



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 3 – B.9 (PPP) PROBLAD **PLUS**

Ecotoxicology

Great Britain

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Background information

The active substance ‘aqueous extract from the germinated seeds of sweet *Lupinus albus*’, is a plant extract with fungicidal properties that can be used on food and non-food crops. It is extracted from the germinated seeds of sweet *Lupinus albus* and manufactured into PROBLAD PLUS. In general, botanical active substances are complex mixtures comprising of numerous components, therefore, the whole technical grade is regarded as the active substance which is described as a UVCB substance (Substance of Unknown or Variable composition, Complex reaction product or Biological material).

A major component of the aqueous extract from the germinated seeds of sweet *Lupinus albus* is the BLAD protein. BLAD is a naturally occurring seed storage protein present in germinated sweet lupins. It is a 20 kDa¹ polypeptide that is comprised of 173 amino acid residues and is a stable intermediate of the catabolism of β -conglutin², or characterised as a fragment of the amino acid sequence of β -conglutin, therefore, BLAD has no specific molecular or structural formula. BLAD is present in the aqueous extract from the germinated seeds of sweet *Lupinus albus* as a 210 kDa glyco-oligomer which is mainly composed of the 20 kDa polypeptide. The published literature (Monteiro et al. 2015; see Volume 3 CA B8 for summary) indicates that the 210 kDa protein present in PROBLAD PLUS is a glyco-oligomer composed of several polypeptides, the major ones exhibiting molecular masses of 14, 17, 20 (BLAD) 32, 36, 48 and 50 kDa. It is noted that BLAD contains a high proportion of the nitrogen-rich amino acids, which is consistent with its role as a seed storage protein.

BLAD forms 20% w/w of the PROBLAD PLUS formulation and is not isolated during the preparation of the product. The ecotoxicology data has been submitted with PROBLAD PLUS (sometimes also named PROBLAD). The active substance

¹ Unit of molecular mass, 1 Dalton (Da) = 1 g/mol

² Major *Lupinus* seed storage protein

aqueous extract from the germinated seeds of sweet *Lupinus albus* and the plant protection product 'PROBLAD PLUS' are identical in composition and are derived from the same continuous manufacturing process. Therefore all toxicity data has been submitted and evaluated in the Volume 3 CA B9 dossier. Additionally, a range of published literature studies have been provided, which are summarised and evaluated in the Volume 3 CA B9.

This section summarises the ecotoxicological effects of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) and evaluates the potential risk to non-target organisms.

The applicant has confirmed that formulation is intended to be marketed as 'PROBLAD' in GB.

Proposed GAP

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is proposed for use as a fungicide for use on strawberries and tomatoes.

The critical use pattern is summarised in the following table.

Table 9.0-1: Critical use pattern for aqueous extract from the germinated seeds of sweet *Lupinus albus*.

Use	Maximum application rate		Application method	Number of applications	Minimum application interval (days)	Application timing
	L product/ha	Kg a.s/ha ¹				
Strawberries, professional field	3.2	4.016	Foliar spraying	1-6	8	BBCH 61-89
Strawberries, professional greenhouse	3.2	4.016	Foliar spraying	1-6	8	BBCH 61-89
Tomatoes, professional field	3.2	4.016	Foliar spraying	1-6	8	BBCH 61-89
Tomatoes, professional greenhouse	3.2	4.016	Foliar spraying	1-6	8	BBCH 61-89

¹ Based on density of 1.255 g/mL.

The risk assessments that follow will consider field uses as the worst-case use pattern, which will be protective of the risks for greenhouse applications.

B.9.1. Effects on birds and other terrestrial vertebrates

B.9.1.1. Effects on birds

Toxicity

No standard toxicity studies have been submitted with aqueous extract from the germinated seeds of sweet *Lupinus albus*. However, lupin seeds are commonly used as a feedstuff in poultry rearing and several published literature studies have been provided investigating the inclusion of lupins in bird diets. These have been

evaluated in detail in Volume 3 CA B9.1 with respect to their relevance and reliability. The available studies are summarised in Table 9.1.1-1 below.

Table 9.1.1-1: Summary of literature data for birds

Reference	Test substance	Test species	Parameters measured	Exposure Duration	Result	Relevance	Reliability
K-CA 8.1.1.1/01 Arslan & Seeker (2002)	White Lupin seed, <i>Lupinus albus</i> , inclusion at 15% in diet	Japanese quail, <i>Coturnix japonica</i>	Feed consumption, live weight gain, feed conversion efficiency	42 days	No adverse effect on feed consumption, live weight gain or feed conversion efficiency at 15% in diet	Relevant: dietary method of exposure, standard test species and same lupin species	Reliable with reservations
K-CA 8.1.1.1/02 Rubio et al. (2003)	Sweet Lupin, <i>Lupinus angustifolius</i> , seed meal, included raw at 400 g/kg or dehulled at 320 g/kg	Broiler chickens	Body weight, feed intake, gain:feed ratio, liver weight, liver RNA composition	21 days	Reduced body weight following consumption of 400 g/kg diet. No significant effect on body weight from dehulled seed meal at 320 g/kg.	Relevant: dietary method of exposure. Physiological parameters not considered relevant.	Reliable with reservations
K-CA 8.1.1.1/03 Viveros et al. (2007)	Sweet Lupin, <i>Lupinus albus</i> var. Multolupa, seed meal included in diet at 200 and	Broiler chickens	Body weight, feed consumption, gain:feed ratio, liver weight, liver fat, caecal pH,	21 days	No overt toxic effects or mortality at 200 or 400 g/kg seed meal but statistically significant reduction in body weight.	Relevant: dietary method of exposure, same lupin species used.	Reliable with reservations

	400 g/kg, with and without cholesterol		intestinal viscosity, blood parameter changes		Reduction in food consumption observed but not statistically significant.	Dietary cholesterol supplementation not relevant.	
K-CA 8.1.1.1/04 Kaczmarek et al. (2016)	Sweet lupin, <i>Lupinus albus</i> , 100, 150, 200, 250, 300 g/kg	Broiler chickens	Mortality, body weight, food intake, food conversion ratio, ileal digestibility, intestinal morphology	35 days	Significant reduction in body weight gain at 200 g/kg and above (up to 300 g/kg), food intake rate significantly reduced at 250 and 300 g/kg	Relevant: dietary method of exposure, same species of lupin used.	Reliable with reservations
K-CA 8.1.1.1/05 Ravindran, Ravindran & Bryden (2006)	24 feedstuffs, including <i>Lupinus albus</i> and <i>Lupinus angustifolius</i>	Broiler chickens	Ileal digestibility coefficient of tryptophan	42 days	Digestibility of <i>L. albus</i> was higher than other grain legumes, most cereals and oilseed meals tested (excluding wheat, sunflower and soybean)	Limited relevance: dietary method of exposure and same species of lupin used but parameters measured not considered particularly useful	Reliable with reservations

K-CA 8.1.1.1/06 Olkowski et al. (2005)	Lupin seeds: <i>Lupinus</i> <i>angustifolius</i> , <i>Lupinus luteus</i> and <i>Lupinus albus</i> . Raw at 400 g/kg or dehulled at 350 g/kg.	Broiler chickens	Body weight, blood parameters, ileal digesta	21 days	Reduced feed intake and growth rate compared to control at 350–400 g/kg.	Relevant: dietary method of exposure and same species of lupin used.	Reliable with reservations
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None of the available literature studies indicated overt acute toxicity of lupin seeds to birds. Reduction in body weight was observed in several of the studies, particularly in treatments with whole/raw lupin seeds, but this was often attributed to reduced feed intake due to the presence of large amounts of alkaloids (Arslan & Seker (2002)); insoluble lupin fibre (Rubio et al. (2003); Kaczmarek et al. (2007)); or poor palatability (Viveros et al. (2007); Olkowski et al. (2005)).

As the available literature data did not indicate any toxic effects of lupin to birds it is considered acceptable to base the toxicity assessment on the available literature studies. All studies were considered to be 'reliable with reservations'. They could not be considered as fully reliable due to lack of defined test item (batch no., purity etc.), lack of reporting on the source of test organisms or housing conditions. Of the available studies, only one was conducted with the standard OECD 223 test species, Japanese quail (Arslan & Seker, 2002). The test item was seeds of *Lupinus albus* (the same species from which the active substance under assessment is derived) and exposure was via the diet at a concentration of 15%. It is acknowledged that there is some uncertainty in using data with seeds to read across to the active substance, despite both being obtained from the same lupin species. The active substance is derived from germinated seeds, and the germination process would result in changes in the chemical composition. However, all other studies were conducted with broiler chickens and/or with seeds from different species of lupin. The study by Arslan & Seker (2002) is therefore considered to be most relevant for assessing the risk to birds from aqueous extract of the germinated seeds of sweet *Lupinus albus*.

Acute oral toxicity endpoint

Considering the study by Arslan & Seker (2002), the potential amount of raw lupin ingested by each bird each day has been calculated. The total dietary feed per day in the raw lupin treatment group was 15.17 g feed/quail/day. As lupins comprised 15% of the diet, this would equate to 2275.5 mg/quail/day. This has been converted to a dose in terms of mg/kg body weight/day (as required in EFSA 2009) using the average weight of Japanese quail over the study duration, which was 87.58 g. The resulting dose was 25982 mg/kg bw/d. Since no mortality was observed at this dose, the LD₅₀ can be estimated to be > 25982 mg/kg bw/d. The OECD 223 acute avian toxicity study guideline recommends a maximum tested dose of 2000 mg/kg bw, so the dose received in this study exceeded this by more than a factor of 10.

Since the published studies indicated low toxicity, but could not produce a quantitative endpoint for the risk assessment, the acute mammalian endpoint will be used. This endpoint (> 5000 mg/kg bw) is lower than the estimated average dose with no effects over 42 days in Japanese Quail. The relevant acute endpoint is therefore summarised below.

Table 9.1.1-2: Acute toxicity endpoint for use in avian risk assessment

Study type	Test substance	Species	Endpoint	Value
Acute oral toxicity	PROBLAD PLUS	Rat	LD ₅₀	> 5000 mg/kg bw

Chronic oral toxicity


Studies assessing the reproductive effects of the aqueous extract from the germinated seeds of sweet *Lupinus albus* on birds have not been conducted. A variety of literature studies have been provided which investigate the effects of feeding lupins to birds (broiler chicken and quail). Although these studies did not assess any reproductive parameters, there were no indications of toxicity to adult birds over extended time periods (up to 42 days). Additionally, the lead component, BLAD, is a naturally occurring protein which is formed during the germination process of *Lupinus albus*. It has a mode of action specific to fungi, whereby it binds to chitin and chitosan, leading to weakened cell wall structure. BLAD is known to be susceptible to proteolytic degradation (Monteiro et al. 2015; see Volume 3 CA B8.1.1.2 for summary). It is therefore considered unlikely that BLAD would have toxic effects in a reproductive or developmental capacity. The remaining components comprise proteins and carbohydrates that would already occur naturally in the environment and would comprise a large portion of avian diets. Overall, the potential for chronic/reproductive toxicity is considered to be low, and further assessment is not considered to be necessary.

B.9.1.2. Effects on terrestrial vertebrates other than birds

Acute oral toxicity

An acute mammalian toxicity study has been carried out with PROBLAD PLUS. Full details of this study are provided in Volume 3CA B6 (Toxicology). The endpoint is presented in the table below.

Table 9.1.2-1: Acute toxicity endpoint for use in risk assessment for mammals

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

The study was considered valid and suitable for use in risk assessment. The LD₅₀ of > 5000 mg/kg bw/d will be used in the acute risk assessment that follows.

In addition, several published literature studies have been presented which investigate the inclusion of lupin seeds in mammalian diets. These have been summarised and evaluated with respect to their relevance and reliability in Volume 3 CA B9. A summary of the studies is presented in the table below.

Table 9.1.2-2: Summary of literature data for mammals.

Reference	Test substance	Test species	Parameters measured	Exposure Duration	Result	Relevance	Reliability
K-CA 8.1.2.2/01 Ephrem et al. (2015)	Sweet blue lupin seeds <i>Lupinus angustifolius</i> . Inclusion of 195, 245 or 295 g sweet lupin seed with 150 g wheat bran.	Washera lambs	Daily feed intake, daily weight gain, feed conversion efficiency, apparent digestibility of nutrients.	90-day feeding and 7-day digestibility	Significant increase in average daily gain in treatments supplemented with sweet blue lupin seeds.	Limited relevance; dietary method of exposure is relevant but different lupin species tested, non-standard test species and digestibility trial not relevant.	Reliable with reservations
K-CA 8.1.2.2/02 Kim, J.C. (2012)	<i>Lupinus angustifolius</i> (whole and dehulled seeds). Inclusion of whole or de-hulled lupins at 60, 120, 180 and 240 g/kg diet.	Pigs, species not stated	Feed conversion ratio, faecal consistency and moisture content, plasma urea nitrogen	14 days	Slower growth in 240 g/kg dehulled lupin treatment, attributed to decreased feed intake. Significant increase in faecal-haemolytic <i>E. coli</i> score. 240 g/kg whole/ 180 g/kg dehulled lupin significantly	Limited relevance; dietary method of exposure is relevant but different species of lupin tested and non-standard test species. Digestibility trials not relevant to mammalian dietary risk assessment.	Reliable with reservations

					increased plasma urea nitrogen levels.		
K-CA 8.1.2.2/03 Volek & Marounek (2009)	Whole white lupin seeds (<i>Lupinus albus</i>). 150 g/kg.	Hyphlus rabbits (<i>Oryctolagus cuniculus</i>)	Feed intake and feed conversion, total tract apparent digestibility,	79 days	No effect on weight gain or feed conversion efficiency in sweet lupin treatment.	Relevant; same species of lupin tested as active substance, dietary method of exposure.	Reliable with reservations

In the available literature studies, no signs of toxicity of lupins to mammals via dietary exposure were observed. All studies were considered to be 'reliable with reservations'. They could not be considered as fully reliable due to lack of defined test item (batch no., purity etc.), lack of reporting on the source of test organisms or housing conditions. All three studies were primarily assessing nutrient digestibility and feed conversion ratio. The assessments of nutrient digestibility are not considered to be relevant to the dietary risk assessment for mammals. Of the available studies, Volek & Marounek (2009) is considered to be the most relevant to the dietary risk assessment for mammals because the test item was the same species of lupin from which the active substance is derived. In this study, no significant effect on weight gain or feed conversion efficiency was observed following inclusion of lupin seeds in the diet at 150 g/kg. These studies may be considered as supporting information in the risk assessment for mammals.

Chronic oral toxicity

No standard laboratory data is available for the assessment of the chronic risks to mammals. However, there were no indications in the 90 day oral study in the rat, of adverse effects on reproductive organs (see Volume 3CA B6.3.2). A variety of literature studies have been provided which investigate the effects of feeding lupins to mammals. Although these studies did not assess any reproductive parameters, there were no indications of toxicity to mammals over extended time periods (up to 90 days). Additionally, the lead component, BLAD, is a naturally occurring protein which is formed during the germination process of *Lupinus albus*. It has a mode of action specific to fungi, whereby it binds to chitin and chitosan (these targets are absent in mammalian biological systems), leading to weakened cell walls structure. BLAD is known to be susceptible to proteolytic degradation (Monteiro et al. 2015; see Volume 3CA B8.1.1.2 for summary). As stated in Volume 3CA B6.1.1, BLAD will 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. The remaining components comprise proteins and carbohydrates (see Volume 4) that would already occur naturally in the environment and would comprise a large portion of mammalian diets. Overall the potential for chronic/reproductive toxicity is considered to be low, and further assessment is not considered to be necessary.

B.9.2. Risk assessment for birds and other terrestrial vertebrates

B.9.2.1. Risk assessment for birds

Exposure

The proposed uses are summarised in Table 9.0-1. Due to negligible exposure of birds, no consideration is required for the uses in glasshouses. For the field uses, exposure of birds will be predominantly dietary, through the consumption of residues on food items. Exposure is calculated according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

Screening step

The proposed application is on tomatoes and strawberries between BBCH 61–89. The proposed use is at 4.016 kg/ha with a maximum 6 applications and an interval of 8 days. EFSA 2009 does not provide multiple application factor (MAF) values for 8 day application intervals, so to provide a conservative risk assessment the MAF value for 6 applications with a 7 day application interval has been used. Results of the acute screening step are summarised in the table below.

Table 9.2.1-1: Screening assessment of the acute risk for birds due to use in tomatoes and strawberries (BBCH 61-89) at 4.016 kg/ha

Intended use	Tomatoes and strawberries BBCH 61 – 89 (field uses)				
Test substance					
Application rate (kg/ha)					
Acute toxicity (mg/kg bw)	> 5000				
TER criterion					
	10				
Crop scenario	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Fruiting vegetables, Strawberries	Small omnivorous bird	158.8	1.9	1211.7	4.12

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The acute TER value for the intended use is below the trigger value of 10. The MAF value used from EFSA 2009 was a conservative estimate for 6 applications with a 7 day interval. HSE has calculated the true MAF value for 6 applications with an 8 day interval to be 1.8. It is noted that the screening step would still fail if using this value. An acceptable risk has not been demonstrated, further consideration is required. A first-tier risk assessment is conducted below.

Table 9.2.1-2: First-tier assessment of the acute risk for birds due to use in tomatoes and strawberries (BBCH 61-89) at 4.016 kg/ha

Intended use	Tomatoes and strawberries BBCH 61 – 89 (field uses)				
Test substance					
Application rate (kg/ha)					
Acute toxicity (mg/kg bw)					
TER criterion	10				
Crop scenario	Generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Tomatoes					
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	Frugivorous bird “starling”	49.4	1.9	376.94	13.3

Fruit stage BBCH 71 – 89					
Fruiting vegetables BBCH ≥ 20	Small insectivorous bird “wagtail”	25.2	1.9	192.29	26.0
Strawberries					
Strawberries BBCH ≥ 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 ≥ 20	Small insectivorous bird “wagtail”	25.2	1.9	192.29	26.0

The TER values all exceed the trigger value of 10. Therefore an acceptable acute risk to birds has been demonstrated at first tier for the proposed uses on tomatoes and strawberries.

Risk to birds through drinking water

Leaf scenario

EFSA Bird and Mammal Guidance (2009) states the leaf scenario should be considered for leafy vegetables that form heads or have a morphology that facilitates the collection of rain/irrigation water in reservoirs that are large enough and easily accessible to attract birds. Since aqueous extract from the germinated seeds of

sweet *Lupinus albus* is intended to be applied to tomatoes and strawberries, which are not leafy vegetables that form heads, the leaf scenario does not require consideration.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

Estimates of soil adsorption for the majority of components (water, proteins, carbohydrates) are not considered necessary as these components already occur in agricultural soils in large concentrations. For the lead component, BLAD, a default K_{oc} of 10,000 L/kg has been proposed (see Volume 3 CA B8.1.4.1). As the K_{oc} values of the components are > 500 L/kg, this indicates they are more sorptive substances and the appropriate trigger value for comparison is 3000. As a worst-case first step, HSE has used the maximum total application rate (6 applications at 4016 g/ha = 24096 g/ha). Given that PROBLAD PLUS and the lead component BLAD have been shown to be readily biodegradable (see Volume 3 CA B8.2.2.1), it is considered that only the acute risks from drinking water exposure require consideration.

Table 9.2.1-3: Ratio of application rate to toxicity for risk to birds consuming drinking water from puddles contaminated by aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)

Test substance	K_{oc}	Max. application rate (g/ha)	Acute LD ₅₀ (mg/kg bw)	Ratio of AR _{eff} / LC ₅₀	Ratio trigger
PROBLAD PLUS	1287 – 10000 L/kg	24096	> 5000	4.8	3000

The calculated ratio is below the relevant trigger of 3000. Therefore no calculations are required. An acceptable acute risk to birds from consumption of contaminated drinking water following the proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) may be concluded.

Effects of secondary poisoning

According to EFSA 2009, substances with a $\log K_{ow} \geq 3$ have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains.

Aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS), and the lead component BLAD, have been demonstrated to be readily biodegradable according to the conditions of OECD 301 D and 301 B (see Volume 3 CA B8.2.2.1). Therefore the exposure and potential for bioaccumulation will be limited due to the rapid breakdown and no consideration of secondary poisoning is considered to be necessary. This is discussed in further detail below. Furthermore, PROBLAD PLUS is a complex mixture and none of the components are isolated during preparation of the product. It is therefore not considered relevant to derive a $\log K_{ow}$ for PROBLAD PLUS.

Risk to earthworm-eating birds

An assessment for secondary poisoning is not considered to be necessary. BLAD has been demonstrated to be readily biodegradable (see Volume 3 CA B8.2.2.1) and is also known to be susceptible to proteolytic degradation, including by enzymes naturally present in soil and in the digestive tract (Monteiro et al. 2015; see Volume 3 CA B8.1.1.2 for summary). Therefore given the breakdown in soil and by digestive enzymes, bioaccumulation is considered unlikely to occur. Additionally, the high K_{oc} of BLAD indicates a strong adsorption to soil which would result in limited bioavailability in soil to earthworms. Uptake by earthworms is therefore expected to be marginal and no assessment for secondary poisoning is considered necessary.

Risk to fish eating-birds

To determine the risk to fish-eating birds, typically a value for residues in fish is calculated using the experimental fish bioconcentration factor (BCF). PROBLAD PLUS has been determined to be readily biodegradable (see Volume 3 CA B8.2.2.1), so limited exposure is expected to fish and as such, an experimental BCF has not been determined. The K_{oc} of the lead component BLAD has been determined to be 10000 L/kg. On this basis, bioavailability in water is expected to be low, since BLAD has a high potential for adsorption to sediment and particulate matter. Overall it is considered that no further assessment of the risk to fish-eating birds is required.

Overall conclusions for birds

Acceptable acute risks to birds have been demonstrated for the proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) on tomatoes and strawberries at first tier. Consideration of the chronic risks to birds was not considered to be necessary, since the main components comprise proteins and carbohydrates (see Volume 4) that would already occur naturally in the environment and would comprise a large portion of avian diet. The lead component, BLAD, has a mode of action specific to fungi, whereby it binds to chitin and chitosan, leading to weakened cell walls structure. It is therefore considered unlikely that BLAD would have toxic effects in a reproductive or developmental capacity. Additionally, lupin seeds are commonly used as a feedstuff for poultry rearing and a variety of published literature is available investigating the inclusion of lupin seeds in avian diets, which do not indicate any adverse effects.

An acceptable risk to birds from contaminated drinking water has been demonstrated when considering the puddle scenario. No consideration of secondary poisoning was considered necessary, given the low bioavailability of BLAD in soil/water, its rapid breakdown and susceptibility to proteolytic degradation.

Overall an acceptable risk to birds may be concluded and no further consideration is required.

B.9.2.2. Risk assessment for other terrestrial vertebrates

Exposure

The proposed uses are summarised in Table 9.0-1. Due to negligible exposure of mammals, no assessment is required for the glasshouse uses on strawberries and tomatoes. For the field uses, exposure of mammals will be predominantly dietary, through the consumption of residues on food items. Exposure is calculated according to the EFSA Guidance Document on Risk Assessment of Birds and Mammals (2009).

Screening step

The proposed application is on tomatoes and strawberries between BBCH 61-89. The proposed use is at 4.016 kg/ha with a maximum of 6 applications and an interval of 8 days. EFSA 2009 does not provide MAF values for 8 day application intervals, so to provide a conservative risk assessment the MAF value for 6 applications with a 7 day application interval has been used. The results of the acute screening step are summarised in the table below.

Table 9.2.2-1: Screening assessment of the acute risk for mammals due to use in tomatoes and strawberries (BBCH 61-89) at 4.016 kg/ha)

Intended use	Tomatoes and strawberries BBCH 61 – 89 (field uses)				
Test substance					
Application rate (kg/ha)					
Acute toxicity (mg/kg bw)	> 5000				
TER criterion					
	10				
Crop scenario	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Strawberries	Small herbivorous mammal	118.4	1.9	903.4	5.5
Tomatoes	Small herbivorous mammal	136.4	1.9	1040.8	4.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The acute TER value for the intended uses on strawberries and tomatoes are below the trigger value of 10. The MAF value used from EFSA 2009 was a conservative estimate for 6 applications with a 7 day interval. HSE has calculated the true MAF value for 6 applications with an 8 day interval to be 1.8. It is noted that the screening step would still fail if using this value. An acceptable risk has not been demonstrated, further consideration is required. A first tier risk assessment is conducted below.

Table 9.2.2-2: First-tier assessment of the acute risk for mammals due to use in tomatoes and strawberries (BBCH 61-89) at 4.016 kg/ha

Intended use		Tomatoes and strawberries BBCH 61 – 89 (field uses)				
Test substance		PROBLAD PLUS				
Application rate (kg/ha)		6 × 4.016				
Acute toxicity (mg/kg bw)		10				
TER criterion						
Crop scenario	Generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Tomatoes						
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” ¹	40.9	1.9	312.1	16.0	
Fruiting vegetables	Small omnivorous mammal “mouse”	5.2	1.9	39.7	125.9	

BBCH \geq 50					
Strawberries					
Strawberries BBCH \geq 20	Small insectivorous mammal “shrew”	5.4	1.9	41.2	121.4
Strawberries BBCH \geq 40	Small herbivorous mammal “vole” ¹	54.6	1.9	416.6	12.0
Strawberries BBCH \geq 40	Large herbivorous mammal “lagomorph”	14.0	1.9	106.8	46.8
Strawberries BBCH \geq 40	Small omnivorous mammal “mouse”	6.9	1.9	52.6	95.1

¹The vole is not a relevant focal species in UK arable crops, however calculations are provided for completeness.

The TER values all exceed the trigger value of 10. Therefore an acceptable acute risk to mammals has been demonstrated at first tier for the proposed uses on tomatoes and strawberries.

Risk to mammals through drinking water

In accordance with EFSA Bird and Mammal Guidance (2009), only the puddle scenario is considered to be a relevant drinking water risk for mammals.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

A default Koc of 10,000 L/kg has been assigned to the lead component BLAD (see Volume 3 CA B8.1.4.1). As the Koc values of the components are > 500 L/kg, this indicates they are more sorptive substances and the appropriate trigger value for comparison is 3000. As a worst-case first step, HSE has used the maximum total application rate (6 applications at 4016 g/ha = 24096 g/ha). Given that PROBLAD PLUS and the lead component BLAD have been shown to be readily biodegradable (see Volume 3 CA B8.2.2.1), it is considered that only the acute risks from drinking water exposure require consideration.

Table 9.2.2-3: Ratio of application rate to toxicity for risk to mammals consuming drinking water from puddles contaminated by aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)

Test substance	Koc	Max. application rate (g/ha)	Acute LD ₅₀ (mg/kg bw)	Ratio of AR _{eff} / LC ₅₀	Ratio trigger
PROBLAD PLUS	1287 – 10000 L/kg	24096	> 5000	4.8	3000

The calculated ratio is below the relevant trigger of 3000 for PROBLAD PLUS. Therefore no calculations are required. An acceptable acute risk to birds from the proposed uses of PROBLAD PLUS may be concluded.

Effects of secondary poisoning

According to EFSA 2009, substances with a log Kow ≥ 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains.

Aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS), and the lead component BLAD, have been demonstrated to be readily biodegradable according to the conditions of OECD 301 D and 301 B (see Volume 3 CA B8.2.2.1). Therefore the exposure and potential for bioaccumulation will be limited due to the rapid breakdown and no consideration of secondary poisoning is considered to be necessary. This is discussed in further detail below. Furthermore, PROBLAD PLUS is a complex mixture and none of the components are isolated during preparation of the product. It is therefore not considered relevant to derive a log Kow for PROBLAD PLUS.

Risk to earthworm-eating mammals

An assessment for secondary poisoning is not considered to be necessary. BLAD has been demonstrated to be readily biodegradable (see Volume 3 CA B8.2.2 1) and is also known to be susceptible to proteolytic degradation, including by enzymes naturally present in soil and in the digestive tract (Monteiro et al. 2015; see Volume 3 CA B8.1.1.2 for summary). As stated in Volume 3CA B6.1.1, under normal mammalian metabolism, the protein will be broken down under enzymatic processes in the gastrointestinal tract, enter the amino acid pool and be consumed into normal metabolic processes. Therefore given the breakdown in soil and by digestive enzymes in mammalian systems, bioaccumulation and secondary poisoning is considered unlikely to occur. Additionally, the high Koc of BLAD indicates a strong adsorption to soil which would result in limited bioavailability in soil to earthworms. Uptake by earthworms is therefore expected to be marginal and no assessment for secondary poisoning is considered necessary.

Risk to fish eating-mammals

To determine the risk to fish-eating birds, typically a value for residues in fish is calculated using the experimental fish bioconcentration factor (BCF). PROBLAD PLUS has been determined to be readily biodegradable, so limited exposure is expected to fish and as such, an experimental BCF has not been determined. The Koc of the lead component BLAD has been determined to be 10000 L/kg. On this basis, bioavailability in water is expected to be low, since BLAD has a high potential for adsorption to sediment and particulate matter. Overall it is considered that no further assessment of the risk to fish-eating mammals is required.

Overall conclusions for mammals

Acceptable acute risks to mammals have been demonstrated for the proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) on tomatoes and strawberries at first tier. Consideration of the chronic risks to mammals was not considered to be necessary, since the main components comprise proteins and carbohydrates (see Volume 4) that would already occur naturally in the environment and would comprise a large portion of mammalian diet. The lead component, BLAD, has a mode of action specific to fungi, whereby it binds to chitin and chitosan, leading to weakened cell wall structure. It is therefore considered unlikely that BLAD would have toxic effects in a reproductive or developmental capacity. Additionally, lupin seeds are commonly used as a feedstuff for livestock rearing and a variety of published literature is available investigating the inclusion of lupin seeds in livestock diets, which do not indicate any adverse effects.

An acceptable risk to mammals from contaminated drinking water has been demonstrated when considering the puddle scenario. No consideration of secondary poisoning was considered necessary, given the low bioavailability of BLAD in soil/water and its rapid breakdown and susceptibility to proteolytic degradation.

Overall an acceptable risk to mammals may be concluded and no further consideration is required.

B.9.3. Effects on aquatic organisms

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Data have been submitted addressing the toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus* (tested as PROBLAD PLUS) to fish, aquatic invertebrates (acute and chronic) and algae. These studies have been summarised and evaluated in Volume 3CA B9.2. No additional studies have been submitted here. In addition, several published literature studies have been presented which investigate the use of lupin seeds as a dietary supplement in aquaculture. These have been summarised and evaluated with respect to their relevance and reliability in Volume 3CA B9.2.

B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

None submitted.

B.9.3.3. Further testing on aquatic organisms

None submitted.

B.9.4. Risk assessment for aquatic organisms

The following risk assessment has been conducted according to the EFSA (2013) guidance document (EFSA Journal 2013;11(7):3290).

Exposure

Exposure estimates have been taken from Volume 3CP B8.5. Predicted Environmental Concentrations (PECs) for spray drift and drainflow exposure have been established by the Environmental Fate evaluator. PEC values for both PROBLAD PLUS and the lead component BLAD have been determined. There are no metabolites for consideration.

The PEC_{sw} values are summarised in the table below:

Table 9.4-1: PECsw spray drift and drainflow values for PROBLAD PLUS and BLAD

Component	PECsw spray drift (µg/L)	PECsw drainflow (µg/L)
PROBLAD PLUS	63.3	14.166
BLAD	24.9	2.873

Toxicity

The laboratory data available to address the toxicity of the active substance, aqueous extract from the germinated seeds of sweet *Lupinus albus* (tested as PROBLAD PLUS), is summarised below (Table 9.4-2). Studies that were not considered suitable for use in risk assessment are also indicated in the table.

Table 9.4-2: Endpoints relevant for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
Acute toxicity to fish					
PROBLAD	Oncorhynchus mykiss	96-hours, Semi-static	LC ₅₀ (nom.)	> 100 Not suitable for use in risk assessment, supporting information only	<div></div> (2011)
Chronic toxicity to fish					
No data submitted					
Bioconcentration in fish					

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
No data submitted					
Acute toxicity to invertebrates					
PROBLAD PLUS	Daphnia magna	48-hours, semi-static	EC ₅₀ (nom.)	159.32 Not suitable for use in risk assessment, supporting information only	Weber, K. (2011)
			NOEC (nom.)	31.3 Not suitable for use in risk assessment	
PROBLAD PLUS	Daphnia magna	48-hours, semi-static	EC ₅₀ (mm)	>75	Gerke, A.K. and Schneider, S.Z. (2019)
Long-term toxicity to invertebrates					
PROBLAD PLUS	Daphnia magna	21-days, static	EC ₅₀ (mm)	> 2.7	Gerke, A.K. and Schneider, S.Z. (2019)
			NOEC (mm)	2.7	
Toxicity to sediment-dwelling organisms					
No data submitted					
Toxicity to algae					

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
PROBLAD	Desmodesmus subspicatus	72- hours, static	E _y C ₅₀ (nom.)	28.7 Not suitable for use in risk assessment	Falk, (2011)
			NOEC	11.1 Not suitable for use in risk assessment	
PROBLAD	Raphidocelis subcapitata	72- hours, static	E _r C ₅₀ (mm)	51	Arnie et al. (2019)
			NOEC (mm)	6.6	
Toxicity to aquatic macrophytes					
No data submitted.					

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

Bold values are recommended for use in risk assessment.

The studies listed as not suitable for use in risk assessment did not conduct analytical measurements of the test item in the test media. The applicant has stated that analytical dose verification would not have been possible in these studies, as the lower dosing levels in these studies would have been below the LOQ of the analytical method. However it is noted that it would have theoretically been possible to verify the higher test concentrations (which would have been most relevant for deriving the endpoints), as this was done in the remaining studies which did make analytical assessments. To account for possible degradation of the test substance, the applicant has proposed using the results of the ready biodegradability test to provide an estimate of degradation in the aquatic systems. Approximately 50% degradation occurred over the first four days, so it was proposed to adjust the endpoints by a factor of 2. HSE does not consider this approach to be appropriate, since the conditions of the ready biodegradability test would not be representative of the conditions experienced in the aquatic studies above. Moreover, additional acute daphnia and algae studies have been provided which did include analytical

measurement of the test item in solution and provided mean-measured endpoints. It is considered more appropriate for these studies to be used in the risk assessment.

In addition to the laboratory studies conducted above, a number of published literature studies have been submitted which investigate the use of lupin seeds as a feedstuff in aquaculture. These studies are considered as supporting evidence for the risk assessment and are summarised in Table 9.4-3 below. The literature studies are discussed in further detail in the selection of endpoints, below.

Table 9.4-3. Summary of literature submitted for aquatics

Reference	Test substance	Test species	Parameters measured	Exposure Duration	Result	Relevance	Reliability
K-CA 8.2.2/01 Farhangi, M. and Carter, C. G. (2001)	Commercial dehulled lupin (<i>Lupinus angustifolius</i>)	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Amino acid profile, growth response, feed utilisation, digestive enzyme activity, immune responses	8 weeks	Dehulled lupin at up to 40% in diet causes no significant effect on growth performance or nutrient utilisation.	Not relevant: dietary method of exposure	Reliable with reservations
K-CA 8.2.2/02 Borquez et al., (2010)	<i>Lupinus albus</i> var. Hamburg	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Growth performance, feed utilisation, histology, digestibility, muscle fatty acid composition	11-week growth trial and 4-week digestibility trial	Whole lupin grain in diet at 50% causes no effect on growth performance and feed intake.	Not relevant: dietary method of exposure	Reliable with reservations
K-CA 8.2.2/03	<i>Lupinus albus</i> seeds 'lupin kernel meal'	Red hybrid tilapia	Growth performance, haematocrite/haemoglobin contents	120 days	Extruded lupin kernel meal at up to 50% in	Not relevant: dietary	Reliable with reservations

Yones, A.M. (2010)	100% purity- no batch number provided	(<i>Oreochromis</i> sp.)			diet can be used without adversely affecting growth performance, digestibility coefficient and blood characteristics.	method of exposure	
K-CA 8.2.2/04 Zhang <i>et al.</i> (2012a)	Lupin protein concentrates (LPC)- White lupine (<i>Lupinus albus</i>)	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Growth and feed utilisation, digestibility, body composition, nutrient retention and metabolic loss	62 days feeding test, followed by a 20-day digestibility experiment.	Inclusion of white lupin protein concentrates at up to 439 g LPC/kg diet, over a 82 day period, does not negatively affect growth of rainbow trout.	Not relevant: dietary method of exposure	Reliable with reservations

K-CA 8.2.2/05 Zhang, Y. et al. (2012)	Lupin protein concentrates (LPC)- White lupine (<i>Lupinus albus</i>)	Black sea bream (<i>Acanthopagrus schlegeli</i>)	Feed utilisation and nutrient retention	60 days	Inclusion of white lupin protein concentrates at up to 500 g LPC/kg diet does not negatively affect growth of black sea bream	Not relevant: dietary method of exposure	Reliable with reservations
K-CA 8.2.2/06 Molina- Poveda, C., Lucas, M., and Jover, M. (2013)	Andean lupin meal (<i>Lupinus mutabilis Sweet</i>)	Whiteleg shrimp (<i>Litopenaeus vannamei</i>) juveniles	Dry matter and protein digestibility and the effects of different test vessels	45 days	Inclusion of lupin kernel meal as a potential alternative protein source replacing at least 50% of protein from fish meal does not negatively affect growth or survival of	Not relevant: dietary method of exposure	Reliable with reservations

					Whiteleg shrimp		
K-CA 8.2.2/07 Hemre et al., (2009)	Proteins in kernel meals of dehulled white (<i>Lupinus albus</i>), sweet (<i>L. angustifolius</i>) and yellow (<i>L. luteus</i>) lupines	Aqua-cultured fish such as Atlantic salmon (<i>Salmo salar</i>), rainbow trout (<i>Onchorhyncus mykiss</i>), Atlantic halibut (<i>Hippoglossus hippoglossus</i>) and Atlantic cod (<i>Gadus morhua</i>)	-	-	Meals containing lupines can positively influence growth of aqua-cultured fish	Not relevant: dietary method of exposure	Reliable with reservations
K-CA 8.2.2/08 Glencross (2001)	NA literature review	Rainbow trout (<i>Onchorhyncus mykiss</i>) and Silver Perch (<i>Bidyanus bidyanus</i>).	-	-	Meals containing lupines can positively influence growth of	Not relevant: dietary method of exposure	Reliable with reservations

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

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					aqua-cultured fish		
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Selection of endpoints for Tier 1 risk assessment for the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)

The following endpoints were selected for use in the Tier 1 risk assessment for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS).

Acute toxicity to fish: One acute toxicity study was conducted with *Oncorhynchus mykiss* (██████ 2011). The submitted study was not accepted for use in the risk assessment due analytical measurement of the test item not being conducted, and it therefore not meeting the validity criteria outlined in OECD 203 (analytical measurement of test concentrations is compulsory). It is therefore not possible to confirm the concentration of test item that the fish were exposed to throughout the study. There are therefore no acceptable acute fish endpoints. A weight of evidence approach has been used to consider the acute toxicity to fish:

- A study without analytical measurements showed no mortality up to 100 mg a.s./L (nominal).
- Two studies were provided for *Daphnia magna* one with analytical measurements, one without. Both showed similar results and had no immobilisation at similar nominal concentrations to the fish acute study.
- The analytical measurements in the *Daphnia magna* study showed mean measured concentrations at nominal 120 mg/L as 75 mg/L and at nominal 60 mg/L as 31 mg/L. The fish acute study is longer, so would expect mean measured concentrations to be a bit lower than these, but these results give an indication of the magnitude of difference between nominal and mean measured concentrations.
- The lowest endpoint for the risk assessment is 2.7 mg a.s./L (mm) from a chronic *Daphnia magna* study. When taking into account the different assessment factor to derive the RAC, this still provides a margin of safety to cover the uncertainty in the acute fish endpoint providing a lower tier risk assessment is sufficient.
- As a vertebrate study a repeat study should only be requested if required. Since there is a demonstration of low acute toxicity to fish (with uncertainty about the exact endpoint) and a margin of safety to the RAC it is not considered necessary to repeat the acute fish toxicity study.

As a result, the RAC can be calculated without using an acute toxicity endpoint for fish as it has been demonstrated the studies on *Daphnia magna* sufficiently cover the acute toxicity to fish.

Long-term toxicity to fish: No chronic fish toxicity tests were submitted. PROBLAD PLUS and the lead component BLAD have been shown to be readily biodegradable (see Volume 3 CA B8.2.2.1) and therefore chronic exposure via water is considered to be unlikely. Additionally, PROBLAD PLUS comprises mainly proteins and carbohydrates, which aquatic organisms would likely already be exposed to and consume as part of their natural diet. Furthermore, sweet lupins are widely used as a feedstuff in aquaculture. Eight literature studies were submitted which investigate the inclusion of lupins in the diets of aquatic organisms, seven of which were considering effects on fish. These have been considered in detail in Volume 3CA B.9.2.2 and are summarised in Table 9.4-3 above. All studies were considered to be reliable with reservations however they are of limited relevance to the aquatic risk assessment due to the exposure being via diet as opposed to a concentration in water. Nonetheless, no adverse effects (main parameter assessed was growth) were seen in any of the available studies. Given that no mortality was seen in the acute fish study, which was dosed via water, the available literature studies provide some support that, once exposed internally, no long-term effects on growth would be expected. HSE therefore considers that no further consideration of the chronic risk to fish is necessary.

Acute toxicity to aquatic invertebrates: Two acute aquatic invertebrate toxicity studies with the active substance were conducted. One of the studies (Weber, 2011) did not conduct analytical measurements of the test solutions and therefore is not considered suitable for use in the risk assessment, as it is not possible to verify the concentration of test item the organisms were exposed to. The other study (Gerke and Schneider, 2019) did provide analytical measurements and was considered to be valid and suitable for use in the risk assessment.

The relevant endpoint is:

EC₅₀ (mm) = > 75 mg PROBLAD PLUS/L.

Chronic toxicity to aquatic invertebrates: one chronic study with aquatic invertebrates was conducted with *D. magna* (Gerke and Schneider, 2019). The study was considered valid and suitable for use in risk assessment.

The accepted endpoint for use in the risk assessment is:

NOEC (growth) (mm) = 2.7 mg PROBLAD PLUS/L.

Toxicity to sediment-dwelling organisms: No studies were submitted with sediment-dwelling organisms. Given that PROBLAD PLUS and BLAD have been demonstrated to be readily biodegradable, it is considered that residues in water will be rapidly degraded. However, 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, HSE will consider the margin of safety in the chronic aquatic invertebrate risk assessment. Additionally, one literature study was available assessing the inclusion of lupin meal in the diet of whiteleg shrimp (Molina-Poveda et al. 2013). There were no effects on growth or survival of whiteleg shrimp following inclusion of lupin kernel meal in the diet. Whilst not a sediment-dwelling species, whiteleg shrimp would feed at the soil-water interface and the results of this study can be considered as supporting evidence for the acceptability of risk to sediment-dwelling organisms.

Toxicity to algae: Two algal toxicity studies with PROBLAD PLUS were conducted. The study by Falk, (2011) did not provide any analytical measurements of the test solutions and therefore is not considered suitable for use in the risk assessment, since it is not possible to confirm the concentration of test item that the algae were exposed to. Additionally, a reliable ErC_{50} could not be determined for this study due to a lack of clear dose-response. The study by Arnie et al. (2019) did provide analytical data and was considered to be valid and suitable for use in risk assessment. The endpoint suitable for use in risk assessment is:

ErC_{50} (mm) = 51 mg PROBLAD PLUS/L.

Toxicity to aquatic macrophytes: No data was submitted. In accordance with assimilated Regulation No 283/2013, effects on aquatic macrophytes are only required for substances with herbicidal mode of action or plant growth regulators.

Table 9.4-4: Regulatory acceptable concentrations (RAC) for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) for each organism group

Test species:	Invertebrates		Algae
	Acute <i>D. magna</i>	Chronic <i>D. magna</i>	<i>R. subcapitata</i>
Endpoint	EC ₅₀	NOEC	ErC ₅₀
[µg a.s./L]	75000	2700	51000
AF	100	10	10
RAC [µg a.s./L]	750	270	5100

Risk assessment

The risk assessment is summarised in Table 9.4-5.

Table 9.4-5: First-tier risk assessment for exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) due to use on strawberries and tomatoes at 6 x 3.2 L product/ha

Scenario	PEC (µg/L)	Aquatic invertebrates acute	Aquatic invertebrates long-term	Algae
		<i>D. magna</i>	<i>D. magna</i>	<i>R. subcapitata</i>
		RAC (EC ₅₀)	RAC (NOEC)	RAC (ErC ₅₀)
		750 [µg/L]	270 [µg/L]	5100 [µg/L]
Spray-drift (1 m)	63.3	0.0844	0.234	0.0124

Drainflow	14.166	0.019	0.052	0.0028
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Values in **bold** are above the trigger of 1

The PEC/RAC ratios for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) are below the trigger value of 1 for both spray drift and drainflow exposure, for all organism groups.

BLAD is the lead component of PROBLAD PLUS and is present at 20.0%. PEC_{sw} and drainflow values have been determined for BLAD (see Volume 3 CP B8), however no toxicity data is available with this substance. The RAC values for PROBLAD PLUS could be expressed in terms of BLAD content by multiplying by 0.20, however, as the PEC values have also been calculated using this proportion, the resulting PEC/RAC ratios would be the same as calculated above for PROBLAD PLUS (Table 9.4-5).

Risk to sediment-dwelling organisms:

With a PEC/RAC of 0.234 for spray drift and 0.052 for drainflow, there is a large margin of safety 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.

Conclusion:

The risk from spray drift and drainflow is acceptable for all organism groups without mitigation for both aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS), and the lead component BLAD. No further consideration is required.

B.9.5. Effects on arthropods

B.9.5.1. Effects on bees

Data have been submitted addressing the toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus* (tested as PROBLAD PLUS) to bees. These studies have been summarised and evaluated in the Volume 3CA Part B9.3.1.

B.9.5.2. Effects on non-target arthropods other than bees

Data have been submitted addressing the toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) to the standard non-target arthropod species, *Typhlodromus pyri* and *Aphidius rhopalosiphi*. Both glass plate and extended laboratory studies have been submitted with these species. An extended laboratory study has also been submitted with the additional species, *Chrysoperla carnea*. These studies have been summarised and evaluated in the Volume 3CA Part B9.3.2.

B.9.6. Risk assessment for arthropods

B.9.6.1. Risk assessment for bees

There is currently not one scheme which clearly uses the data outlined in assimilated Regulations 283/2013 and 284/2013, therefore all submitted studies relevant to the GAP are evaluated. The data requirements for assimilated Regulations 283/2013 and 284/2013 include the submission of an acute oral and contact toxicity study at lower tier. The endpoints for these studies are based on mortality (LD₅₀). For an active substance, a study on the chronic toxicity to bees and a study on the effects on honey bee development are also required.

The risk assessment for bees is based on SANCO/10329/2002 rev 2 final 17 October 2002 guidance, and the data requirements according to assimilated Regulations 283/2013 and 284/2013. The LD₅₀ values from the lower tier acute oral and contact toxicity studies are compared with the application rate to calculate a Hazard Quotient (HQ). If the HQ is lower than the trigger value of 50, then an acceptable acute risk has been determined. Due to a lack of agreed or noted risk assessment guidance, data from studies on the chronic toxicity to bees and effects on honey bee development are not considered in the risk assessment.

Where unacceptable risks (HQ ≥ 50) are found at first tier, higher tier studies may have been submitted. If there is a risk at lower tier but no submitted higher tier data, then mitigation may be implemented. There is currently no noted guidance for higher

tier risk assessments for bees, therefore information from available sources would be taken into account in a weight of evidence approach for the risk assessment.

B.9.6.1.1. Toxicity

A summary of the submitted studies and associated endpoints can be found in Table 9.6.1-1.

Table 9.6.1-1: Summary of bee toxicity endpoints for aqueous extract from the germinated seeds of sweet *Lupinus albus* (tested as PROBLAD PLUS)

Study	Endpoint	Value	Reference
Problad: Acute Oral and Contact Toxicity to the Honeybee, <i>Apis mellifera</i> L., in the laboratory	LD ₅₀ Contact:	> 100 µg product /bee	K-CA 8.3.1.1.1/01 Kling, 2010.
	LD ₅₀ Oral:	> 109.42 µg product /bee	
PROBLAD PLUS: Acute oral toxicity to the Honey bee, <i>Apis mellifera</i> L., under laboratory conditions.	LD ₅₀ 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 ₅₀ Oral :	> 2320.9 µg product /bee	K-CA 8.3.1.1.2/02 Aguilar-Alberola, 2020
	LD ₅₀ Contact :	> 1200.0 µg product /bee	
PROBLAD PLUS: Chronic toxicity test for adult honeybees (<i>Apis mellifera</i> L.	10 day LDD ₅₀ :	42.10 µg PROBLAD PLUS/bee/day. Not suitable for	K-CA 8.3.1.2/01 Harkin, 2015

		use in risk assessment.	
PROBLAD PLUS: Chronic Oral toxicity test (10-day feeding) to the Honey bee (<i>Apis mellifera</i> L.) under laboratory conditions	10 day LDD ₅₀ :	361.9 µg PROBLAD PLUS bee/day	K-CA 8.3.1.2/02 Aguilar-Alberola, 2019.
PROBLAD PLUS: <i>In vitro</i> chronic toxicity to larval stage honey bee (<i>Apis mellifera</i> L.)	7 day NOEC (no reliable EC ₅₀)	155.0 µg PROBLAD PLUS/larva. Not suitable for use in risk assessment.	K-CA 8.3.1.3/01 Harkin, 2015
PROBLAD PLUS: Honey Bee (<i>Apis mellifera</i> L.) larval toxicity test following repeated exposure under laboratory conditions	EC ₅₀ larval:	> 154.0 µg PROBLAD PLUS/larva	K-CA 8.3.1.3/02 Aguilar-Alberola, 2019.

Endpoints in **bold** will be used in the risk assessment.

Acute toxicity endpoints:

Acute oral and contact studies with honeybees have been provided (Kling, 2010). An additional acute oral study has been provided (Aguilar-Alberola, 2019). All studies were valid and considered suitable for use in risk assessment. The endpoints from the studies by Kling (2010) are considered in the risk assessment, since they provide the most conservative assessment.

Acute toxicity studies with bumblebees have also been submitted. Given the lack of noted guidance available for conducting risk assessments for bumblebees, these studies are not considered further, although it is noted that the endpoints exceed those available for honeybees by more than one order of magnitude. This indicates that bumblebees are not more sensitive than honeybees.

Chronic toxicity endpoints:

Two chronic adult honeybee and two larval honeybee studies were submitted. The chronic adult honeybee endpoint from K-CA 8.3.1.2/01 Harkin (2015) was only considered as supporting information. This study was conducted before the finalised OECD 245 (2017) guideline and, as such, had a number of deviations and uncertainties, although it did meet the validity criteria. Additionally, no certificate of analysis was provided with this study, so the test item batch and purity could not be confirmed. Given that a newer study was available it was considered that this study should not be considered further.

The chronic larval study endpoint from K-CA 8.3.1.3/01 Harkin (2015) was only considered suitable as supporting information, since the study was only 7 d duration and no assessments of pupal mortality or adult emergence were made, nor was there any analytical verification of the test item concentrations. Additionally, no certificate of analysis was provided so it was not possible to confirm the purity or batch number of the test item. Given that a newer study was available it was considered that this study should not be considered further.

Given the lack of noted guidance for assessing the chronic risk to bees, these studies are not included in the risk assessment that follows.

B.9.6.1.2. Acute risk assessment

Assessment of the acute risk to bees is conducted in accordance with assimilated Regulation No 1107/2009, and the noted Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). To calculate the HQ for acute oral and contact toxicity studies, the maximum single application rate (4016 g/ha) is divided by the endpoint (LD₅₀) as conducted in the table below.

Table 9.6.1-2: HQ calculations for honeybees; contact and oral exposure for the application to strawberry and tomato (6 x 4016 g product/ha)

Test item	Study	Application rate	Endpoint (µg Product /bee)	HQ (< 50)

		(g product/ha)		
PROBLAD PLUS	Acute contact	4016	> 100	< 40.2
	Acute oral		> 109.42	< 36.7

HQ: Hazard Quotient

The hazard quotients for acute oral and contact toxicity studies on honey bees (*Apis mellifera* L.) are less than the trigger value of 50, therefore the acute risk to bees is acceptable.

B.9.6.1.3. Chronic risk assessment

Due to a lack of agreed or noted risk assessment guidance, and given that acceptable acute risks have been demonstrated, data from studies on the chronic toxicity to bees and effects on honey bee development are not considered in the risk assessment.

B.9.6.2. Risk assessment for non-target arthropods other than bees

The risk assessment for non-target arthropods other than bees (NTA) was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and with consideration of the recommendations of ESCORT II guidance³.

Risk assessment according to ESCORT II is divided into tiers. The first tier requires laboratory ‘glass plate’ data on two indicator species, *A. rhopalosiphum* and *T. pyri*. The endpoint for mortality (LR₅₀) for both species is compared to in- and off-field Predicted Environmental Rates (PER), to calculate a Hazard Quotient (HQ). If the HQ is lower than the trigger value of 2, an acceptable risk can be determined.

Where unacceptable risks (HQ ≥ 2) are found at first tier, extended laboratory data using more realistic substrates and additional crop-relevant species are submitted. For this tier of risk assessment, rather than calculating a HQ, the worst-case endpoint

³ Guidance Document on regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non-Target Arthropods. From the ESCORT 2 Workshop (European Standard Characteristics of Non-target Arthropod Regulatory Testing). Candolfi et al., 2000.

(mortality and/or reproduction) is compared directly to the in- and off-field PER for each species. Where the endpoints are greater than the PER, an acceptable risk can be concluded. If the risk assessment fails at this tier, the usual recourse is to higher tier data, e.g., field studies.

For PROBLAD PLUS, the following uses are requested on the representative GAP:

Table 9.6.2-1: Summary of uses requested for aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)

Use #	Target crop	Application	Outdoor/indoor
1	Strawberry	6 x 3.2 L product/ha (8 day interval)	Indoor (PPFE)* and outdoor (field)
2		6 x 3.2 L product/ha (8 day interval)	
3	Tomatoes	6 x 3.2 L product/ha (8 day interval)	
4		6 x 3.2 L product/ha (8 day interval)	

*Permanent Protection with Full Enclosure

As uses 1 and 3 are for use indoors (PPFE), exposure is considered to be negligible for wild populations of NTA. Therefore provided permanent protection with full enclosure is specified on the product label for these uses, no further risk assessment is required and an acceptable risk can be concluded.

For uses 2 and 4, a full outdoor risk assessment according to ESCORT II is required. This is conducted below.

B.9.6.2.1. Toxicity

Tier I and Tier II toxicity tests on *Aphidius rhopalosiphi* and *Typhlodromus pyri* have been carried out with PROBLAD PLUS. These studies have been evaluated in Volume 3 CA B9.3.2. An additional extended laboratory test on *Chrysoperla carnea* has been submitted. This study has been evaluated in Volume 3 CP B9.5.2. The table below presents the study endpoints.

Table 9.6.2-2: Study endpoints for non-target arthropods other than bees

Species	Endpoint	Value (mL product/ha)	Reference
First tier studies			
A. rhopalosiphi	LR ₅₀	> 10500 mL PROBLAD /ha	K-CA 9.3.2-01 Klug, 2010
T. pyri	LR ₅₀	> 10500 mL PROBLAD /ha	K-CA 9.3.2-02 Klug, 2010
Extended laboratory studies			
C. carnea	LR ₅₀	> 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	
T. pyri	LR ₅₀	> 8000 mL PROBLAD PLUS in 200 L water/ha	K-CA 9.3.2-03 Fallowfield, 2014
	Highest rate observed with < 50% effects on reproduction	> 8000 mL PROBLAD PLUS in 200 L water/ha	
A. rhopalosiphi	LR ₅₀	> 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	

B.9.6.2.2. Exposure

B.9.6.2.2.1. In-field exposure

Non-target arthropods inhabiting the crop can be exposed to residues of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) by direct contact, either as a result of overspray or through contact with residues on plants and soil or in food items. Aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) is applied at a maximum rate of 6 x 3.2 L product/ha to strawberries and tomatoes.

To calculate the $PER_{in-field}$, the application rate is multiplied by the MAF (multi-application factor). The MAF for products applied to foliage according to ESCORT II Appendix III is 3.2 (for 6 x applications).

$$PER_{in-field} = \text{Application rate [mL/ha]} \times \text{MAF}$$

The in-field PER for PROBLAD PLUS is therefore:

$$3200 \times 3.2 \text{ mL} = \mathbf{10240 \text{ mL product/ha.}}$$

B.9.6.2.2.2. Off-field exposure

Risk assessment for areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations. Exposure of non-target arthropods living in off-field areas to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure via soil residues in off-field areas was not considered.

The off-field PER is calculated firstly by multiplying the in-field PER by a drift factor published by the BBA [90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden], which are presented in ESCORT II, Appendix IV.

For field crops (strawberries, 6 applications) the drift value is 1.64% at 1 m. For tomatoes, the drift value for 'vegetables, ornamentals, small fruits, height > 50 cm' has been used, which is 6.41% at 3 m. The drift factor (% drift/100) is therefore 0.0164 and 0.0641 for strawberries and tomatoes, respectively. Secondly, the drift factor is divided by the VDF (vegetative distribution factor) for which a default value of 10 is used. For studies that involve 3D application methods (typically found in extended laboratory studies), the VDF is omitted. Finally, a correction factor (default 10, or 5 if additional species are tested) is applied. The calculation is summarised in the equation below:

$$PER_{\text{Off-field}} = PER_{\text{in-field}} \times (\text{drift factor}/\text{VDF}) \times \text{correction factor}$$

Therefore, the (first tier) off-field PER for PROBLAD PLUS on strawberries is:

$$3200 \times (0.0164/10) \times 10 = \mathbf{167.9 \text{ mL product/ha}}$$

The (first tier) off-field PER for PROBLAD PLUS on tomatoes is:

$$3200 \times (0.0641/10) \times 10 = \mathbf{656.4 \text{ mL product/ha}}$$

B.9.6.2.3. Risk assessment

B.9.6.2.3.1. Tier 1 risk assessment

The first tier risk assessments for the uses on strawberry and tomato are conducted in Tables 9.6.2-3 and 9.6.2-4, respectively.

Table 9.6.2-3: Toxicity data, PER and HQ values for non-target arthropods exposed to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) via use on strawberry

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

The first tier risk assessment for *T. pyri* and *A. rhopalosiphi* passes for the proposed use on strawberry, with both in-field and off-field HQ values < 2.

Table 9.6.2-4: Toxicity data, PER and 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 _{in-field} [mL/ha]	PER _{off-field} [mL/ha]	HQ _{in-field}	HQ _{off-field}	Trigger value
PROBLAD PLUS	<i>Typhlodromus pyri</i> protonymphs	Laboratory test, artificial substrate, 2D exposure	LR ₅₀ > 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 ₅₀ > 10500	10240	656.4	0.97	0.06	HQ < 2 risk is acceptable

The first tier risk assessment for *T. pyri* and *A. rhopalosiphi* passes for the proposed use on tomato, with both in-field and off-field HQ values < 2.

B.9.6.2.3.2. Tier II risk assessment

The applicant has submitted extended laboratory data with *T. pyri*, *A. rhopalosiphi* and *C. carnea*, all of which are considered to not be adverse. As the risk was acceptable at Tier I, a Tier II risk assessment is not required and the extended study endpoints will not be considered further.

B.9.6.2.3.3. Overall conclusions for non-target arthropods

Acceptable in-field and off-field risks to non-target arthropods have been demonstrated for the proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) on strawberries and tomatoes. No further consideration or mitigation measures are required.

B.9.7. Effects on non-target soil meso- and macrofauna

B.9.7.1. Earthworms

One acute and two chronic earthworms studies with aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) have been submitted. Since the acute study does not form part of the data requirements in accordance with assimilated Regulation No 283/2013 and is not required for the risk assessment, this study has not been evaluated. The two chronic studies have been summarised and evaluated in Volume 3 CA B9.4.

B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

No standard laboratories studies have been submitted with the soil macro-organisms *Hypoaspis aculeifer* and *Folsomia candida*. 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*. However, a published literature study assessing the effects of lupin residue on decomposer fauna has been submitted, and is summarised and evaluated in Volume 3 CA B 9.4.2, with the inclusion of HSE comments on the relevance and reliability.

B.9.8. Risk assessment for non-target soil meso- and macrofauna

B.9.8.1. Risk assessment for earthworms

Toxicity

One acute and two chronic earthworms studies with PROBLAD PLUS have been submitted. Since the acute study does not form part of the data requirements in accordance with assimilated Regulation No 283/2013 and is not required for the risk assessment, this study has not been evaluated. The two chronic studies have been summarised and evaluated in Volume 3 CA B9.4.1. Both studies were deemed valid and suitable for use in risk assessment. The endpoints are summarised in the table below. As aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) is a UVCB substance, it was not considered appropriate to derive a log Pow value. In the absence of log Pow, HSE has taken a worst-case approach to assume that the log Pow exceeds 2, and has therefore adjusted the toxicity endpoints by a factor of 2.

Table 9.8.1-1: Endpoints available for use in risk assessment

Test substance	Test organism	Endpoint (mg/kg soil dw)		Reference
PROBLAD PLUS	Eisenia andrei	NOEC (reproduction)	100	Friedrich (2017).
		NOEC _(CORR) ¹	50	KCA 8.4.1/02
		EC ₁₀	>100	
		EC _{10(CORR)} ¹	>50	
PROBLAD PLUS	Eisenia andrei	NOEC (reproduction)	125	Antón (2020). KCA 8.4.1/03
		NOEC _(CORR) ¹	62.5	
		EC ₁₀	161.3	
		EC _{10(CORR)} ¹	80.65	

¹Endpoints are corrected by a factor of 2 due presumed log Pow > 2. Endpoints considered in the risk assessment are highlighted **bold**.

No effects at any test concentration were observed in the Friedrich (2017) study. The NOEC was therefore set at the highest concentration tested (100 mg/kg soil dw). This is supplemented by the Antón (2020) study which tested up to 1000 mg PROBLAD PLUS/kg soil. A dose-response was observed and statistically significant effects on reproductive capacity were observed at concentrations of 250 mg PROBLAD PLUS/kg soil and above, resulting in a NOEC of 125 mg PROBLAD PLUS/kg soil and an EC₁₀ of 161.3 mg PROBLAD PLUS/kg soil. Whilst the EC₁₀ is the more scientifically robust endpoint, in the absence of updated guidance specifying which endpoint to use, the lower of the two can be selected. Combining the results from the two studies it is considered that the corrected NOEC from Antón (2020) should be used in the risk assessment.

Exposure

Estimates of the maximum predicted environmental concentrations in soil (PEC values) of PROBLAD PLUS have been established in Volume 3CP B8.2. The application to strawberry has been considered as the worst-case use and is considered to be protective of the risk from tomatoes. The relevant PEC values considered for toxicity exposure ratio (TER) calculations are summarised in table 9.8.1-2 below.

Table 9.8.1-2: PEC_{Soil} value for 6 x 4016 g/ha application to strawberries

Proposed application	PEC_{Soil} (mg/kg)
6 x 4016 g/ha to strawberries 8 day application interval	12.277

Risk assessment

Earthworm toxicity studies have been submitted in accordance with the data requirements under assimilated Regulation No 283/2013. The assessment of the chronic risk to earthworms has been conducted according to SANCO/10329/2002 guidance. The risk is assessed in terms of Toxicity Exposure Ratios which are calculated using the following equation:

$$\text{TER} = \text{Endpoint (NOEC or EC}_{10}\text{)} / \text{PEC}_{\text{Soil}}$$

In the absence of a log Pow for PROBLAD PLUS, as a worst-case approach the study endpoints are corrected by a factor of two, to account for differences in the organic matter content of the test soil in comparison to artificial soils.

The risk is considered acceptable if the TER is > 5.

The resulting TERs for earthworms are summarised in Table 9.8.1-3 below.

Table 9.8.1-3: TER calculations for earthworms for the proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) on tomatoes and strawberries (6 x 4.016 kg/ha)

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

Conclusion

The TER value exceeds the trigger value of 5. An acceptable chronic risk to earthworms has been demonstrated for the proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) on strawberries and tomatoes. No further consideration is required.

B.9.8.2. Risk assessment for non-target soil meso-and macrofauna (other than earthworms)

No standard toxicity studies have been conducted with *H. aculeifer* and *F. candida*. One literature study has been submitted assessing the effects of lupin residues on decomposer fauna. 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*. Additionally a published literature study is available (van Vliet et al. (2000)) which demonstrated in a six-month litterbag study that lupin residues did not adversely affect soil biota or function. Collembola were the most abundant micro-arthropods present on the lupin residues. Although the study is of limited relevance, due to being conducted under non-representative climatic conditions and with a different species of lupin, it does provide support for the conclusion of acceptable risks to soil macro-organisms from aqueous extract from the germinated seeds of sweet *Lupinus albus*.

Overall it is considered that an acceptable risk to soil macro-organisms may be concluded for the proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus*.

B.9.9. Effects on soil nitrogen transformation

A standard soil nitrogen transformation laboratory study in accordance with OCED 216 has been submitted. This has been summarised and evaluated in Volume 3 CA B9.5. In addition, three published literature studies have been submitted in support of the risk assessment for soil micro-organisms. These studies (K-CA 8.5/02, K-CA 8.5/03 and K-CP 10.6.2/01) have been summarised and evaluated with respect to their relevance and reliability in Volume 3 CA B9.5.

B.9.10. Risk assessment for soil nitrogen transformation

B.9.10.1. Toxicity

One nitrogen transformation study was submitted for assessment, conducted with PROBLAD PLUS. This has been evaluated in Volume 3 CA B9.5 and was considered to be valid and suitable for use in risk assessment. The endpoint is summarised in the table below:

Table 9.10.1-1: Summary of nitrogen transformation study endpoint

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

In addition, three published literature studies have been submitted. These have been evaluated with respect to their relevance and reliability in Volume 3 CA B9.5 and Volume 3 CP B9.9 and are summarised in Table 9.10.1-2 below.

These three studies investigated the effects of incorporated lupin residues on soil micro-organism community abundance and functionality. All studies were considered to be reliable with reservations. The studies by Lelei and Onwonga (2014) and Cookson et al. (1998) indicated that populations of soil micro-organisms (bacteria and fungi) increased in soils with incorporated lupin residues. Additionally, the study by Elgharably et al. (2011) indicated that soil respiration increased with incorporation of lupin residues and the study by Cookson et al (1998) demonstrated functionality of decomposer communities, since mass loss was highest for litterbags containing lupin residues. Overall, these literature studies provide support that lupin residues do not adversely affect soil micro-organism community abundance or function.

Table 9.10.1-2: Summary of available literature studies on soil micro-organisms

Reference	Test substance	Test soil	Parameters measured	Exposure Duration	Result	Relevance	Reliability
K-CA 8.5/02 Elgharably and Marscher (2011)	Residues of <i>Lupinus albus</i> incorporated in soil at 2% w/w.	Sampled from Monarto, South Australia. 75% sand, 5% silt and 20% clay	Soil respiration rate, cumulative respiration, microbial biomass C, N, P	45 days	Soil respiration and microbial biomass C, N and P significantly increase in lupin residue treatment.	Relevant: same species of lupin used, measurements of microbial community functionality taken	Reliable with reservations
K-CA 8.5/03 Lelei and Onwonga (2014)	White lupin <i>Lupinus albus</i> seeds and residues (incorporated at depth of 15 cm)	Located Njoro sub-county, Kenya. 36% sand, 29.6% silt and 34% clay	Enumeration of fungi and bacteria	Two years: 2010-2011	Soil bacterial and fungal populations were increased in lupin-maize cropping systems	Limited relevance- same species of lupin used but did not investigate functionality of microbial communities e.g. nitrogen/ carbon	Reliable with reservations

						transformation or respiration	
K-CP 10.6.2/01 Cookson, W.R et al. (1998)	White lupin <i>Lupinus albus</i> residues; litterbag study	Wakanui silt loam (USDA)	Substrate-induced respiration, quantification of fungal and bacterial populations, nitrogen and lignin content of residues	90 days	Highest mass loss in lupin residue litterbags, correlated with greater populations of bacteria and fungi on residues of lupin.	Relevant: same species of lupin used, measurements of microbial community functionality taken	Reliable with reservations

B.9.10.2. Exposure

Estimates of the maximum predicted environmental concentrations in soil (PEC_{Soil}) of PROBLAD PLUS have been estimated in Section B8.2. The application to strawberry has been considered as the worst-case use and is considered to be protective of the risk from tomatoes. The relevant PEC value is summarised in table 9.10.2-1 below.

Table 9.10.2-1: PEC_{Soil} value for 6 x 4016 g/ha application to strawberries

Proposed application	PEC_{Soil} (mg/kg)
6 x 4016 g/ha to strawberries 8-day application interval	12.277

B.9.10.3. Risk assessment

According to SSANCO Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final), the trigger for acceptable risk is a < 25% difference (±) in activity compared to the control treatment. A comparison has been made of the study endpoints and the maximum PEC_{Soil} values in the table below:

Table 9.10.3-1: Risk assessment for soil micro-organisms

Test substance	Species	Endpoint (mg /kg dry soil)	PEC_{Soil} max (mg/kg)	Acceptable risk?
PROBLAD PLUS	Soil micro-organisms (nitrogen transformation)	52	12.277	Yes

Overall conclusion for soil micro-organisms:

According to SANCO/1039/2002, the outcome of the soil micro-organism test is directly assessed in terms of risk. The decisive parameter is the magnitude of effect (±) compared to the untreated control and the time-course of recovery. The critical level is ± 25% after 100 days. No effects were observed at greater than or equal to a 25% difference from the control condition at any test concentration, indicating a low risk to soil micro-organisms. Additionally, the published literature summarised in

Table 9.10.1-2 did not indicate any adverse effects on populations of soil micro-organisms, or the functionality of these populations.

No further refinement is necessary, as an acceptable risk has been demonstrated.

B.9.11. Effects on terrestrial non-target higher plants

B.9.11.1. Summary of screening data

None submitted.

B.9.11.2. Testing on non-target plants

A study assessing the toxicity of PROBLAD PLUS has been conducted with six plant species according to vegetative vigour methods. Additionally, a second study assessing the toxicity of PROBLAD PLUS to six plant species according to seedling emergence and vegetative vigour methods has been conducted. These studies are summarised and evaluated in Volume 3CA Part B9.6.2.

B.9.11.3. Extended laboratory studies on non-target plants

None submitted.

B.9.11.4. Semi-field and field tests on non-target plants

None submitted.

B.9.12. Risk assessment for terrestrial non-target higher plants

This risk assessment is based on the 'Guidance Document on Terrestrial Ecotoxicology', (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are defined as non-crop plants located outside the treated area. Spray drift from the treated areas may lead to deposition of the applied product on plants in adjacent off-crop areas, or else onto ground where non-target plants will shortly germinate and emerge.

B.9.12.1. Toxicity

A study with six plant species was conducted in order to assess effects on vegetative vigour (Peterek (2011)). This study is summarised and evaluated in Volume 3CA, Part B9.6.2. The endpoints are summarised in Table 9.12.1-1 below. The study was considered to be valid and did not indicate any adverse effects on any of the measured parameters. This study was conducted at an application rate of 2.0 L/ha (equivalent to 2.51 kg/ha, based on density of 1.255 g/mL).

A second study assessing the toxicity of PROBLAD PLUS to six plant species according to both seedling emergence and vegetative vigour methods has been conducted (Huerta (2020)). This study is summarised and evaluated in Volume 3CA, Part B9.6.2. The endpoints are summarised in Table 9.12.1-1 below. This study was conducted at the maximum proposed application rate of 3.2 L/ha (equivalent to 4.016 kg/ha, based on density of 1.255 g/mL), covered both seedling emergence and vegetative vigour, had appropriate analytical measurements and was considered valid.

Table 9.12.1-1: Summary of non-target plant testing data for aqueous extract from the germinated seeds of sweet *Lupinus albus* (tested as PROBLAD PLUS)

Study reference	Test species	Vegetative vigour	Seedling emergence
		ER ₅₀ 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	> 3.2	> 3.2

	esculentum (tomato)		
	Zea mays (maize)	> 3.2	> 3.2
	Allium cepa (onion)	> 3.2	> 3.2

B.9.12.2. Exposure (spray drift)

The risks to non-target plants from spray drift are determined based on SANCO/10329/2002. The proposed use is summarised in the table below.

Table 9.12.2-1: Proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS)

Use #	Target crop	Application	Outdoor/indoor
1	Strawberry	6 x 3.2 L product/ha (4016 g a.s./ha)	Indoor (PPFE)* and outdoor (field)
2		6 x 3.2 L product/ha (4016 g a.s./ha)	
3	Tomatoes	6 x 3.2 L product/ha (4016 g a.s./ha)	
4		6 x 3.2 L product/ha (4016 g a.s./ha)	

*Permanent protection with full enclosure.

Exposure to non-target plants from crops grown under permanent protection with full enclosure is expected to be negligible, but the risk assessment for outdoor exposure will be protective of any potential risk to protected crops.

In accordance with SANCO 10329/2002, for the assessment of strawberries a drift value of 2.77% is considered for a distance of 1 m from the edge of the crop. For the assessment of tomatoes a drift value of 8.02% is considered for a distance of 3 m from the edge of the crop. The off-field PER is calculated according to the following equation.

$$\text{PER}_{\text{off-field}} = \text{Application rate (g/ha)} \times \text{drift factor}$$

The calculated PER values are summarised in the table below:

Table 9.12.2-2: PER values for application to tomatoes and strawberries

Crop	Maximum single application (g PROBLAD PLUS/ha)	Drift factor (% drift/100)	PER (g PROBLAD PLUS/ha)
Strawberry	4016	0.0277	111.24
Tomato	4016	0.0802	322.08

B.9.12.3. Risk assessment

The risk to non-target plants has been assessed in accordance with SANCO/10329/2002 by calculating TER values using the following formula:

$$\text{TER} = \text{ER}_{50} \text{ (g/ha)} \div \text{PER}_{\text{off-field}} \text{ (g/ha)}$$

The TER values are then compared to a trigger value of 5.

The TER values are presented below:

Table 9.12.3-1: TER values for non-target plants exposed to aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) following application to strawberries and tomatoes

Application rate (g PROBLAD PLUS/ha)	Crop	Endpoint (g PROBLAD PLUS/ha)	PER_{off-field} (g PROBLAD PLUS/ha)	TER	Trigger value
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

The TER values exceed the trigger value of 5, indicating acceptable risk to non-target plants from the proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) on strawberry and tomato.

Overall conclusions for non-target plants

Overall, acceptable risks to non-target plants from the proposed uses of aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS) on tomatoes and strawberries have been demonstrated without need for risk mitigation. No further consideration is required.

B.9.13. Effects on other terrestrial organisms (flora and fauna)

A published literature study was presented investigating the bactericidal effects of lupin alkaloids. This has been summarised and evaluated in Volume 3 CA B9.7. The study was deemed to be of limited relevance to the assessment of aqueous extract from the germinated seeds of sweet *Lupinus albus* since testing was conducted with individual compounds rather than a whole lupin extract. Additionally it is considered that effects on soil bacteria will have been accounted for in the assessment of soil micro-organisms, so no further information is required.

B.9.14. Risk assessment for other terrestrial organisms (flora and fauna)

Not required.

B.9.15. References relied on

All available data has been submitted in the Volume 3 CA B9 document. For references relied upon, please refer to the reference list in Volume 3 CA B9.11.