoes**

Application rate	Situation	Worker activity	Clothing/ PPE Requirements	% of systemic AOEL	PHI / Re-entry restriction
6 x 4.016 kg a.s./ha	Outdoor	Reaching/picking	Workwear (no PPE)	79.7	0
6 x 4.016 kg a.s./ha	Protected	Searching/Reaching/picking	Workwear (no PPE)	80.8	0

The predicted short term worker exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to outdoor and protected tomatoes and outdoor strawberries at an application rate of 6 x 4.016 kg a.s./ha is calculated to be within acceptable limits with the proposed 1 day PHI.

The predicted short term worker exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to protected strawberries at an application rate of 6 x 4.016 kg a.s./ha is calculated to be within acceptable limits with a 6 day PHI.

The predicted short term worker exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to protected strawberries at reduced application rate of 6 x 3.5 kg a.s./ha is calculated to be within acceptable limits with a 1-day PHI.

Estimates of operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to outdoor and protected tomatoes and strawberries have been calculated using the EFSA Calculator (Version: 30<sup>th</sup> March 2015, EFSA 2014 Guidance), EUROPOEM and the online EFSA OPEX Model (Version 1.0.2, EFSA 2022 Guidance). The results are summarised in the tables below:

**Table 2.6.16-1: Summary of short term operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD PLUS' to outdoor tomatoes and strawberries. Model: EFSA Calculator**

Application Equipment	PPE Requirements	% of systemic AOEL
Vehicle mounted boom sprayer	Workwear (No PPE)	11



Manual-handheld (tank and lance) sprayer	Workwear (No PPE)	5
Handheld knapsack	Workwear (No PPE)	2

**Table 2.6.16-2: Summary of short term operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD-PLUS' to outdoor tomatoes and strawberries. Model: Online EFSA OPEX Model**

Application Equipment	PPE Requirements	% of systemic AOEL
Vehicle mounted boom sprayer	Workwear (No PPE)	9.6
Manual-handheld (tank and lance) sprayer	Workwear (No PPE)	5.5
Handheld knapsack	Workwear (No PPE)	1.8

**Table 2.6.16-3: Summary of short term operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD-PLUS' to protected tomatoes and strawberries. Model: EFSA Calculator and EUROPOEM**

Application Equipment	Crop culture	PPE Requirements	% of systemic AOEL
Manual-handheld (tank and lance) sprayer*	Not Applicable	Workwear (No PPE)	12
Handheld knapsack*	Not Applicable	Workwear (No PPE)	13

\*Calculated using EUROPOEM Database.

**Table 2.6.16-4: Summary of short term operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD-PLUS' to protected tomatoes and strawberries. Model: Online EFSA OPEX Model**

Application Equipment	Crop culture	PPE Requirements	% of systemic AOEL
Manual-handheld (tank and lance) sprayer	Normal	Workwear (No PPE)	6.1
Handheld knapsack	Normal	Workwear (No PPE)	6.3
Manual trolley sprayer	Normal	Workwear (No PPE)	2.4

Manual-handheld (tank and lance) sprayer	Dense	Workwear (No PPE)	95.9
Handheld knapsack	Dense	Workwear (No PPE)	96.1

The predicted short term operator exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD-PLUS' to outdoor and protected tomatoes and strawberries is calculated to be within acceptable limits.

It is considered that application to protected crops via vehicle mounted equipment is likely to be within acceptable limits with the following additional operator protection phases:

- Broadcast air-assisted sprayers must only be used where the operator's normal working position is within a closed cab with a suitable in-cab filtration system\* during application in protected situations.  
\*Closed cabin meeting at least EN 15695 category 3.
- Vehicle-mounted or trailed horizontal or vertical boom sprayers must only be used where the operator's normal working position is within a closed cab with a suitable in-cab filtration system\* or suitable respiratory protective equipment\*\* must be worn during application in protected situations.  
\*Closed cabin meeting at least EN 15695 category 3  
\*\*Disposable filtering facepiece respirator to at least EN149 FFP3 or equivalent.

The product 'PROBLAD-PLUS' is not classified for human health effects. Thus, there are no additional PPE requirements.

#### Bystander and Resident Exposure

For the proposed uses of 'PROBLAD-PLUS' on tomatoes and strawberries grown outdoors, estimates of resident exposure using the EFSA Calculator (Version: 30<sup>th</sup> March 2015) predict that short term exposure to a child and adult is within acceptable limits for all exposure pathways, with the (mean) sum for all pathways equivalent to 5% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a child resident and 3% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for an adult resident.

Note: The EFSA 2014 guidance and associated EFSA Calculator does not consider resident exposure from indoor applications.

The short term exposure of bystanders is covered by the resident exposure assessment.

For the proposed uses of 'PROBLAD-PLUS' on tomatoes and strawberries grown in outdoor and protected environments, estimates of resident exposure using the online EFSA OPEX Model (Version 1.0.2) predict that short term exposure to a child and adult is within

acceptable limits for all exposure pathways, with the (mean) sum for all pathways equivalent to:

- 5.4% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a child resident and 2.7% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for an adult resident for the proposed application to tomatoes and strawberries grown outdoors.
- 4% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a child resident and 2.1% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for an adult resident for the proposed application to tomatoes and strawberries grown in protected environments.

The short term exposure of bystanders is covered by the resident exposure assessment.

#### Worker Exposure

For the proposed uses of 'PROBLAD-PLUS' on outdoor and protected tomatoes and strawberries, estimates of worker exposure using the EFSA Calculator (Version: 30<sup>th</sup> March 2015) predict that short term exposure is equivalent to:

- 32% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a re-entry worker wearing workwear (covering the arms, body and legs) whilst conducting reaching/picking activities in tomato crops grown outdoors that have been previously treated with 'PROBLAD-PLUS'.
- 38% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a re-entry worker wearing workwear (covering the arms, body and legs) whilst conducting reaching/picking activities in strawberry crops grown outdoors that have been previously treated with 'PROBLAD-PLUS'.
- 74% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a re-entry worker without workwear conducting reaching/picking activities in tomato and strawberry crops grown indoors that have been previously treated with 'PROBLAD-PLUS'.

The predicted short term worker exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD-PLUS' to outdoor and protected tomatoes and strawberries at an application rate of 6 x 4.016 kg a.s./ha is calculated to be within acceptable limits.

For the proposed uses of 'PROBLAD-PLUS' on outdoor and protected tomatoes and strawberries, estimates of worker exposure using the online EFSA OPEX Model (Version 1.0.2) and HSE Decline Model predict that short term exposure is equivalent to:

- 31.9% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a re-entry worker wearing workwear (covering the arms, body and legs) whilst conducting reaching/picking activities in tomato crops grown outdoors that have been previously treated with 'PROBLAD-PLUS'.

- 38.3% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a re-entry worker wearing workwear (covering the arms, body and legs) whilst conducting reaching/picking activities in strawberry crops grown outdoors that have been previously treated with 'PROBLAD-PLUS'.
- 75.1% of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a re-entry worker without workwear conducting reaching/picking activities in tomato crops grown indoors that have been previously treated with 'PROBLAD-PLUS'.
- 161%\* of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a re-entry worker without workwear conducting searching, reaching and picking activities in strawberry crops grown indoors that have been previously treated with 'PROBLAD-PLUS'.
- 46%\* of the AOEL of aqueous extract from the germinated seeds of sweet *Lupinus albus* for a re-entry worker wearing workwear (covering the arms, body and legs) conducting searching, reaching and picking activities 21 days (expressed as 3 weeks) post application in strawberry crops grown indoors that have been previously treated with 'PROBLAD-PLUS'.

\*Calculated using the HSE Decline Model.

Therefore, the predicted short term worker exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for application of 'PROBLAD-PLUS' to outdoor and protected tomatoes and strawberries at an application rate of 6 x 4.016 kg a.s./ha is calculated to be within acceptable limits.

## **2.7. Residue**

### **2.7.1. Summary of storage stability of residues**

Samples analysed as part of the residue trials were stored for over 30 days therefore storage stability should be addressed. No storage stability data were submitted. The uncertainty regarding the stability of residues in stored samples has been taken into consideration in the assessment (see section 2.7.4). No further data are required at this time. Further data may be required in a future consideration where results from samples stored for extended time periods are relied upon.

### **2.7.2. Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish**

The applicant did not submit radiolabelled studies according to OECD guidelines. Since it is often not possible to radiolabel complex botanical active substances, it is reasonable that it is not feasible to perform studies based on radioactive detection. A range of field trials including analysis of treated crops and literature data was provided to address the nature of residues in treated crops.

The evidence provided shows that the lead component of the aqueous extract from the germinated seeds of sweet *Lupinus albus* is a 20 kDa protein termed BLAD. This is a seed storage protein formed upon the germination of sweet lupin seeds. The data evaluated provides some supporting evidence that low residues of the aqueous extract from the germinated seeds of sweet *Lupinus albus* are expected in treated crops.

The applicant provided a position paper in addition to the submitted data to further address the requirements.

As stated in the botanicals guidance, SANCO/11470/2012 rev. 8, if the proposed botanical active substance is considered to be the same material that is reasonably expected to be or to become a component of food, this provides considerable reassurance for consumer exposure. As stated in the provided literature, sweet lupin varieties are consumed by humans. The constituent proteins are naturally occurring plant proteins which are expected to break down into constituent amino acids. Data showing the 'background' levels of these proteins in plants have not been provided. However due to the UVCB nature of the substance and nature of the analytical methods used to determine the content of the lead component BLAD, it is recognised that this would be challenging to determine and reliably quantify.

It is noted in the botanicals guidance that for many botanical active substances residue data may not be required if it has been determined that residue levels are unlikely to exceed natural exposure and that the residues are not of toxicological concern. This can be demonstrated by a scientific rationale. This is considered

demonstrated based on the origin of the active substance (the aqueous extract from the germinated seeds of sweet *Lupinus albus*, hence formed from proteins expected to be present in sweet lupin plants which are consumed by humans). Proteins present within plants may be utilised as part of respiration, being hydrolysed into their component amino acids. The evidence provided supports the degradation of the proteins into naturally occurring amino acids, simple carbohydrates and lipids which are indistinguishable from the breakdown of other naturally occurring substances. It is therefore not possible or necessary to identify the components formed following treatment of crops with PROBLAD PLUS. These components are not considered to be of any concern for consumers given they are natural components of foodstuffs.

Quinolizidine alkaloids (QAs) mainly occur in lupin species and other plants of the Genisteae tribe. They are secondary metabolites for defence, are biosynthesized from lysine in green tissues of the plant and are stored in all organs of the plant, including seeds. These may be of toxicological concern, therefore have been considered in the context of risks for animal and human health resulting from consumption of lupin and lupin-derived products, not from the use of PPPs.

The following summary is presented in the EFSA scientific opinion on the presence of QAs in food and feed (EFSA, 2019):

‘Information on the toxicokinetics and metabolism of QAs in humans and experimental animals is limited to sparteine and lupanine. Both compounds are rapidly absorbed, widely distributed and rapidly eliminated in urine mainly unchanged or as oxidised metabolites. Genetic polymorphisms in CYP2D6 (occurring in 5–10% of Caucasians, also called poor metabolisers) may affect sparteine oxidation in humans leading to a slower elimination. Similarly, scarce data are available on ‘absorption, distribution, metabolism, and excretion’ (ADME) in farm animals, horses and companion animals. In ruminants, there is extensive absorption and slow elimination of lupanine and 5,6-dehydrolupanine. In pigs, lupanine is extensively absorbed and excreted in urine mainly unmodified, together with the metabolites isolupanine and 13-OH-lupanine. There is no evidence of the formation of conjugated metabolites of sparteine, lupanine or their metabolites in any species. No information is available to assess transfer rates of QAs in food of animal origin; however, based on indirect evidence, possible transfer to milk should be considered.’

Therefore, there is no evidence that QAs metabolise to any compounds other than related alkaloid structures, so no unexpected toxicologically relevant components are anticipated from the consumption of either lupins or food of plant origin treated with aqueous extract from the germinated seeds of sweet *Lupinus albus* which may contain low levels of QAs.

Overall, based on the available literature and limited data presented (in Volume 3 CA B7 and also Volume 4), the nature of residues in treated crops is expected to be the unchanged applied substance, aqueous extract from the germinated seeds of sweet

*Lupinus albus*, with the lead component BLAD, and some breakdown products which are smaller naturally occurring components such as amino acids, simple carbohydrates and lipids, which are indistinguishable from the composition of the treated plants.

### 2.7.3. Definition of the residue

As outlined in the botanicals guidance, SANCO/11470/2012 rev. 8, in the case of botanical active substances listed as food and feed, information on the nature and magnitude of residues is usually not necessary. The following entry is included in Part 1 of assimilated Regulation No. 396/2005:

MRL code number: 0260050-001, Common name: Lupins/lupini beans, Scientific name: *Lupinus albus* subsp. *albus*.

It is recognized that the MRL code applies to the beans/seeds, rather than the whole plant, or specifically the germinated seed (and extracts from this), however, the inclusion of this specific species in Part 1, provides significant support for residue definitions not being required. The active substance is derived from food grade sweet lupin seeds; it is not a food item itself as it is an extract from the germinated seeds which has been heated to evaporate and concentrate the material, but the germinated seeds and other crop fractions from lupins are used as a food item consumed by humans and livestock. The biological composition of the aqueous extract from the germinated seeds of sweet *Lupinus albus* is unlikely to be significantly different to the biological components present in the traded commodities which the above MRL code applies to. It should be noted that a range of proteins are present in the aqueous extract from the germinated seeds of sweet *Lupinus albus* but only BLAD as the lead component was identified and quantified in the submitted residue trials. A residue definition is not meaningful as the major component is a naturally occurring seed storage protein, and the range of other proteins present in the active substance are naturally occurring plant proteins, all of which are likely to degrade into amino acids. Additionally, the lead component and other components present in aqueous extract from the germinated seeds of sweet *Lupinus albus* are not unique to the application of the PPP therefore are not suitable for a residue definition (for either enforcement or risk assessment).

The evidence provided indicates that low levels of residues of either the aqueous extract from the germinated seeds of sweet *Lupinus albus* or the lead component BLAD are expected. Although there is uncertainty regarding the exact LOQ of the method used to determine levels of BLAD in treated crops, there is confidence that levels will be sufficiently low to not be a concern.

Based on the information provided in the residues section, **a residue definition for risk assessment is not appropriate**. It should be noted that this conclusion relates to the uses considered in this assessment only.

It should be noted that data comparing the levels of the proteins which are present in the aqueous extract from the germinated seeds of sweet *Lupinus albus* in untreated crops (natural background levels) has not been provided. This information would be helpful to support the cases made in support of this assessment and may be a way to support future uses. This is also of relevance for any future uses which comprise a significant portion of livestock diet and may trigger the consideration of possible residues in products of animal origin.

For inclusion of the substance in Part 4 of the GB MRL statutory register, the criteria outlined in the SANCO/11188/2013 rev. 2 guidance has been considered:

- The active substance is approved as a basic substance (criterion 1)
- The compounds is listed in Part 1 of assimilated Regulation 396/2005 (criterion 2)
- The compound has no identified hazardous properties (criterion 3)
- Natural exposure is higher than the one linked to the use as a PPP (criterion 4)
- No consumer exposure is foreseen linked to the mode of application of the PPP (criterion 5)

Criterion 1 and 5 are not met for this substance.

Regarding criterion 2, the aqueous extract from the germinated seeds of sweet *Lupinus albus* originates from Lupins/lupini beans listed in Part 1. Although the substance is not identical to what is listed in Part 1, as explained above, this provides support for inclusion in Part 4.

Regarding criterion 3, whilst a chronic toxicological reference value (ADI) has been established, it is a very high value demonstrating the low potency of this substance. An acute reference dose has not been established.

With regards to criterion 4, as explained in section 2.7.9, the exposure to residues of the aqueous extract from the germinated seeds of sweet *Lupinus albus* are likely to be lower than exposure to the naturally occurring components present in crops and the consumption of lupins/lupini beans.

The assessment of aqueous extract from the germinated seeds of sweet *Lupinus albus* does not fully meet the criteria for inclusion in Part 4 of the GB MRL statutory Register. However, the active substance does in part meet criterion 2, 3 and 4. Therefore it is proposed that aqueous extract from the germinated seeds of sweet *Lupinus albus* is included in Part 4 of the GB MRL statutory Register. The aqueous extract from the germinated seeds of sweet *Lupinus albus* **is exempt from MRLs and a residue definition for enforcement is not applicable.**

The guidance on the Part 4 criteria indicate that the assessment for inclusion on Part 4 should consider future uses where possible. A range of uses were proposed as



requiring the exemption from MRLs in addition to the representative uses. The conclusions above are applicable to these uses also; the total application rate is the same or less critical than the representative uses. The same justifications can be made regarding the UVCB substance and natural occurrence of the components. Extensions of uses beyond those currently considered for inclusion of the aqueous extract from the germinated seeds of sweet *Lupinus albus* in Part 4 of the GB MRL statutory Register will require further consideration. This may require the submission of additional information, including residue trials, so that the background exposure and exposure resulting from the uses of the PPP can be fully assessed.

A chronic toxicological reference value (ADI) has been set for the aqueous extract from the germinated seeds of sweet *Lupinus albus*. Therefore, an indicative risk assessment has been performed. This has been based on the residues of aqueous extract from the germinated seeds of sweet *Lupinus albus* in treated crops. As the toxicological reference value is based upon data generated with 'PROBLAD PLUS', this has been used as the residue definition for risk assessment for the purposes of the indicative consumer risk assessment within this evaluation only. It should be noted that further consideration of an appropriate residue definition for risk assessment may be required to support further uses of the aqueous extract from the germinated seeds of sweet *Lupinus albus*.

It is noted that the SANCO/11188/2013 rev. 2 guidance also states: "Careful consideration should be given to the appropriateness of including food and/or feed items into Annex IV which are known allergens. In such cases, a consumer exposure risk assessment might be warranted (see criterion 4)." A consideration of the aqueous extract from the germinated seeds of sweet *Lupinus albus* as an allergen has been made in the toxicology section of the evaluation (Volume 3 CA B6.5.8).

#### **2.7.4. Summary of residue trials in plants and identification of critical GAP**

A limited data set of field trials were submitted. The submitted trials were not conducted in line with the OECD guidelines and were not relevant to the representative GAPs under consideration: the trials were performed outdoors in the USA (therefore not clearly representative of the agronomic and climatic conditions of GB) and only two independent trials were provided for tomatoes and strawberries.

There is uncertainty regarding the stability of residues in crop samples upon storage. No storage stability data is available. No samples are stored for extremely extended time periods (maximum 117 days), and some samples are stored for less than 30 days. Comparable samples stored for less than or more than 30 days give mostly similar results, but there are more 'positive' results from the samples stored for less than 30 days. Overall, there is some uncertainty with the stability of residues in crop samples upon storage and therefore the results determined in trials where samples were stored for more than 30 days.

There is uncertainty regarding the validity of the analytical method used in the field trials. A validated LOQ was not able to be concluded upon. Low procedural recoveries were reported in the field trials (33.4-89.7%). The ELISA method used is not clearly appropriate for determining quantitative results. Additionally, the method used determined the content of the lead component BLAD, rather than the whole aqueous extract from the germinated seeds of sweet *Lupinus albus*. Overall, there is some uncertainty regarding the quantitation of the method and the LOQ.

However, the trials provide supporting information demonstrating that low residues are expected following the representative uses. Two of the trials were significantly overdosed (~4N) and result in low residues of BLAD (approximately < 0.03 0.6 mg/kg). **The field trials support residues of BLAD and the aqueous extract from the germinated seeds of sweet *Lupinus albus* being low in treated crops.**

For the QAs, secondary metabolites of known concern in lupin species, natural levels in sweet *Lupinus albus* seeds are outlined in the EFSA scientific Opinion on the presence of QAs in food and feed (EFSA, 2019) and are presented in Table 2.7.4-1.

**Table 2.7.4-1: Summary of levels of QAs in sweet *Lupinus albus***

Component	Range of levels determined (mg/kg)
Lupanine	26-1,378
13 $\alpha$ -OH-Lupanine	3-157
13 $\alpha$ -Angeloyloxylupanine	Not detected-88
Albine	10-162
Angustofoline	Not detected-65
13 $\alpha$ -Tigloyloxylupanine	Not detected-44
$\alpha$ -Isolupanine	Not detected-22
Tetrahydrorhombifoline	1.0-13
Total	50-1,656

The levels of all the QAs in the aqueous extract from the germinated seeds of sweet *Lupinus albus* are given in Volume 4. These are within the levels reported in the EFSA scientific Opinion. Therefore, as the possible levels of QAs in treated crops are

well below the levels which may occur naturally in sweet *Lupinus albus*, no further consideration of residue levels in crops treated with PROBLAD PLUS are required.

#### **2.7.5. Summary of feeding studies in poultry, ruminants, pigs and fish**

No livestock metabolism or feeding studies have been submitted or are required.

The proposed representative uses of PROBLAD PLUS are on strawberries and tomatoes which are not used as feed for livestock, therefore metabolism data is not required considering these representative uses. The uses proposed to support MRLs beyond the representative uses also do not comprise a significant portion of livestock diets.

#### **2.7.6. Summary of effects of processing**

No data to address the effects of processing on the residues have been provided. Assimilated Regulation No. 283/2013 outlines that if residues in products of plant origin are < 0.01 mg/kg, further consideration of processing is not required. In this case, there is confidence that the expected residues will be low, although there is uncertainty regarding the validated LOQ.

It is noted from the literature that BLAD is stated to have ‘high stability against denaturation, withstanding boiling, treatment with organic solvents and detergents, and exposure to high concentrations of strong acids, as long as they do not cleave its peptide bonds’. This suggests that BLAD will remain stable during processing under these conditions, however this is not further supported by data or evidence, such as data on the physical and chemical properties of the material. There is also confidence from the assessment that residues of the aqueous extract from the germinated seeds of sweet *Lupinus albus* will be readily biodegraded when present in/on treated plants, due to naturally occurring proteases/ biodegradation. There is confidence that the aqueous extract from the germinated seeds of sweet *Lupinus albus* will break down into naturally occurring amino acids. Low levels of BLAD are expected to remain in the treated crops, which may be stable upon typical processing conditions.

Given the low levels expected in treated crops, and nature of the residue, there is sufficient information and confidence that there are no concerns with residues in processed commodities. No further information is required to address the effects of processing on residues of aqueous extract from the germinated seeds of sweet *Lupinus albus*.

#### **2.7.7. Summary of residues in rotational crops**

Although a moderate to high persistence in soil is suggested from the DT<sub>50</sub> estimates presented in B.8.1.4.1 (which is based on calculations from estimates of the chemical structure rather than data), in reality, as demonstrated in the biodegradation studies, aqueous extract from the germinated seeds of sweet *Lupinus albus* will be subject to biological degradation (likely via proteases) into natural components (amino acids) in

the soil after application, and not persist in the natural environment. Therefore, PROBLAD PLUS is considered to be readily biodegradable in soil (see Volume 3 CA B8.2.2.1) and no further consideration of residues in rotational crops is required.

#### **2.7.8. Summary of other studies**

The proposed GAP does permit application during flowering (BBCH 61-89). In accordance with SANTE/11956/2016 rev.9, tomatoes are not considered melliferous therefore no further consideration is required. Strawberries are considered melliferous therefore exposure to honeybees cannot be ruled out on this basis.

Based on the low levels of BLAD found in plants in the studies provided to address plant metabolism and magnitude of residues in plants, significant residues are unlikely to be found in the flowering parts of strawberry plants. Hence, significant residues are not expected to be present in bee products. Additionally, the aqueous extract from the germinated seeds of sweet *Lupinus albus* is comprised of naturally occurring seed storage proteins found in lupin plants, which are themselves foraged by bees. Therefore, the components that bees are potentially exposed to are not a concern in terms of potential residues in honey for the assessment of consumer exposure.

Overall, no further consideration of residues in bee products is required.

#### **2.7.9. Estimation of the potential and actual exposure through diet and other sources**

An acute reference value is not necessary as there is no evidence of acute effects following the ingestion of aqueous extract from the germinated seeds of sweet *Lupinus albus*. Therefore, an acute consumer risk assessment is not required.

As a chronic toxicological reference value has been set for the aqueous extract from the germinated seeds of sweet *Lupinus albus*, an indicative risk assessment has been performed. As the toxicological reference value is based upon data generated with 'PROBLAD PLUS', this has been used as the residue definition for risk assessment.

This has been based on the residues of aqueous extract from the germinated seeds of sweet *Lupinus albus* in treated crops. There is some uncertainty in the quantification of results from the field trials provided. The data indicate residues of BLAD < 0.03 0.6 mg/kg but there are uncertainties with the data (storage stability, validated LOQ, representativeness of trials). Therefore, a worst-case estimate of potential residues and intakes has been calculated.

To exceed the acceptable daily intake of 5 mg/kg bw/day, considering the chronic consumption of tomatoes and strawberries in both the UK and EU PRIMo models, over 1000 mg/kg of residue of the aqueous extract from the germinated seeds of sweet *Lupinus albus* would need to be present on the treated crops. Note: This

estimate results from considering the highest chronic consumption of commodities and inputting arbitrary residue levels into the consumer exposure models.

There are multiple orders of magnitude between the levels observed in the field trials data and the levels required to pose any adverse health effects. Therefore, there is sufficient confidence that no adverse health effects are expected as a result of the proposed uses.

It should be noted for context that lupin can be harvested as a legume (fresh) or as a pulse (dry). There is no specific consumption data for lupin as a legume or a pulse in either the UK or EU PRIMo consumer risk assessment models. However, if lupin was harvested as a legume, the MRL commodity code for lentils (0260050) would apply; there is consumption data for fresh lentils with critical consumption reported as 40 mg/kg bw/day (which would include fresh lupins). If lupin was harvested as a pulse, lupin has its own commodity code (0300040) but there is no consumption data associated with this code. The critical consumption of the similar crop dry lentils (commodity code 0300020) is 226.8 mg/kg bw/day; the consumption of dry lupins is expected to be significantly below this.

~~It should be noted for context, that the consumption of lentils (encompassing the commodity code for lupin) reported in the EU PRIMo model is 40 mg/kg bw/day. There is no specific consumption data for lupin in either the UK or EU PRIMo consumer risk assessment models.~~

Considering the other uses proposed to support setting MRLs for uses beyond the representative uses, similar conclusions can be made. To exceed the acceptable daily intake of 5 mg/kg bw/day, considering the chronic consumption of the commodities for which uses are proposed in both the UK and EU PRIMo models, over 300 mg/kg of residue of the aqueous extract from the germinated seeds of sweet *Lupinus albus* would need to be present on the treated crops. As above, there are multiple orders of magnitude between the levels observed in the field trials data and the levels required to pose any adverse health effects. Note: This estimate results from considering the highest chronic consumption of commodities and inputting arbitrary residue levels into the consumer exposure models.

For future uses, if expected residues are higher than the low levels presented in this assessment, reliable quantitative data may be required to support the consumer risk assessment.

#### **2.7.10. Proposed MRLs and compliance with existing MRLs**

It is proposed that aqueous extract from the germinated seeds of sweet *Lupinus albus* is exempt from MRLs and therefore it is proposed that it is included in Part 4 of the GB MRL Statutory Register.

### 2.7.11. Proposed import tolerances and compliance with existing import tolerances

The other uses proposed to support setting MRLs for uses beyond the representative uses relate to possible future uses in GB. The residues assessment has considered these uses also. The aqueous extract from the germinated seeds of sweet *Lupinus albus* is proposed to be included in Part 4 of the GB MRL Statutory Register in relation to these uses also.

## 2.8. Fate and behaviour in the environment

Standard laboratory studies to determine the route of degradation in soil, surface water and sediment have not been provided. The rate of degradation in these environmental compartments has been estimated based upon standard ready biodegradability tests and following ECHA Guidance (ECHA, 2017). Aqueous extract from the germinated seeds of sweet *Lupinus albus* and the major component BLAD were demonstrated to be readily biodegradable.

Based upon the taxonomy and current knowledge the legume plant family is understood to contain quinolizidine alkaloids (QAs) which are components that contain the quinolizidine structure. The QAs have been identified as relevant impurities (SANCO/10597/2003 –rev. 10.1) and SANCO/11470/2012 rev. 8, indicates these alkaloids as components of possible concern for humans, animals and/or the environment and hence the aqueous extract from the germinated seeds of sweet *Lupinus albus* is considered a 'group 2' botanical active substance. There are many quinolizidine alkaloids present at very low levels within the active substance and lupanine has been indicated as a marker compound for these impurities. Whilst it is noted that this marker compound is not considered to be a relevant impurity according to the final HSE assessment, the predicted levels in groundwater have been detailed by the applicant and included for completeness.

### 2.8.1. Summary of fate and behaviour in soil

The degradation of the aqueous extract from the germinated seeds of sweet *Lupinus albus* has been addressed by the submission of a ready biodegradability study that indicates the active substance and the lead component BLAD are readily biodegradable. The ECHA 2017 guidance notes that substances that demonstrate high sorption properties can be subject to a longer soil DT50. Based on this a DT50 of 300 days in soil for the aqueous extract from the germinated seeds of sweet *Lupinus albus* and the lead component BLAD has been used in the risk assessment determined. Therefore, the aqueous extract from the germinated seeds of sweet *Lupinus albus* potentially could exhibit moderate to high persistence in soil considering the ECHA Guidance (ECHA, 2017).

Whilst this DT50 would trigger the need for an accumulation assessment it is noted that there is additional qualitative evidence to indicate that the aqueous extract from



the germinated seeds of sweet *Lupinus albus* would likely be subject to biological degradation in soil and not persist in the natural environment. Following ECP advice the applicant provided further supporting information demonstrating protease levels in the soil, and likely rapid degradation of similar sized peptides in the soil environment. Based upon a weight of evidence approach using the submitted ready biodegradability data, the additional supporting information from the literature and the in vitro testing submitted by the applicant a proposal of 30 days DT50 can be supported for future assessments of the aqueous extract from germinated seed of sweet *Lupinus albus*. Therefore, soil accumulation studies are not required. The PEC values presented below are acceptable to address the risks as will be conservative values.

Standard laboratory studies were not available for the determination of the mobility of aqueous extract from the germinated seeds of sweet *Lupinus albus* in soil. A quantitative structure property relationships (QSPR) model estimation of adsorption was made for protein BLAD. ECP independent expert advice was sought on this methodology. Members agreed that this approach is not appropriate for the determination of K<sub>oc</sub> for a large polypeptide such as BLAD. However, based on the substance properties it was agreed that the BLAD is likely an immobile substance and the use of a default K<sub>oc</sub> to address this in the modelling was accepted. For the marker compound lupanine, QSPR and Molecular Connectivity Index (MCI) estimations of adsorption were made. The outcome indicated that BLAD would likely be an 'immobile' substance while lupanine was indicated to would exhibit moderate to low mobility in soil.

### 2.8.2. Summary of fate and behaviour in water and sediment

The degradation of the aqueous extract from the germinated seeds of sweet *Lupinus albus* has been addressed by the submission of a ready biodegradability study that indicates the active substance and the lead component BLAD are readily biodegradable. Therefore, the active substance and the lead component BLAD are considered to have low persistence in surface waters and sediment and biological degradation is considered to be the most significant route of dissipation.

### 2.8.3. Summary of fate and behaviour in air

HSE has concluded that this high molecular weight compound will likely have a low vapour pressure and there is additional information in the published literature indicating that the lead component BLAD can withstand prolonged boiling which would support the indicated low volatility of this substance (Monteiro et al. 2015). In addition, the aqueous extract from the germinated seeds of sweet *Lupinus albus* has a boiling point at 100°C (Volume 3 CA B.2), which would indicate a low vapour pressure for this aqueous extract. Volatilisation to air following application to soil and plant surfaces is not anticipated, and route and rate of degradation in air studies are not considered necessary.

#### **2.8.4. Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products**

No monitoring data are provided for this new active substance.

#### **2.8.5. Definition of the residues in the environment requiring further assessment**

The following compounds are to be considered for the environmental risk assessment:

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

Groundwater: BLAD and lupanine

Surface Water: Aqueous extract from the germinated seeds of sweet *Lupinus albus*

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

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

#### **2.8.6. Summary of exposure calculations and product assessment**

Soil: PEC<sub>Soil</sub> values for the proposed use on strawberry have been calculated using standard methodology (i.e. 5 cm depth and 1.5 g/cm<sup>3</sup> bulk density) and based on the proposed use at 6 applications of 4016 g/ha, an 8 day treatment interval, 60% crop interception and a DT50 of 300 days. On this basis the PEC<sub>Soil</sub> initial value would be 12.28 mg/kg (equivalent to 2.46 mg/kg of the lead component BLAD based on an assumed BLAD content of 20%). These exposures will be protective of the proposed use on tomato.

It is noted that the degradation endpoint used here is determined from a screening study, and there is supporting evidence presented in Volume 3 CA B.8.1 that this substance will biodegrade and be subject to microbial degradation in soil. The use of a DT50 of 300 days is therefore understood as being highly conservative and consideration of accumulation in the terrestrial environment is not considered necessary.

Groundwater: Leaching simulations for BLAD and lupanine were conducted with the FOCUS groundwater scenarios relevant to the UK in FOCUS PEARL (version 4.4.4) and FOCUS PELMO (version 5.5.3). The simulations were based on application of PROBLAD PLUS to strawberry and tomatoes. Six applications at a rate of 4016 g a.s/ha with an interval of eight days were simulated. Crop interception values



according to the EC groundwater guidance were used resulting in 60% interception for strawberry at BBCH 61 and 80% interception for tomato at BBCH 61.

For the lead component BLAD a DT50 of 300 days in soil was used and based upon on the **substance properties-QSPR-modelling** a Kfoc of 10,000 was used to represent an 'immobile' compound. It is noted that lupanine is not indicated as a relevant impurity in the aqueous extract from the germinated seeds of sweet *Lupinus albus*. However, quinolizidine alkaloids are **considered to be relevant impurities according to SANCO/10597/2003 rev.10.1 and are** detailed as substances of potential concern and in accordance with SANCO/11470/2012 rev. 8, the aqueous extract from the germinated seeds of sweet *Lupinus albus* is considered a 'group 2' botanical. Therefore, the groundwater modelling for lupanine was assessed using the calculated Kfoc values of 57.3 and 1287 and DT50 values of 30 and 300 days respectively. In all cases a default 1/n of 1 was used.

Based upon the above approaches the predicted 80th percentile average annual concentrations of BLAD and lupanine in groundwater from both PEARL and PELMO were at < 0.001 µg/L in all scenarios.

Surface water and sediment: PEC<sub>sw</sub> values via spray drift for the proposed uses on strawberry and tomato have been calculated for the aqueous extract from the germinated seeds of sweet *Lupinus albus* and the lead component BLAD using standard methodology for broadcast application via horizontal boom sprayer. PEC<sub>sw</sub> values for a single and multiple applications have been considered resulting in PEC<sub>sw</sub> values for the aqueous extract from germinated seeds of sweet *Lupinus albus* of 37.01 µg/L and 63.3 µg/L respectively. This would equate to PEC<sub>sw</sub> spraydrift values for the lead component BLAD at 7.42 µg/L and 12.66 µg/L for a single and multiple applications respectively.

The PEC<sub>sw</sub> via drainflow values have been determined for this multiple application use by deriving the application rate from the peak PEC<sub>soil</sub> initial value. Based upon the proposed GAP on strawberry an initial PEC<sub>soil</sub> value for the aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) of 12.277 mg/kg was calculated. A Koc value of 1000 mL/g, was used as a conservative value to cover the range of Koc values representing the constituents of the aqueous extract, at 1287-10,000 L/kg. This tier 1 drainflow assessment would predict a PEC<sub>sw</sub> (drainflow) value for the aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) at 14.166 µg/L which would equate to PEC<sub>sw</sub> drainflow value of 2.83 µg/L for the lead component BLAD.

As the available ecotoxicological data provided did not include sediment dosed studies no PEC<sub>sed</sub> values have not been determined.

Air: Predicted exposures in air have not been determined.

Other routes of exposure: Studies estimating concentrations for other routes of exposure of the plant protection product are not required.

## 2.9. Effects on non-target species

### 2.9.1. Summary of effects on birds and other terrestrial vertebrates

#### Birds

No toxicity data is available to address the acute and long-term toxicity to birds for the active substance. A variety of published literature studies have been provided which investigate the inclusion of lupin seeds in avian diets. These are summarised and evaluated with respect to their relevance and reliability in Volume 3 CA B9. To achieve a quantitative risk assessment, the acute mammalian endpoint has been used in the acute risk assessment for birds as the literature studies indicate this LD<sub>50</sub> would be protective for birds.

**Table 2.9.1-1: Summary of endpoints used to assess risk from aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) to birds**

Test substance	Test type	Test Species	Endpoint	Value	Reference (Author, date)
PROBLAD PLUS	Acute Oral	Rat	LD <sub>50</sub>	> 5000 mg a.s./kg bw	██████████ (2012a)

No consideration of chronic oral toxicity to birds was considered to be necessary, since the lead component BLAD has a mode of action specific to fungi and has been demonstrated to be readily biodegradable and susceptible to proteolytic degradation. It is therefore considered unlikely that BLAD would have toxic effects in a reproductive or developmental capacity. Additionally, the available literature data did not indicate adverse effects on adult birds over extended time periods (up to 42 days).

#### Mammals

Acute toxicity data have been provided and considered within the human health assessment (Volume 3 CA B6). Endpoints for use in the mammalian risk assessment have been established for acute toxicity only. No consideration of the long-term toxicity was deemed necessary. The following endpoint has been used in the risk assessment.

**Table 2.9.1-2: Summary of endpoints used to assess risk from aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) to mammals**

Test substance	Test type	Test Species	Endpoint	Value	Reference (Author, date)
PROBLAD PLUS	Acute Oral	Rat	LD <sub>50</sub>	> 5000 mg a.s./kg bw/d	██████████ (2012a)

No consideration of chronic oral toxicity to mammals was considered to be necessary, since the lead component BLAD has a mode of action specific to fungi and has been demonstrated to be readily biodegradable and susceptible to proteolytic degradation. BLAD would be broken down under enzymatic processes in the gastrointestinal tract, enter the amino acid pool and be consumed into normal metabolic processes. It is therefore considered unlikely that BLAD would have toxic effects in a reproductive or developmental capacity. Additionally, the available literature data did not indicate adverse effects on mammals over extended time periods (up to 90 days).

### **Endocrine disruption assessment for birds and mammals**

No endocrine disruptor studies were conducted or were considered necessary in the human health assessment (Volume 3CA B6), nor are they considered necessary for the ecotoxicology assessment. The main components are naturally occurring proteins, lipids and carbohydrates and will already contribute a large portion of the diet of terrestrial vertebrates. No indications of toxicity or sub-lethal effects were demonstrated in literature studies investigating inclusion of lupins in bird and mammal diets and there were no indications in the 90-day oral study in the rat, of adverse effects on the endocrine system. HSE considered the literature review acceptable for the endocrine disruption ecotoxicology assessment. Overall, no further consideration was required for endocrine disruption in birds and mammals.

### **2.9.2. Summary of effects on aquatic organisms**

Toxicity data to address aqueous extract from the germinated seeds of sweet *Lupinus albus* (tested as PROBLAD PLUS) have been provided. First tier toxicity data used in the risk assessment are summarised here (Table 2.9.2-1).

**Table 2.9.2-1: First tier aquatic toxicity data relevant to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS).**

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
Acute toxicity to fish					
PROBLAD PLUS	Oncorhynchus mykiss	96 hours, semi-static	LC <sub>50</sub>	> 100 Supporting information only	<div></div> (2011)
Chronic toxicity to fish					
No data submitted or considered necessary. PROBLAD PLUS and lead component BLAD have been demonstrated to be readily biodegradable so chronic exposure via water was considered unlikely. Additionally, lupins are widely used as a feedstuff in aquaculture and available literature studies do not indicate adversity.					
Acute toxicity to invertebrates					
PROBLAD PLUS	Daphnia magna	48-hours, semi-static	<u>EC<sub>50</sub></u> (mm)	<u>&gt; 75</u>	Gerke, A.K. and Schneider, S.Z. (2019)
Long-term toxicity to invertebrates					
PROBLAD PLUS	Daphnia magna	21-days, static	EC <sub>50</sub> (mm)	> 2.7	Gerke, A.K. and Schneider, S.Z. (2019)
			<u>NOEC</u> (mm)	<u>2.7</u>	
Toxicity to sediment dwelling organisms					
No data submitted or considered necessary. PROBLAD PLUS and lead component BLAD have been demonstrated to be readily biodegradable so residues in water will be rapidly degraded. It is noted that the high Koc of BLAD would indicate that partitioning to sediment would be likely to occur. In the absence of specific data with sediment-dwelling organisms, the margin of safety in the chronic aquatic invertebrate risk assessment is considered.					

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
Toxicity to algae					
PROBLAD	Raphidocelis subcapitata	72- hours, static	<u>E<sub>r</sub>C<sub>50</sub></u> <u>(mm)</u>	<u>51</u>	Arnie et al. (2019)
			NOEC (mm)	6.6	
Toxicity to aquatic macrophytes					
No data submitted or considered necessary. The active substance is not a herbicide or plant growth regulator.					

nom. = nominal; m.m. = arithmetic mean measured; g.m. = geometric mean measured.

Underlined values are recommended for use in risk assessment.

### Endocrine disruption assessment for aquatic organisms:

Two literature studies were identified as potentially relevant to endocrine disruption as a result of literature search CEV/02/01-LRR3, 2019 (see Volume 3 CA B.9.11 for summary). Having reviewed the studies, HSE considers them both to be reliable with reservations, but of limited relevance for the assessment of aqueous extract from the germinated seeds of sweet *Lupinus albus*. Both studies focussed on the effects of specific alkaloids found in lupins, rather than a whole lupin extract. Additionally, the exposure was via diet, rather than via water. Histopathological examinations were made of liver tissues, but the measured parameters would not be sufficient for investigation into EATS-mediated activity.

As previously stated, the main components of aqueous extract from the germinated seeds of sweet *Lupinus albus* are water, proteins and carbohydrates which are already likely to form part of the diet of aquatic organisms. Although there is some uncertainty in relying on dietary exposure studies to assess the risk via aquatic exposure, the published literature does not indicate any adverse effects from inclusion of lupin seeds in fish diets. Therefore, HSE considers that no further consideration of endocrine disruption is required.

### 2.9.3. Summary of effects on arthropods

#### Bees

The available toxicity endpoints for bees are summarised in the table below.

**Table 2.9.3-1: Effects on bees relevant to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)**

Study	Endpoint	Value	Reference
PROBLAD: Acute Oral and Contact Toxicity to the Honeybee, <i>Apis mellifera</i> L., in the laboratory	LD <sub>50</sub> Contact:	<u>&gt; 100 µg product /bee</u>	K-CA 8.3.1.1.1/01 Kling, 2010.
	LD <sub>50</sub> Oral:	<u>&gt; 109.42 µg product /bee</u>	
PROBLAD PLUS: Acute oral toxicity to the Honey bee, <i>Apis mellifera</i> L., under laboratory conditions.	LD <sub>50</sub> Oral:	> 196.8 µg product /bee	K-CA 8.3.1.1.1/02 Aguilar-Alberola, 2019.
PROBLAD PLUS: Acute oral and contact Toxicity to the Bumblebee <i>Bombus terrestris</i> L., under Laboratory Conditions	LD <sub>50</sub> Oral :	> 2320.9 µg product /bee	K-CA 8.3.1.1.2/02 Aguilar-Alberola, 2020
	LD <sub>50</sub> Contact :	> 1200.0 µg product /bee	
PROBLAD PLUS: Chronic Oral toxicity test (10-day feeding) to the Honey bee ( <i>Apis mellifera</i> L.) under laboratory conditions	10-day LDD <sub>50</sub> :	361.9 µg PROBLAD PLUS bee/day	K-CA 8.3.1.2/02 Aguilar-Alberola, 2019.
PROBLAD PLUS: Honeybee ( <i>Apis mellifera</i> L.) larval toxicity test following repeated	EC <sub>50</sub> larval:	> 154.0 µg PROBLAD PLUS/larva	K-CA 8.3.1.3/02 Aguilar-Alberola, 2019.

exposure under laboratory conditions			
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Endpoints underlined will be used in the risk assessment.

### Non-target arthropods other than bees

The toxicity endpoints for non-target arthropods other than bees are summarised in the table below.

**Table 2.9.3-2: Effects on non-target arthropods other than bees relevant to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)**

Species	Endpoint	Value (mL product/ha)	Reference
<b>First tier studies</b>			
<i>A. rhopalosiphi</i> , glass plate 2D exposure,	LR <sub>50</sub>	> 10500 mL PROBLAD /ha	K-CA 9.3.2-01 Klug, 2010
<i>T. pyri</i> , glass plate exposure, 2D	LR <sub>50</sub>	> 10500 mL PROBLAD /ha	K-CA 9.3.2-02 Klug, 2010
<b>Extended laboratory studies</b>			
<i>C. carnea</i> , French bean leaf discs, 2D exposure	LR <sub>50</sub>	> 10240 mL PROBLAD PLUS in 200 L water/ha	K-CP 9.5.2-1 Vaughan, 2017
	Highest rate observed with < 50% effects on reproduction	> 10240 mL PROBLAD PLUS in 200 L water/ha	
<i>T. pyri</i> , French bean leaf discs, 2D exposure	LR <sub>50</sub>	> 8000 mL PROBLAD PLUS in 200 L water/ha	K-CA 9.3.2-03 Fallowfield, 2014

Species	Endpoint	Value (mL product/ha)	Reference
	Highest rate observed with < 50% effects on reproduction	> 8000 mL PROBLAD PLUS in 200 L water/ha	
A. rhopalosiphi, Barley seedlings, 3D exposure	LR <sub>50</sub>	> 8000 mL PROBLAD PLUS in 400 L water/ha	K-CA 9.3.2-04 Stevens, 2014
	Highest rate observed with < 50% effects on reproduction	> 8000 mL PROBLAD PLUS in 400 L water/ha	

#### 2.9.4. Summary of effects on non-target soil meso- and macrofauna

##### Earthworms and other soil macro-organisms

Earthworm toxicity data has been provided with the aqueous extract from the germinated seeds of sweet *Lupinus albus* (tested as PROBLAD PLUS). This is summarised in Table 2.9.4-1.

No toxicity data has been provided with the soil macro-organisms *Folsomia candida* or *Hypoaspis aculeifer*. In accordance with assimilated Regulation No 283/2013, for plant protection products applied as a foliar spray, “if data are available on both *Aphidius rhopalosiphi* and *Typhlodromus pyri* these may be used in an initial risk assessment” for assessing the risks to soil organisms other than earthworms. Given that an acceptable risk to both *A. rhopalosiphi* and *T. pyri* was demonstrated using standard Tier I glass plate studies, it is considered that no data are required on the soil macro-organisms *F. candida* and *H. aculeifer*.



**Table 2.9.4-1: First tier earthworm toxicity data relevant to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS).**

<b>Test substance</b>	<b>Test organism</b>	<b>Endpoint (mg/kg soildw)</b>		<b>Reference</b>
PROBLAD PLUS	Eisenia andrei	NOEC (reproduction)	100	Friedrich (2017). KCA 8.4.1/02
		NOEC <sub>(CORR)</sub> <sup>1</sup>	50	
		EC <sub>10</sub>	> 100	
		EC <sub>10(CORR)</sub> <sup>1</sup>	> 50	
PROBLAD PLUS	Eisenia andrei	NOEC (reproduction)	125	Antón (2020). KCA 8.4.1/03
		<u>NOEC<sub>(CORR)</sub><sup>1</sup></u>	<u>62.5</u>	
		EC <sub>10</sub>	161.3	
		EC <sub>10(CORR)</sub> <sup>1</sup>	80.65	

<sup>1</sup>Endpoints are corrected by a factor of 2 due to worst case assumption of log Pow > 2. Endpoints considered in the risk assessment are underlined.

### **2.9.5. Summary of effects on soil nitrogen transformation**

A study on nitrogen transformation in soil is available for aqueous extract from the germinated seeds of sweet *Lupinus albus* (tested as PROBLAD PLUS).

**Table 2.9.5-1: First tier soil nitrogen transformation rate study relevant to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS).**

<b>Test substance</b>	<b>Reference</b>	<b>Endpoint</b>
PROBLAD PLUS	Ganssmann (2010b)	No effects on soil nitrogen transformation (< 25% deviation from control) at concentrations up to <u>52 mg PROBLAD PLUS/kg dry soil</u>

#### **2.9.6. Summary of effects on terrestrial non-target higher plants**

A study assessing the toxicity of PROBLAD PLUS has been conducted with six plant species according to vegetative vigour methods. A second study assessing the toxicity of PROBLAD PLUS according to both seedling emergence and vegetative vigour methods was submitted. The endpoints used in the risk assessment are summarised below.

**Table 2.9.6-1: Summary of effects on non-target plants following exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)**

Study reference	Test species	Vegetative vigour	Seedling emergence
		ER <sub>50</sub> L PROBLAD PLUS/ha	
KCA 8.6.1/01 Peterek S. (2011)	Brassica napus (oilseed rape)	> 2.0	-
	Cucumis sativus (cucumber)	> 2.0	-
	Lactuca sativa (lettuce)	> 2.0	-
	Lycopersicon esculentum (tomato)	> 2.0	-
	Zea mays (maize)	> 2.0	-
	Allium cepa (onion)	> 2.0	-
KCA 8.6.2/01 Huerta, F. (2020)	Brassica napus (oilseed rape)	> 3.2	> 3.2
	Cucumis sativus (cucumber)	> 3.2	> 3.2
	Lactuca sativa (lettuce)	> 3.2	> 3.2
	Lycopersicon esculentum (tomato)	> 3.2	> 3.2
	Zea mays (maize)	> 3.2	> 3.2
	Allium cepa (onion)	> 3.2	> 3.2

### **2.9.7. Summary of effects on other terrestrial organisms (flora and fauna)**

No additional data were submitted or considered to be necessary.

### **2.9.8. Summary of effects on biological methods for sewage treatment**

No study was considered necessary to assess the potential risk to biological sewage treatment systems since PROBLAD PLUS and the lead component, BLAD, were demonstrated to be readily biodegradable.

### **2.9.9. Summary of product exposure and risk assessment**

#### **2.9.9.1. Risk assessment for birds and mammals**

#### **Birds**

##### **Acute dietary risk**

The results of the risk assessment for the active substance are summarised here. Risk assessments were conducted according to EFSA Bird and Mammal Guidance Document (2009). The risk to birds from the active substance was assessed based on the proposed uses on tomatoes and strawberries. Six applications at 4.016 kg/ha between BBCH 61-89 were assessed. Only acute risks were assessed quantitatively. No consideration of the chronic risk was considered necessary since PROBLAD PLUS and BLAD have been demonstrated to be readily biodegradable, and the major components are naturally occurring proteins, lipids and carbohydrates that are already present in avian diets.

The screening step did not demonstrate an acceptable acute risk to birds. The DDD was determined to be 1211.7 mg/kg bw/d and the TER was 4.12. An acceptable risk was demonstrated at first tier since the TER values exceeded the trigger value of 10, as shown below:

**Table 2.9.9.1-1: Summary of the first tier acute bird risk assessment for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)**

<b>Intended use</b>		Tomatoes and strawberries BBCH 61-89 (field uses)				
<b>Test substance</b>		PROBLAD PLUS				
<b>Application rate (kg/ha)</b>						
<b>Acute toxicity (mg/kg bw)</b>						
<b>TER criterion</b>		10				
<b>Crop scenario</b>	<b>Generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>a</sub></b>	
<b>Tomatoes</b>						
Fruiting vegetables Fruit stage BBCH 71-89	Frugivorous bird “crow”	57.4	1.9	437.98	11.4	
Fruiting vegetables BBCH ≥ 50	Small granivorous bird “finch”	7.4	1.9	56.46	88.6	
Fruiting vegetables BBCH ≥ 50	Small omnivorous bird “lark”	7.2	1.9	54.94	91.0	
Fruiting vegetables Fruit stage BBCH 71-89	Frugivorous bird “starling”	49.4	1.9	376.94	13.3	

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Fruiting vegetables BBCH $\geq$ 20	Small insectivorous bird “wagtail”	25.2	1.9	192.29	26.0
<b>Strawberries</b>					
Strawberries BBCH $\geq$ 40	Small omnivorous bird “lark”	9.6	1.9	73.25	68.3
Strawberries Late (Flowering/ development of fruit/ maturity of fruit) BBCH 61-89	Frugivorous bird “starling”	27	1.9	206.02	24.3
Strawberries BBCH $\geq$ 20	Small insectivorous bird “wagtail”	25.2	1.9	192.29	26.0

**Drinking water**

An acceptable acute risk to birds from contaminated drinking water was established for the puddle scenario by considering the ratio of effective application rate to relevant endpoint. The leaf scenario was not relevant for the proposed use pattern. Chronic drinking water exposure was not considered relevant (see Volume 3 CP B9 for discussion).

**Secondary poisoning**

Consideration of the risks to earthworm-eating and fish-eating birds was not considered to be necessary (see Volume 3 CP B9 for discussion).

**Overall conclusion for birds**

The risk to birds from aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) is considered to be acceptable for the proposed use.

**Mammals**

The results of the risk assessment for the active substance are summarised here. Risk assessments were conducted according to EFSA Bird and Mammal Guidance Document (2009). The risk to mammals from the active substance was assessed based on the proposed uses on tomatoes and strawberries. Six applications at 4.016 kg/ha between BBCH 61-89 were assessed. Only acute risks were assessed quantitatively. No consideration of the chronic risk was considered necessary since PROBLAD PLUS and BLAD have been demonstrated to be readily biodegradable, and the major components are naturally occurring proteins, lipids and carbohydrates that are already present in mammalian diets.

### **Acute dietary risk**

The screening step did not demonstrate an acceptable acute risk to mammals for either the use on tomatoes or the use on strawberries. For strawberries, the DDD was determined to be 903.4 mg/kg bw/d and the TER was 5.5. For tomatoes, the DDD was determined to be 1040.8 mg/kg bw/d and the TER was 4.8. An acceptable risk was demonstrated at first tier since the TER values exceeded the trigger value of 10, as shown in below:

**Table 2.9.9.1-2: Summary of the first-tier acute mammalian risk assessment for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)**

<b>Intended use</b>		Tomatoes and strawberries BBCH 61-89 (field uses)				
<b>Test substance</b>						
<b>Application rate (kg/ha)</b>						
		6 × 4.016				
<b>Acute toxicity (mg/kg bw)</b>		> 5000				
<b>TER criterion</b>						
		10				
<b>Crop scenario</b>	<b>Generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>a</sub></b>	
<b>Tomatoes</b>						
Fruiting vegetables Fruit stage BBCH 71-89	Frugivorous mammal “rat”	45.2	1.9	344.9	14.5	
Fruiting vegetables BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1.9	41.2	121.4	
Fruiting vegetables BBCH ≥ 50	Small herbivorous mammal “vole”	40.9	1.9	312.1	16.0	
Fruiting vegetables BBCH ≥ 50	Small omnivorous mammal “mouse”	5.2	1.9	39.7	125.9	



<b>Strawberries</b>					
Strawberries BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4	1.9	41.2	121.4
Strawberries BBCH ≥ 40	Small herbivorous mammal “vole”	54.6	1.9	416.6	12.0
Strawberries BBCH ≥ 40	Large herbivorous mammal “lagomorph”	14.0	1.9	106.8	46.8
Strawberries BBCH ≥ 40	Small omnivorous mammal “mouse”	6.9	1.9	52.6	95.1

### Drinking water

An acceptable acute risk to mammals from contaminated drinking water was established for the puddle scenario by considering the ratio of effective application rate to relevant endpoint. Chronic drinking water exposure was not considered relevant (see Volume 3 CP B9 for discussion).

### Secondary poisoning

Consideration of the risks to earthworm-eating and fish-eating mammals was not considered to be necessary (see Volume 3 CP B9 for discussion).

### Overall conclusion for mammals

The risk to mammals from aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) is considered to be acceptable for the proposed use.

#### 2.9.9.2. Risk assessment for aquatic organisms

The result of the risk assessment for the active substance is summarised here. Risk assessments were conducted according to Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters: EFSA journal 2013;11(7):3290.

**Tier 1 aquatic risk assessment for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)**

Table 2.9.9.2-1 shows the aquatic risk assessment for surface water and drainflow for the proposed uses on tomatoes and strawberries at 6 x 3.2L/ha.

**Table 2.9.9.2-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)**

Scenario	PEC (µg/L)	Aquatic invertebrates acute	Aquatic invertebrates long-term	Algae
		D. magna	D. magna	R. subcapitata
		RAC (EC <sub>50</sub> )	RAC (NOEC)	RAC (E <sub>r</sub> C <sub>50</sub> )
		750 [µg/L]	270 [µg/L]	5100 [µg/L]
<b>Spray-drift (1 m)</b>	63.3	0.0844	0.234	0.0124
<b>Drainflow</b>	14.166	0.019	0.052	0.0028

Based on the assessment above, an acceptable risk from spray drift and drainflow exposure was demonstrated for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) for the proposed uses without the need for risk mitigation.

**Risk to sediment-dwelling organisms:**

With a PEC/RAC of 0.234 µg/L for spray drift and 0.052 µg/L for drainflow, there is a large margin of safety (an order of magnitude or higher) in the chronic invertebrate risk assessment. Sediment-dwelling organisms would need to be ~ 4.2 x more sensitive than *Daphnia magna* before an unacceptable spray drift risk was demonstrated and ~19 x more sensitive for an unacceptable drainflow risk to be demonstrated. Additionally, lupin seeds are used as a feedstuff in aquaculture, including for aquatic invertebrates like whiteleg shrimp which feed at the water-soil interface. A published literature study investigated the inclusion of lupin kernel meal in the diet of whiteleg shrimp (Molina-Poveda et al. 2013). No adverse effects on survival or growth were observed. Taken together, these results indicate that, should PROBLAD PLUS or BLAD be bioavailable in sediment, no adverse effects would be

expected for sediment-dwelling organisms when exposed via either water or when consumed as part of the diet.

### **Overall conclusion for aquatic organisms**

An acceptable risk to aquatic organisms for the proposed uses can be concluded.

### **2.9.9.3. Risk assessment for bees**

The acute risk to adult honeybees was assessed in accordance with the SANCO Terrestrial Guidance Document (SANCO/10329/2002). The acute contact and oral LD<sub>50</sub> values are compared to the maximum single application rate to derive a hazard quotient (HQ). HQ values ≤ 50 indicate a low acute risk to honeybees. The acute contact and oral risk assessments are summarised below.

**Table 2.9.9.3-1: HQ calculations for honeybees: contact and oral exposure for proposed uses on tomatoes and strawberries at 6 x 4016 g/ha**

Test item	Study	Application rate (g product/ha)	Endpoint (µg product /bee)	HQ (< 50)
PROBLAD PLUS	Acute contact	4016	> 100	< 40.2
	Acute oral		> 109.42	< 36.7

The HQ values are below the trigger value of 50, indicating acceptable acute risks to honeybees.

Acute bumblebee and chronic adult and larval honeybee endpoints were available in addition to the above. These have not been used in the risk assessment due to the lack of noted guidance. However, it was noted that these studies did not show any indications of toxicity of the active substance to bees.

**Overall conclusion for bees**

Overall, the risk to bees from the proposed uses is considered to be acceptable.

**2.9.9.4. Risk assessment for non-target arthropods**

The risk assessment for non-target arthropods other than bees was conducted in accordance with SANCO Terrestrial Guidance Document (SANCO/10329/2002) and recommendations of ESCORT II guidance.

The risk assessment was conducted for both the use on tomatoes and the use on strawberries. Tier I endpoints available for *A. rhopalosiphii* and *T. pyri* were used in the first tier in- and off-field risk assessment. All calculated HQ values were below the trigger value of 2, indicating acceptable risks. The risk assessments are summarised below:

**Table 2.9.9.4-1: HQ values for non-target arthropods exposed to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) via use on strawberries**

Test substance	Species/ Life stage	Test type	Endpoint [mL/ha]	PER <sub>in-field</sub> [mL/ha]	PER <sub>off-field</sub> [mL/ha]	HQ <sub>in-field</sub>	HQ <sub>off-field</sub>	Trigger value
PROBLAD PLUS	<i>Typhlodromus pyri</i> protonymphs	Laboratory test, artificial substrate, 2D exposure	LR <sub>50</sub> > 10500	10240	167.9	0.97	0.015	HQ < 2 risk is acceptable
	<i>Aphidius rhopalosiphii</i> adults	Laboratory test, artificial substrate, 2D exposure	LR <sub>50</sub> > 10500	10240	167.9	0.97	0.015	HQ < 2 risk is acceptable

**Table 2.9.9.4-2: HQ values for non-target arthropods exposed to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) via use on tomatoes**

Test substance	Species/ Life stage	Test type	Endpoint [mL/ha]	PER <sub>in-field</sub> [mL/ha]	PER <sub>off-field</sub> [mL/ha]	HQ <sub>in-field</sub>	HQ <sub>off-field</sub>	Trigger value
PROBLAD PLUS	<i>Typhlodromus pyri</i> protonymphs	Laboratory test, artificial substrate, 2D exposure	LR <sub>50</sub> > 10500	10240	656.4	0.97	0.06	HQ < 2 risk is acceptable
	<i>Aphidius rhopalosiphi</i> adults	Laboratory test, artificial substrate, 2D exposure	LR <sub>50</sub> > 10500	10240	656.4	0.97	0.06	HQ < 2 risk is acceptable

Extended laboratory studies were also available with *A. rhopalosiphi*, *T. pyri* and *C. carnea* but a Tier II risk assessment was not required, since the Tier I HQ values were below the trigger value of 2.

#### **Overall conclusion for non-target arthropods**

Overall, the risk to non-target arthropods is considered to be acceptable for the proposed uses.

#### **2.9.9.5. Risk assessment for soil meso- and macro-fauna**

##### **Earthworms**

The risk assessment is performed according to the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (SANCO/10329/2002 rev 2 final). The standard risk assessment is based on TER values. If the long-term TER is below 5 further consideration of the risk is required. In the absence of information on the log Pow for PROBLAD PLUS, as a worst-case assumption the toxicity endpoints have been corrected by a factor of 2. Data were only available to conduct a quantitative risk assessment for earthworms. This is summarised in the table below.

**Table 2.9.9.5-1: TER calculations for earthworms for proposed use of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) on strawberries and tomatoes**

Test substance	Study type	Endpoint (mg PROBLAD PLUS/kg soil dw)	Proposed use	PEC <sub>Soil</sub> (mg PROBLAD PLUS/kg soil dw)	TER	Trigger
PROBLAD PLUS	E. andrei, reproduction	62.5	Strawberry and tomato	12.277	5.1	5

Since the TER exceeds the trigger value of 5, the risk to earthworms from the proposed uses is considered to be acceptable.

#### **Soil macro-organisms**

In accordance with assimilated Regulation No 283/2013, for plant protection products applied as a foliar spray, “if data are available on both *Aphidius rhopalosiphi* and *Typhlodromus pyri* these may be used in an initial risk assessment” for assessing the risks to soil organisms other than earthworms. Given that an acceptable risk to both *A. rhopalosiphi* and *T. pyri* was demonstrated using standard Tier I glass plate studies, it is considered that no data is required on the soil macro-organisms *F. candida* and *H. aculeifer*. Therefore acceptable risks to soil macro-organisms may be concluded.

#### **Overall conclusion for soil meso- and macro-organisms**

Overall, the risk to soil meso-and macro-fauna can be concluded to be acceptable.

#### **2.9.9.6. Risk assessment for soil micro-organisms**

The risk assessment is performed according to the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (SANCO/10329/2002 rev 2 final). The magnitude of effect is compared to the untreated control. PEC<sub>soil</sub> values have been compared to concentrations at which < 25% effects on nitrogen transformation were observed in Table 2.9.9.6-1.

**Table 2.9.9.6-1. Risk assessment for soil micro-organisms**

<b>Test substance</b>	<b>Species</b>	<b>Endpoint (mg /kg dry soil)</b>	<b>PEC<sub>Soil</sub> max (mg/kg)</b>	<b>Acceptable risk ?</b>
<b>PROBLAD PLUS</b>	Soil micro-organisms (nitrogen transformation)	52	12.277	Yes

Since the PEC<sub>Soil</sub> is below the endpoint at which 25% effects occurred, the risk to soil micro-organisms from the proposed uses is considered to be acceptable.

#### **Overall conclusion for soil micro-organisms**

Overall, the risk to soil micro-organisms is considered to be acceptable.

#### **2.9.9.7. Risk assessment for terrestrial non-target higher plants**

The risks to non-target plants were determined based on the Working Document for terrestrial ecotoxicology, SANCO 10329/2002 rev 2 final and are shown in tables 2.9.9.7-1 (seedling emergence and vegetative vigour) below for the risk from spray drift.

**Table 2.9.9.7-1. Risk assessment for non-target plants:**

<b>Application rate (g PROBLAD PLUS/ha)</b>	<b>Crop</b>	<b>Endpoint (g PROBLAD PLUS/ha)</b>	<b>PER<sub>off-field</sub> (g PROBLAD PLUS/ha)</b>	<b>TER</b>	<b>Trigger value</b>
4016	Strawberry	> 2510	111.24	> 22.56	5
4016	Tomato	> 2510	322.08	> 7.79	5
4016	Strawberry	> 4016	111.24	> 36.10	5
4016	Tomato	> 4016	322.08	> 12.46	5

Since the TERs exceed the trigger of 5, an acceptable risk to non-target plants from the proposed uses may be concluded.

### **Overall conclusion for non-target plants**

Overall, the risk to non-target plants is considered to be acceptable.

## **2.10. Classification and labelling**

A mandatory classification and labelling report has been prepared under GB CLP by HSE and is publicly available. This report concluded that aqueous extract from the germinated seed of sweet *Lupinus albus* is not classified for any hazard classes, and therefore an entry on the GB MCL list is not required.

## **2.11. Relevance of metabolites in groundwater**

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is a naturally occurring plant extract and is a UVCB substance. The active substance and its major component BLAD were demonstrated to be readily biodegradable. No metabolites have been determined in soil, therefore consideration of metabolites in groundwater is not necessary, see section 2.8.6.

## **2.12. Consideration of isomeric composition in the risk assessment**

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is a naturally occurring plant extract and is a UVCB substance not containing isomers. No consideration of isomeric composition is necessary.

## **2.13. Residue definitions**

### **2.13.1. Definition of residues for exposure/risk assessment**

**Food of plant origin:** Not required

**Food of animal origin:** Not required

**Soil:** Not required

**Groundwater:** Not required

**Surface water:** Not required

**Sediment:** Not required

**Air:** Not required

### **2.13.2. Definition of residues for monitoring**

**Food of plant origin:** Not required

**Food of animal origin:** Not required

**Soil:** Not required

**Groundwater:** Not required



**Surface water:** Not required

**Sediment:** Not required

**Air:** Not required

## **Level 3**

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

## 3. Proposed decision with respect to the application

### 3.1. Background to the proposed decision

#### 3.1.1. Proposal on acceptability against the decision-making criteria – Article 4 and annex II of assimilated Regulation No 1107/2009

3.1.1.1. Article 4			
		Yes	No
i)	It is considered that Article 4 of assimilated Regulation No 1107/2009 is complied with. Specifically, the competent authority considers that authorisation in at least one plant protection product containing the active substance for at least one of the representative uses.	X	HSE currently consider that Article 4 of assimilated Regulation No 1107/2009 may be complied with for aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> , for use as a fungicide on strawberry and tomatoes (refer to Level 1 1.5.1 for details of the representative uses).
3.1.1.2. Submission of further information			
		Yes	No

i)	It is considered that a complete dossier has been submitted	X	X	<p>The literature search submitted did not adequately cover the period before submission. An update has been requested to cover the required more recent period before submission. As HSE propose that this substance meets the criteria for approval as a low-risk active substance (and is approved as such in the EU), we do not anticipate there will be any new information that will impact the assessment. However, no conclusion of the authority will be reached until HSE confirm this following the submission of the updated literature review.</p> <p>Literature review top up has been received and assessed by HSE.</p>
ii)	<p>It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because:</p> <p>(a) the data requirements have been amended or refined after the submission of the dossier; or</p> <p>(b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.</p>		X	N/A

3.1.1.3. Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of assimilated Regulation No 1107/2009 approval should be subject to conditions and restrictions.		X	
3.1.1.4. Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		ADI = 5 mg/kg bw/day ARfD = Not required AOEL = 5 mg/kg bw/day AAOEL = Not required
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or	X		Available information on residues are sufficient for approval of the active substance for all representative uses. However, further data may be required in a future consideration where results from samples stored for extended time periods are relied upon.  Based on the information provided a residue definition for risk assessment is not appropriate.

	<p>feed). In particular it is considered that the dossier:</p> <p>(a) permits any residue of concern to be defined;</p> <p>(b) reliably predicts the residues in food and feed, including succeeding crops</p> <p>(c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing;</p> <p>(d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals;</p> <p>(e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.</p>			<p>Based on the assessment, aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> can be considered exempt from MRLs and a residue definition for enforcement is not applicable.</p> <p>Sufficient information has been provided to predict the effects of processing.</p> <p>See level 2 section 2.7 for detail.</p>
	<p>It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.</p>	X		<p>Yes, for all representative uses.</p>
<b>Efficacy</b>				

		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		The applicant has satisfactorily addressed all of the Efficacy requirements for a new active substance. Effectiveness against the proposed pests has been adequately demonstrated. Crop safety of PROBLAD PLUS to the proposed crops has been supported. Additionally, the resistance risk has been appropriately addressed. Further information will be examined at the product authorisation stage to ensure that the product itself fully complies with the data requirements for Efficacy.
<b>Relevance of metabolites</b>				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		
<b>Composition</b>				
		Yes	No	

	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		<p>Noting the UVCB nature of the substance, sufficient data have been provided to support the manufacturing site and proposed specification.</p> <p>None of the impurities identified in the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> are considered to be of toxicological or ecotoxicological relevance at the levels found.</p>
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	N/A		There is currently no FAO specification for the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> .
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted	N/A		There is currently no FAO specification for the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> .
<b>Methods of analysis</b>				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater	X		<p>Acceptable methods have been submitted for the determination of the lead component BLAD.</p> <p>None of the impurities identified in the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> are</p>



	than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.			considered to be of toxicological or ecotoxicological relevance at the levels found.
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.	X		Acceptable methods have been submitted for the determination of BLAD in various studies in support of the ecotoxicology and toxicology areas of the risk assessment. For the residues risk assessment, the method is not considered acceptably validated but is indicative of the possible levels of BLAD in treated crops.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of assimilated Regulation No 1107/2009.	X		
<b>Impact on human health</b>				
<b>Impact on human health – ADI, AOEL, ARfD</b>				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate	X		<u>ADI</u> : In the 90-day study, a NOAEL of 500 mg/kg bw/d was established, based on vacuolation in the brain and

	safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.			<p>spinal cord in a single individual animal at the limit dose of 1000 mg/kg bw/d. The dose level of 500 mg/kg bw/d is considered to be a suitable starting point for derivation of the ADI. After application of an assessment factor of 10 x 10, an ADI of 5 mg/kg bw/d is obtained.</p> <p><u>AOEL</u>: A systemic AOEL can be derived on the same basis and rationale by which the ADI has been established. A correction factor for oral bioavailability is not required, therefore the resulting AOEL is 5 mg/kg bw/d.</p> <p><u>ARfD</u>: Not required</p> <p><u>AAOEL</u>: Not required</p>
<b>Impact on human health – proposed genotoxicity classification</b>				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, <b>the substance SHOULD BE classified or proposed for classification</b> , in accordance with the provisions		X	<p>See section 2.6.4 above.</p> <p>Overall, it is concluded that aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> is not genotoxic in vivo, and the data requirements of assimilated Regulation 283/2013 have been met.</p>

	of assimilated Regulation No 1272/2008, <b>as mutagen category 1A or 1B.</b>			Therefore, classification for mutagenicity is not warranted.
<b>Impact on human health – proposed carcinogenicity classification</b>				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist in relation to the relevant constituent territory and other available data and information, including a review of the scientific literature, reviewed by the Authority, <b>the substance SHOULD BE classified or proposed for classification</b> , in accordance with the provisions of assimilated Regulation No 1272/2008, <b>as carcinogen category 1A or 1B.</b>		X	See section 2.6.5 above.  The active substance is comprised of substances naturally occurring in botanical extracts and no components give rise to toxicological concern. Based on data from repeated dose testing, published literature and the lack of mutagenic potential in vivo, it is concluded that chronic exposure to the active substance will not result carcinogenicity. Therefore, classification is not required.
ii)	Linked to above classification proposal.  It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or	N/A	N/A	Not applicable.

	synergist concerned on food and feed do not exceed the default value set in accordance with Article 18 (1b) of assimilated Regulation No 396/2005.			
<b>Impact on human health – proposed reproductive toxicity classification</b>				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists in relation to the relevant constituent territory and other available data and information, including a review of the scientific literature, <b>the substance SHOULD BE classified or proposed for classification</b> , in accordance with the provisions of assimilated Regulation No 1272/2008, <b>as toxic for reproduction category 1A or 1B.</b>		X	See section 2.6.6 above.  The active substance is comprised of substances naturally occurring in botanical extracts and no components give rise to toxicological concern. Based on data from repeated dose testing, published literature and the lack of effects in reproductive organs in the repeat dose short term database, it is concluded that exposure to the active substance will not result reproductive or developmental toxicity. Therefore, classification is not required.
ii)	Linked to above classification proposal.  It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions	N/A	N/A	Not applicable.

	excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18 (1b) of assimilated Regulation No 396/2005.			
<b>Impact on human health – proposed endocrine disrupting properties classification</b>				
		Yes	No	
i)	It is considered that <b>the substance SHOULD BE classified or proposed for classification</b> in accordance with the provisions of assimilated Regulation No 1272/2008, as <b>carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties</b>		X	See section 2.6.8 above.  There are no indications that the active substance or its metabolites require classification for carcinogenicity or reproductive toxicity. Therefore, it is not considered to have ED properties on this basis.
ii)	It is considered that <b>the substance SHOULD BE classified or proposed for classification</b> in accordance with the provisions of assimilated Regulation No 1272/2008, as <b>toxic for reproduction category 2 and</b> in addition the competent authority considers the substance <b>has toxic effects on the endocrine organs and on</b>		X	See sections 2.6.3 and 2.6.6 above.  There are no concerns regarding reproductive toxicity, nor are there any indications of adverse effects on ED-sensitive targets in published literature and the available repeat dose studies.

	that basis shall be considered to have endocrine disrupting properties			
iii)	<p>Linked to either i) or ii) immediately above.</p> <p>It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of assimilated Regulation No 396/2005.</p>	N/A	N/A	N/A
<b>Fate and behaviour in the environment</b>				
<b>Persistent organic pollutant (POP)</b>				
		Yes	No	
	<p>It is considered that the active substance <b>FULFILS</b> the criteria of a persistent organic</p>		X	A substance is deemed to meet the P criterion in a POP assessment if the DT <sub>50</sub> is > 2 months in water, > 6 months in sediment or > 6 months in soil.

	<p>pollutant (POP) as laid out in assimilated Regulation No 1107/2009 Annex II Section 3.7.1.</p>		<p>The aqueous extract from the germinated seed of sweet <i>Lupinus albus</i> and its lead component BLAD are readily biodegradable.</p> <p>The ECHA 2017 guidance notes that substances that demonstrate high sorption properties can be subject to a longer soil DT<sub>50</sub>. Based on this a DT<sub>50</sub> of 300 days in soil for the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> and the lead component BLAD has been determined. Which would indicate moderate to high persistence in soil considering the ECHA Guidance (ECHA, 2017). There is additional qualitative evidence to indicate that the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> would likely be subject to biological degradation in soil and not persist in the natural environment. In addition the applicant provided further supporting information demonstrating protease levels in the soil, and likely rapid degradation of similar sized peptides in the soil environment. Based upon a weight of evidence approach using the submitted ready biodegradability data, the additional supporting information from the literature and the in vitro testing submitted by the applicant a proposal of 30 days DT<sub>50</sub> can be supported for future assessments of the aqueous extract from germinated seed of sweet <i>Lupinus albus</i>.</p>
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				<p>Therefore, it is concluded that the persistence criteria in soil <b>unlikely to be</b> <b>is not</b> met.</p> <p>The aqueous extract from the germinated seed of sweet <i>Lupinus albus</i> and its lead component BLAD is not considered persistent in surface water with a DT<sub>50</sub> of 15 days indicated from the ready biodegradability studies.</p> <p>The aqueous extract from the germinated seed of sweet <i>Lupinus albus</i> and its lead component BLAD do not meet the potential for long range transport criteria.</p> <p>Based on the above, the HSE evaluator is of the opinion that the aqueous extract from the germinated seed of sweet <i>Lupinus albus</i> and its lead component BLAD do not fulfil the P criterion.</p>
<b>Persistent, bioaccumulative and toxic substance (PBT)</b>				
		Yes	No	
	<p>It is considered that the active substance <b>FULFILS</b> the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in assimilated Regulation No 1107/2009 Annex II Section 3.7.2.</p>		X	<p>The aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> and its lead component BLAD are not considered a PBT substance as none of the three criteria are met.</p> <p>The aqueous extract from the germinated seed of sweet <i>Lupinus albus</i> and its lead component BLAD are readily biodegradable and are not considered</p>



				<p>persistent. As such it was not considered necessary to investigate bioaccumulative properties.</p> <p>The aqueous extract from the germinated seed of sweet <i>Lupinus albus</i> and its lead component BLAD are not classed as toxic see Volume 3 CA B.6.</p>
<b>Very persistent and very bioaccumulative substance (vPvB).</b>				
		Yes	No	
	<p>It is considered that the active substance <b>FULFILS</b> the criteria of a very persistent and very bioaccumulative substance (vPvB) as laid out in assimilated Regulation No 1107/2009 Annex II Section 3.7.3.</p>		X	<p>A substance is deemed to meet the P criterion in a vPvB assessment if the half-life in soil is &gt; 180 days.</p> <p>As indicated above, the aqueous extract from the germinated seed of sweet <i>Lupinus albus</i> and its lead component BLAD are not considered to be persistent and therefore do meet the vP criterion. See Volume 3 CA B.8. It was not considered necessary to investigate bioaccumulative properties.</p>
<b>Ecotoxicology</b>				
		Yes	No	

	<p>It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) in relation to the relevant constituent territory under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The competent authority is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.</p>	X	<p>Birds: based on the available data and reasoned case provided, an acceptable risk to birds was demonstrated for all proposed uses (see Section 2.9.9.1).</p> <p>Mammals: based on the available data and reasoned case provided, an acceptable risk to birds was demonstrated for all proposed uses (see Section 2.9.9.2).</p> <p>Aquatic organisms: based on the available data and reasoned case provided, an acceptable risk to aquatic organisms was demonstrated for all proposed uses (see Section 2.9.9.3).</p> <p>Bees: based on the available data an acceptable risk to bees was demonstrated for all the proposed uses (see Section 2.9.9.4).</p> <p>Non-target arthropods (NTAs): based on the available data an acceptable risk to NTAs was demonstrated for all proposed uses (see Section 2.9.9.5).</p> <p>Soil meso- and macro-fauna: based on the available data an acceptable risk to earthworms was demonstrated for all the proposed uses. An acceptable risk to soil macro-organisms was concluded on the basis of acceptable risks to NTAs at Tier I (see Section 2.9.9.6).</p>
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				<p>Soil micro-organisms: based on the available data an acceptable risk to soil micro-organisms was demonstrated for all the proposed uses (see Section 2.9.9.7).</p> <p>Non-target terrestrial plants (NTTPs): based on the available data an acceptable risk to NTTPs have been demonstrated for all proposed uses (see Section 2.9.9.8).</p> <p>Sewage treatment: no data was provided or considered necessary because the lead component BLAD has been demonstrated to be readily biodegradable (see Section 2.9.9.9).</p>
	<p>It is considered that, on the basis of the assessment of nationally or internationally agreed test guidelines, the substance <b>HAS</b> endocrine disrupting properties that may cause adverse effects on non-target organisms.</p>		X	<p>Birds and mammals: No endocrine disruptor studies were conducted or considered necessary for the human health assessment or ecotoxicology assessment. No indications of toxicity or sub-lethal effects were demonstrated in literature studies investigating inclusion of lupins in bird and mammal diets. The active substance is not considered to have endocrine disruptor properties on this basis.</p> <p>Aquatic organisms: the published literature does not indicate any adverse effects from inclusion of lupin seeds in fish diets. The active substance is not</p>

				considered to have endocrine disruptor properties on this basis.
	<p>Linked to the consideration of the endocrine properties immediately above.</p> <p>It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.</p>		X	<p>The proposed uses are not considered likely to result in negligible exposure. However, aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> is not considered to be an endocrine disruptor, as described above.</p>
	<p>It is considered that it is established following an appropriate risk assessment on the basis of nationally or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist:</p> <ul style="list-style-type: none"> <li>— will result in a negligible exposure of honeybees, or</li> <li>— has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.</li> </ul>	X		<p>Based on the available data an acceptable risk to bees was demonstrated for all the proposed uses (see Section 2.9.9.4).</p>

Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		As detailed in section 2.7.3, above, a residue definition for risk assessment is not appropriate. The aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> is exempt from MRLs and a residue definition for enforcement is not applicable. It should be noted that this conclusion relates to the uses considered in this assessment only.
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of assimilated Regulation No 1107/2009.	X		The predicted concentrations of BLAD and lupanine in groundwater following assessment of the proposed uses were below the parametric drinking water limit of 0.1 µg/L in all representative scenarios.

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### 3.1.2. Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	Not relevant

**3.1.3. Proposal – Low risk active substance**

Low-risk active substances			
		Yes	No
	<p>It is considered that the active substance <b>shall be considered of low risk.</b></p> <p>In particular it is considered that the substance <b>should NOT be classified or proposed for classification</b> in accordance with assimilated Regulation No 1272/2008 as at least one of the following:</p> <ul style="list-style-type: none"> <li>— carcinogenic category 1A, 1B or 2,</li> <li>— mutagenic category 1A, 1B or 2,</li> <li>— toxic to reproduction category 1A, 1B or 2,</li> <li>— skin sensitiser category 1,</li> <li>— serious damage to eye category 1,</li> <li>— respiratory sensitiser category 1,</li> <li>— acute toxicity category 1, 2 or 3,</li> <li>— specific Target Organ Toxicant, category 1 or 2,</li> </ul>	X	<p>The substance is not classified with any of the classification in the low-risk criteria. Whilst the half life in soil used in the assessment is more than 60 days (EFSA default values were used), aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> is a naturally occurring active substance which does not correspond to any of points (a) to (d) of point 5.1.1 therefore it may be considered as being of low-risk, even if it is persistent (half-life in soil is more than 60 days) or its bio-concentration factor is higher than 100.</p> <p>Therefore, aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> can be considered to meet all the criteria for approval as a low-risk active substance.</p>

<p>— toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests,</p> <p>— explosive,</p> <p>— skin corrosive, category 1A, 1B or 1C;</p> <p>(b) it has been identified as priority substance and is listed in Annex 10 to Directive 2000/60/EC substance Directive 2000/60/EC;</p> <p>(c) it is deemed to be an endocrine disruptor;</p> <p>(d) it has neurotoxic or immunotoxic effects.</p> <p>In addition it is considered that <b>the substance is NOT:</b></p> <p>— persistent (half-life in soil is more than 60 days) or its bio-concentration factor is higher than 100.</p> <p>However, a naturally occurring active substance which does not correspond to any of points (a) to (d) above may be considered as being of low-risk, even if</p>			
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	it is persistent (half-life in soil is more than 60 days) or its bio-concentration factor is higher than 100.			
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**3.1.4. List of studies to be generated, still ongoing or available but not peer reviewed**

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1. Identity of the active substance or formulation				
None				
3.1.4.2. Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
None				
3.1.4.3. Data on uses and efficacy				
None				

<b>3.1.4.4. Data on handling, storage, transport, packaging and labelling</b>				
None				
<b>3.1.4.5. Methods of analysis</b>				
None				
<b>3.1.4.6. Toxicology and metabolism</b>				
None				
<b>3.1.4.7. Residue data</b>				
None				
<b>3.1.4.8. Environmental fate and behaviour</b>				
None				

3.1.4.9. Ecotoxicology				
None				
Literature search				
The submitted literature search does not cover the required period before submission.	All		X	

### 3.1.5. Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in assimilated Regulation No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	Relevance in relation to representative use(s)
None	N/A

### 3.1.6. Critical areas of concern

An issue is listed as a critical area of concern:

- (a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of assimilated Regulation No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
- (b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in assimilated Regulation No 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	Relevance in relation to representative use(s)
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None	N/A
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**3.1.7. Overview table of the concerns identified for each representative use considered**

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)

The material tested in the toxicological studies has been demonstrated to be representative of the technical specification.

## Volume 1 – Level 3

Representative use		Strawberry (X <sup>1</sup> )	Tomato (X <sup>1</sup> )
Operator risk	Risk identified		
	Assessment not finalised		
Worker risk	Risk identified		
	Assessment not finalised		
Bystander risk	Risk identified		
	Assessment not finalised		
Consumer risk	Risk identified		
	Assessment not finalised		
Risk to wild non target terrestrial vertebrates	Risk identified		
	Assessment not finalised		
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified		
	Assessment not finalised		
Risk to aquatic organisms	Risk identified		
	Assessment not finalised		
Groundwater exposure active substance	Legal parametric value breached		
	Assessment not finalised		
Groundwater exposure metabolites	Legal parametric value breached		
	Parametric value of 10µg/L <sup>(a)</sup> breached		
	Assessment not finalised		
Comments/Remarks			

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.

(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 11-final, European Commission, 2021

### 3.1.8. Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	Justification
<p>Effects on Human Health (Relevant impurities: Chemistry and consumer exposure)</p> <p>Quinolizidine alkaloids</p>	<p>Quinolizidine alkaloids (QAs) are found in the legume plant family and are components of possible concern for human health. These may be present in the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> due to lupin being a member of the legume plant family and these components potentially being present in the aqueous extract from germinated legume seeds.</p> <p>The QAs are limited in food regulations in Australia and New Zealand. Additionally, in 1996, the UK Advisory Committee on Novel Food and Processes published a report on seeds from <i>Lupinus angustifolius</i> (FSA, 1996) in which they, considering advice from the Food Advisory Committee and the Committee on Toxicity (COT), concluded that seeds and products from <i>L. angustifolius</i> are safe for use provided that an alkaloid level of 200 mg/kg is not exceeded in seeds or products thereof. However, the QAs are not currently limited in the GB or EU food regulations (GB: assimilated Regulation No 1881/2006).</p> <p>It is noted that the source of lupin seeds at the start of the manufacturing process is limited to containing a maximum content of 200 mg/kg of QAs in the sweet lupin seeds. The observed level of lupanine (considered a marker component for total QAs) in commercial scale batches was a maximum of 31 mg/kg, with other individual QAs, at a maximum of 8.31 mg/kg; the majority of samples showed levels &lt; 0.1 mg/kg. These levels</p>



	<p>support the applicant's proposed specification of maximum 50 mg/kg. However, based on the available toxicological information, the impurities would only need to be included in the specification as relevant impurities if the content of total QAs in the extract exceeded 60 mg/kg.</p> <p>Therefore, to address potential future sources of active substance which may contain higher levels of these components, HSE propose to include a footnote in the notification of inclusion in the GB active substance approvals register:</p> <p>'Quinolizidine alkaloids (QAs) may be present in lupin seeds, which subsequently may then be found in the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> at low levels. If the content of the total QAs in the extract exceeds 0.006% w/w, which may be indicated by the content of lupanine, they are designated as relevant impurities and a clause may be required to limit their concentration.'</p> <p>This footnote would be included in the GB active substance approvals register, GB Conclusion, Volume 1 and reference specification in Volume 4.</p> <p>ECP advice is sought on whether there is sufficient confidence that the quinolizidine alkaloids will be limited to appropriate levels by the inclusion of the suggested footnote, rather than inclusion as relevant impurities in the reference specification, considering they are known to be of possible concern for human health and their potential presence as a contaminant in food.</p>
<p>Effects on Human Health (consumer exposure and MRLs)</p> <p>Limited data, reliance on literature and residue definitions</p>	<p>Limited data have been provided, with none of the study reports being generated in accordance with the relevant OECD guidelines, noting that the data package provided was the same as used for the EU approval. The assessment relies upon published literature and limited data. The field trials data provided have uncertainties: trials performed in USA and not in accordance with GAP application rates, storage stability of residues in samples has not been addressed and the LOQ of the analytical method used is not reliable. However, despite the</p>

	<p>uncertainties, the trials give assurance that residues will be low.</p> <p>Regarding the nature of the residue, there is confidence that the range of proteins present in the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> are naturally occurring plant proteins, all of which are likely to degrade into amino acids.</p> <p>The inclusion of <i>Lupinus albus</i> in Part 1 of assimilated Regulation No. 396/2005 provides support for residue definitions not being required. The biological composition of the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> is unlikely to be significantly different to the biological components present in the traded commodities to which MRLs apply. A residue definition is not meaningful as the major component is a naturally occurring seed storage protein, and the range of other proteins present in the active substance are naturally occurring plant proteins, all of which are likely to degrade into amino acids. Additionally, the lead component and other components present in aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> are not unique to the application of the PPP, therefore these components are not suitable for a residue definition for enforcement. Based on the information summarised in Volume 1, section 2.7.3 and ECP summary, a residue definition for risk assessment or enforcement is not appropriate; this is confirmed by the indicative chronic risk assessment.</p> <p>An indicative chronic risk assessment was undertaken considering the proposed chronic toxicological endpoint and estimating an equivalent highest anticipated residue assuming consumption of treated crops, rather than a conventional risk assessment considering actual residue levels in treated crops. This is presented in Volume 1 section 2.7.9. Even taking into account the uncertainties identified, residue exposures are not expected to exceed the chronic toxicological endpoint, and therefore harmful effects to human health are not expected. It should be noted that this conclusion relates to the uses considered in this assessment (the representative uses and additional MRL assessment uses) only.</p>
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	ECP advice is sought on how reasonable it is to rely on literature information and the unconventional approach to consumer risk assessment taken by HSE to conclude that residue definitions for this substance are not required.
Effects on Human Health (consumer exposure and MRLs)  Inclusion on Part 4 of the GB MRL statutory Register	<p>For inclusion of the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> in Part 4 of the GB MRL statutory Register, the criteria outlined in the SANCO/11188/2013 rev. 2 guidance has been considered. None of the criteria listed are fully met. However, the substance partly meets criteria 2, 3 and 4 and HSE has taken a weight of evidence approach in concluding that for the assessed uses, the active substance should be exempt from MRL setting:</p> <ul style="list-style-type: none"> <li>• Regarding criterion 2, the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> originates from Lupins/lupini beans listed in Part 1 of assimilated Regulation 396/2005. Although the substance is not identical to what is listed in Part 1, as explained in Volume 1 section 2.7.3, this provides support for inclusion in Part 4.</li> <li>• Regarding criterion 3, whilst a chronic toxicological reference value (ADI) has been established, it is a high value demonstrating the low potency of this substance. An acute reference dose has not been established.</li> <li>• Regarding criterion 4, as explained in Volume 1 section 2.7.9, the exposure to residues of the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> are likely to be lower than exposure to the naturally occurring components present in crops and the consumption of lupins/lupini beans.</li> </ul> <p>The guidance on inclusion in Part 4 indicates that the decision applies only to assessed uses. A wider range of uses related to future GB uses of the substance were requested as requiring the exemption from MRLs in addition to the representative uses. HSE has provided an assessment for both the new active substance representative uses and the proposed additional MRL assessment uses, and has concluded that the substance</p>

	<p>is included in Part 4; the total application rate for the additional MRL assessment uses is the same or less critical than the representative uses. The same justifications can be made regarding the natural occurrence of the components. Extensions of uses beyond those currently considered in the assessment report for inclusion of the aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> in Part 4 of the GB MRL statutory Register will require further consideration.</p> <p>The opinion of ECP is sought on the way HSE have taken a weight of evidence approach to inclusion in Part 4 of the GB MRL statutory Register, considering that individually the inclusion criteria have not strictly been met.</p>
Fate and behaviour	<p>In order to demonstrate the susceptibility of BLAD protein to proteolysis the applicant conducted a laboratory study in which BLAD protein was mixed with common proteolytic enzymes, namely pronase, trypsin, proteinase K, <math>\alpha</math>-chymotrypsin and subtilisin. Analysis via SDS-PAGE was then used to assess the levels of protein left in the sample after a given incubation period. The analysis indicates that the BLAD protein is degraded by all five proteolytic enzymes, with the BLAD protein completely degraded after 2 hours.</p> <p>The relevance of this testing depends on the extent of occurrence of the tested proteolytic enzymes in soil and it is noted that pronase, proteinase K and subtilisin are found in commonly occurring soil bacteria or fungi. HSE would welcome ECP thought on the interpretation of these data to a field situation. The applicant states “although it is difficult to provide endogenic concentrations of these enzymes in soil, the enormous concentrations of bacteria and fungi in agricultural soil would indicate that concentrations of these enzymes would be significant. For example, the approximate range of biomass in a typical temperate grassland soil is 1-2 t ha<sup>-1</sup> for bacteria (1-4 t ha<sup>-1</sup> if actinomycetes are included) and 2-5 t ha<sup>-1</sup> for fungi.” No reference to the source of this information was provided.</p>

	ECP advice is sought on the microbial biomass in agricultural soils and the relevance of the in vitro study to the likely exposure of the active substance to proteolytic breakdown in the field.
Ecotoxicology	<p>No data has been submitted to address the risk to sediment-dwelling organisms on the basis that PROBLAD PLUS and PROBLAD have been demonstrated to be readily biodegradable and thus residues in water would be readily degraded. However, HSE has noted that the high Koc of BLAD would indicate that partitioning to sediment would likely occur. In the absence of specific data with sediment-dwelling organisms, HSE has considered the margin of safety in the chronic aquatic invertebrate risk assessment. With a PEC/RAC of 0.234 for spray drift and 0.052 for drain flow, there is a large margin of safety in the chronic invertebrate risk assessment when considering the trigger value of 1. Thus, HSE considers an acceptable risk to sediment-dwelling organisms can be concluded.</p> <p>The ECP is invited to advise on whether, in the absence of specific toxicity data, the risk to sediment-dwelling organisms can be considered as having been sufficiently addressed by considering the margin of safety in the chronic invertebrate risk assessment.</p>

### 3.2. Proposed decision

It is proposed that:

**Aqueous extract from the germinated seeds of sweet *Lupinus albus* can be approved under assimilated Regulation No 1107/2009**

It is considered that it should be specified that conditions of use shall include risk mitigation measures, where appropriate.

### 3.3. Rational for the conditions and restrictions to be associated with the approval or authorisation(s), as appropriate

**3.3.1. Particular conditions proposed to be taken into account to manage the risks identified**

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
None	N/A

**3.4. APPENDICES****GUIDANCE DOCUMENTS USED IN THIS ASSESSMENT****Identity, Physical chemical properties, method of analysis**

- Manual on development and use of FAO and WHO specifications for pesticides, 1st edition, 3<sup>rd</sup> revision; World Health Organisation and Food and Agriculture Organisation of the United Nations, Rome 2016
- Guidance document on significant and non-significant changes of the chemical composition of authorised plant protection products under Regulation (EC) No 1107/2009 of the EU Parliament and Council on placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. SANCO/12638/2011, rev. 2, 20 November 2012
- Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013. SANCO/3030/99 rev. 5, 22 March 2019
- Guidance document for the generation and evaluation of data on the physical, chemical and technical properties of plant protection products under Regulation (EC) No. 1107/2009 of the EU Parliament and Council on placing plant protection products on the market, Final Draft. HSE, 13 July 2018.
- OECD, 2007, Guidance document on the pesticide residue analytical methods, (ENV/JM/MONO(2007)17), Series on testing and assessment No. 72 and Series on pesticides No. 39
- Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, SANTE/2020/12830 rev. 1., 24 February 2021

- Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods. SANTE/2017/10632 rev. 3, 22 November 2017
- Guidance Document On Botanical Active Substances Used In Plant Protection Products, SANCO/11470/2012– rev. 8, 20 March 2014

### **Toxicology (Human Health)**

- ECHA document agreed upon at the Biocidal Products Committee (BPC)-31 on interpreting the definition of relevant impurities (June 2019)
- EFSA Guidance on dermal absorption. EFSA Journal 2017;15(6):4873
- EFSA Guidance on the Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092
- Guidance document on the assessment of the equivalence of technical materials of substances regulated under regulation (EC) No 1107/2009 – Working document, SANCO/10597/2003 – rev. 10.1 (13/7/12)
- Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA/ECHA, 2018). EFSA Journal, Vol 16, Issue 6, June 2018
- Guidance on a strategy for testing of chemicals. Committee on mutagenicity of chemicals in food, consumer products and the environment (COM), 22 December 2021

### **Residues**

- FAO (Food and Agriculture Organization of the United Nations), 2009. Submission and evaluation of pesticide residues data for the estimation of Maximum Residue Levels in food and feed. Pesticide Residues. 2nd Ed. FAO Plant Production and Protection Paper 197, 264 pp.
- OECD, 2009, Guidance document on the definition of residue, (ENV/JM/MONO(2009)30), Series on testing and assessment No. 63 and Series on pesticides No. 31
- OECD, 2013, Guidance document on residues in livestock, (ENV/JM/MONO(2013)8), Series on pesticides No. 73
- OECD, 2016, Guidance document on crop field trials, (ENV/JM/MONO(2011)50/REV1), Series on testing and assessment No. 164 and Series on pesticides No. 66
- OECD, 2018, Guidance document on residues in rotational crops, (ENV/JM/MONO(2018)9), Series on testing and assessment No. 279 and Series on pesticides No. 97

- EFSA guidance on the Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA Journal 2011;9(2):2092)
- Guidance Document On Botanical Active Substances Used In Plant Protection Products, SANCO/11470/2012– rev. 8, 20 March 2014
- Guidance document on criteria for the inclusion of active substances into Annex IV of Regulation (EC) N° 396/2005, SANCO/11188/2013 Rev. 2, 14 September 2015

#### **Environmental Fate and behaviour**

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