



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.6 (AS)

Toxicology & Metabolism Data

Great Britain

February 2025

Version History

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

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B.6. Toxicology and metabolism data

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

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

The active substance contains the impurity quinolidizine alkaloids (QAs). QAs, at a sufficiently high dose, can cause symptoms of toxicity in humans, typically affecting the nervous, circulatory and digestive systems (BfR Opinion No. 003/2017 'Risk assessment of the occurrence of alkaloids in lupin seeds'). These properties are not possessed by the active substance. Therefore, QAs are toxicologically relevant impurities. The maximum level of 50 mg/kg (0.005% w/w) determined from the 5-batch analysis is a suitable limit for these impurities.

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

B.6.1. Absorption, distribution, metabolism and excretion in mammals

B.6.1.1. Absorption, distribution, metabolism and excretion by oral route

The aqueous extract from the germinated seeds of sweet *Lupinus albus* is a plant extract and contains the naturally occurring polypeptide component, Banda de *Lupinus albus* doce, known as BLAD (the lead component). Oral absorption is expected to be complete and The polypeptide component BLAD is known to be susceptible to proteolytic degradation; therefore the oral absorption of intact BLAD is practically negligible. However, it is most likely that the other components within the aqueous extract from the germinated seeds of sweet *Lupinus albus* are well absorbed. Consequently Radiolabelling of the test article is neither possible nor cost

effective. For these reasons, studies on absorption, distribution, metabolism and excretion in mammals were not undertaken and an oral absorption value of 100% is proposed for the aqueous extract from the germinated seeds of sweet *Lupinus albus*, with the exception of BLAD supported. Following oral absorption, the protein will be broken down under enzymatic processes in the gastrointestinal tract. Any amino acids resulting from the proteolysis of BLAD will be absorbed, enter the amino acid pool and being consumed into normal metabolic processes.

For discussion of the ADME properties of other relevant components in the active substance, please refer to Volume 4 as this is considered to be confidential information.

B.6.1.2. Absorption, distribution, metabolism and excretion by other routes

The HSE considers that the likelihood of absorption and systemic exposure via the inhalation and dermal routes to aqueous extract from the germinated seeds of sweet *Lupinus albus* is low, therefore, specific toxicokinetic studies by these routes are not required.

The dermal absorption of the active substance is addressed in Volume 3 CP B6, where a dermal absorption value of 2510% is proposed for the concentrate and diluted product.

B.6.1.3. Summary of ADME

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

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

B.6.2. Acute toxicity

The acute toxicity, skin and eye irritation and skin sensitisation of the aqueous extract from the germinated seeds of sweet *Lupinus albus* (identified as 'PROBLAD PLUS' on all study reports) was investigated in accordance with GLP and OECD compliant

guideline studies; validated methods of analysis are not required for these studies. The following studies have been submitted:

- Acute oral toxicity ‘Up and down’ procedure (OECD 425)
- Acute dermal toxicity study (OECD 402)
- Acute inhalation toxicity study (OECD 403)
- Acute skin irritation (OECD 404)
- Acute eye irritation (OECD 405)
- Acute skin sensitisation study (OECD 406) supplemented with position paper and published literature

B.6.2.1. Oral

Reference:	CA 5.2.1
Report Title:	PROBLAD PLUS: Acute oral toxicity up and down procedure in rats (amended report)
Author(s) & Year:	██████
Document No, Authority registration No	██████ (2012a) Unpublished report No.: 31002
Substance used:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as PROBLAD PLUS in the study report. Material no.: 201009, batch: 7 Undiluted
Guideline(s):	OECD TG No. 425 (2008)
Deviations:	No, and none in comparison to latest version of the OECD TG 425 (2022)
GLP or GEP:	Yes, GLP
Acceptability:	Yes

Study relied upon:	Yes
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Method

The acute oral toxicity of the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus* was investigated in female SD rats in a GLP and OECD 425 guideline compliant study. The rats were tested in an 'up and down' procedure at the limit dose of 5000 mg/kg bw. Test animals were acclimatised and bodyweights measured after a designated fasting period. A single dose was administered via gavage to 1 female rat and food was replaced shortly afterwards. As no adverse clinical signs or observations were noted, a further 2 rats were dosed at the same dose level of 5000 mg/kg bw. Bodyweights were measured at Day 7 and 14, and cage-side observations were made several times during this observation period. Terminal necropsy was performed on all 3 animals at the end of the observation period.

Results

There were no mortalities, no reports of adverse clinical signs, no effects on bodyweight and no reported adverse pathological findings.

Conclusion

Under the conditions of this GLP and OECD test guideline compliant study, the acute oral LD₅₀ of aqueous extract from the germinated seeds of sweet *Lupinus albus* was greater than 5000 mg/kg bw in female rats. Aqueous extract from the germinated seeds of sweet *Lupinus albus* is not acutely toxic by the oral route and, in accordance with assimilated Regulation No 1272/2008, does not meet the criteria for classification for acute oral toxicity.

B.6.2.2. Dermal

Reference:	CA 5.2.2
Report Title:	PROBLAD PLUS: Acute dermal toxicity study in rats – limit test (amended report)
Author(s) & Year:	██████████

Document No, Authority registration No	<div style="background-color: black; width: 80px; height: 1.2em; display: inline-block;"></div> (2012b) Unpublished report No.: 31003
Substance used:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as PROBLAD PLUS in the study report. Material no.: 201009, batch: 7 Undiluted
Guideline(s):	OECD TG No. 402 (1987)
Deviations:	No, and none in comparison to latest version of the OECD TG 402 (2017)
GLP or GEP:	Yes, GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The acute dermal toxicity of the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus* was investigated in a GLP and OECD 402 guideline compliant study, in male and female SD rats (5/sex). On the day prior to application, a dosing area of approx. 10% body surface area was prepared by clipping the dorsal area and the trunk. After clipping and prior to application, the animals were examined for health, weighing (initial) and the skin checked for any abnormalities. A dose of 2000 mg/kg bw was applied evenly at a volume of ~1.6 mg/kg bw and covered with a secured gauze pad. After 24 h exposure, the gauze pad was removed and the test site was gently cleaned. All animals were observed for mortality, signs of gross toxicity and clinical signs at least once daily for 14 days after dosing. Body weights were recorded prior to administration and again on days 7 and 14 (termination) following dosing. Terminal necropsy was performed on all animals on Day 14.

Results

There were no mortalities, no substance-related effects on bodyweight and no adverse pathological findings were noted at necropsy. No adverse dermal reactions

at the test site were attributed to the test substance. A single female presented with an approx. 2% decrease in bodyweight at Day 7. However, due to the minimal extent of the weight loss and the finding that this individual gained weight at a greater rate during the interval 7 to 14 days compared to the other animals, HSE does not view the decrease in bodyweight as being of toxicological significance. A few animals exhibited signs of red nasal discharge. This was limited to a single day (Day 2 observation in 3 males and 1 female) and in the absence of corresponding pathology, the finding was dismissed from further consideration.

Conclusion

Under the conditions of this GLP and OECD test guideline compliant study, the acute dermal LD₅₀ of aqueous extract from the germinated seeds of sweet *Lupinus albus* in rats was greater than 2000 mg/kg bw. Aqueous extract from the germinated seeds of sweet *Lupinus albus* is not acutely toxic by the dermal route and, in accordance with assimilated Regulation No 1272/2008, does not meet the criteria for classification for acute dermal toxicity.

B.6.2.3. Inhalation

Reference:	CA 5.2.3
Report Title:	PROBLAD PLUS: Acute inhalation toxicity study in rats – limit test (amended report)
Author(s) & Year:	██████████
Document No, Authority registration No	██████████ (2012c) Unpublished report No.: 30998
Substance used:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as PROBLAD PLUS in the study report. Material no.: 201009, batch: 7 (pre-test trial), batch 1 (main test)
Method of analysis:	Gravimetric
Guideline(s):	OECD TG No. 403 (2009)
Deviations:	No

GLP or GEP:	Yes, GLP
Acceptability:	Yes
Study relied upon:	Yes

A GLP and OECD 403-compliant acute inhalation toxicity study was conducted in rats, to determine the potential for aqueous extract from the germinated seeds of sweet *Lupinus albus* to produce toxicity from a single concentration of inhaled test article.

Method

Male and female SD rats (5/sex) were exposed, nose-only, to an aerosol of diluted aqueous extract from the germinated seeds of sweet *Lupinus albus*, at a single concentration of 5.34 mg/L, measured gravimetrically, for a 4 h continuous exposure period. A pre-test trial had been conducted to establish the aerosol generation procedures to achieve the desired chamber concentration (5 mg/L of air) and particle size distribution (MMAD 1-4 µm diameter achieved). During the pre-test trial, it was determined that the test item, as supplied i.e. without dilution, was too viscous to be aerosolised. Therefore, in the main study, the test article was diluted 50:50 in distilled water. In order to compensate for the presence of the vehicle in the test atmosphere, the gravimetric chamber concentration was multiplied by 50%. The test atmospheres were sampled for gravimetric analysis from the breathing zone of the animals six times during the 4 h exposure period. This was considered sufficient in terms of a method of analysis. Particle size distribution was assessed twice during the exposure period.

Results

There were no mortalities, no substance-related effects on bodyweight and no adverse pathological findings were noted at necropsy.

Clinical signs of toxicity e.g. rales and irregular respiration were observed in some animals immediately after exposure and predominantly during the first three days post-exposure. All animals appeared healthy and active by day 8 and in the absence of any pathological abnormalities, these clinical signs were not considered to be the result of test substance-specific toxicity.

Conclusion

Under the conditions of this GLP and OECD test guideline compliant study in SD rats, a 4-hr LC₅₀ of > 5.34 mg/L (aerosol) was calculated for males and females, combined. Therefore, aqueous extract from the germinated seeds of sweet *Lupinus albus* is not acutely harmful by the inhalation route and does not meet the criteria for classification for acute inhalation toxicity in accordance with assimilated Regulation No 1272/2008.

B.6.2.4. Skin irritation

Reference:	CA 5.2.4
Report Title:	PROBLAD PLUS: Primary skin irritation study in rabbits (amended report)
Author(s) & Year:	██████ (2012d)
Document No, Authority registration No	Unpublished report No.: 31000
Substance used:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> Material no.: 201009, batch: 7 20% BLAD (the lead component) (80% other ingredients)
Guideline(s):	Yes, OECD 404 (2002)
Deviations:	No
GLP or GEP:	Yes, GLP
Acceptability:	Yes
Study relied upon:	Yes

Method

Three young male adult New Zealand rabbits were exposed via the dermal route to 0.5 mL of PROBLAD PLUS/animal. The test material was administered as supplied (undiluted) to a clipped area of intact skin measuring ~6 cm², and semi-occluded for 4 hours, at which point the patch was removed and the skin was gently washed.

Animals were observed at 30 to 60 minutes, 24, 48 and 72 h after patch removal. Irritation was scored using the Draize scheme.

Results

Initial mild or slight erythema and/or mild oedema was observed in all animals at the 30-60 minute observation timepoints. All indications of oedema were resolved by 24 h, and erythema was resolved by 48 h. There were no effects on bodyweight or clinical signs of systemic toxicity.

Conclusion

Under the conditions of this GLP and OECD test guideline compliant study, aqueous extract from the germinated seeds of sweet *Lupinus albus* did not induce skin irritation in rabbits above the threshold for classification. Therefore, it is concluded that aqueous extract from the germinated seeds of sweet *Lupinus albus* does not meet the criteria for classification as a skin irritant in accordance with assimilated Regulation No 1272/2008.

B.6.2.5. Eye irritation

Reference:	CA 5.2.5
Report Title:	PROBLAD PLUS: Primary eye irritation study in rabbits (amended report)
Author(s) & Year:	██████ (2012e)
Document No, Authority registration No	Unpublished report No.: 30999
Substance used:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> Material no.: 201009, batch: 7 20% BLAD (the lead component) (80% other ingredients)
Guideline(s):	Yes, OECD 405 (1987)
Deviations:	No
GLP or GEP:	Yes, GLP

Acceptability:	Yes
Study relied upon:	Yes

Method

Three young female adult New Zealand rabbits were exposed via the right eye only, to 0.1 mL of PROBLAD PLUS, also known as the undiluted aqueous extract from the germinated seeds of sweet *Lupinus albus*. The other eye of each rabbit remained untreated and served as a control. Observations of the cornea, iris and conjunctiva (erythema and oedema) were made at 1, 24, 48 and 72 h and at 4 and 7 d post-instillation, and scored according to the Draize system.

Results (Table B.6.2-1)

There were no effects on bodyweight nor were there any clinical signs of systemic toxicity. Corneal opacity (grade 1), conjunctival erythema (grade 2) and chemosis (grade 2-3) were noted in all three animals at the 1 hour examination. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by day 4, confirming the effects were slight and fully reversible. Individual animal mean scores for corneal opacity, iritis, conjunctival redness and chemosis (average over 24-72 h) did not meet the grading criteria in accordance with the assimilated CLP Regulation No 1272/2008.

Table B.6.2-1: Eye effects in rabbits 24, 48 and 72 hours after ocular exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus*

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

Conclusion

Under the conditions of this GLP and OECD test guideline compliant in vivo study, aqueous extract from the germinated seeds of sweet *Lupinus albus* did not induce eye irritation in rabbits above the threshold for classification in accordance with assimilated Regulation No 1272/2008. The aqueous extract from the germinated seeds of sweet *Lupinus albus* is not an eye irritant.

B.6.2.6. Skin sensitisation

The potential for aqueous extract from the germinated seeds of sweet *Lupinus albus* to induce skin sensitisation was investigated in a Buehler assay on guinea pigs. In addition to this, the Applicant has submitted a position paper to support the lack of dermal penetration of BLAD, the lead component in aqueous extract from the germinated seeds of sweet *Lupinus albus*. The Applicant has also provided an additional assessment of the potential for oral allergenicity of BLAD which is summarised in Section B.6.8 below.

Although the Buehler assay is not the preferred method for investigating skin sensitisation, the Applicant has also provided an acceptable justification which is reproduced below:

“The skin sensitisation endpoint was assessed using the Buehler assay which is known to be less sensitive than the Magnusson & Kligman (M&K) method or the local lymph node assay (LLNA). The M&K test involves intradermal injection, which represents artificial and unrealistic conditions. Due to the size of the protein, skin penetration is unlikely, with the skin providing an effective barrier to absorption. However, for animal welfare reasons further in vivo testing was not considered justifiable. The M&K method has been replaced by the LLNA assay as part of the 3Rs criteria, with less animals used with laboratories in Europe now not able to offer the M&K study. The potential use of the LLNA to assess skin sensitisation is considered not justifiable scientifically as the protocol has not been validated for high molecular weight compounds, as is the case for the protein-containing PROBLAD PLUS. It should be noted that assimilated Regulation No 283/2013 clearly states that 'Where a guinea pig (Maximisation or Buehler) meeting OECD guidance and providing a clear result is available, further testing shall not be carried out for animal welfare reasons.'". Overall, HSE agrees with the Applicant and consider that this endpoint has been adequately addressed.

Study 1

Reference:	CA 5.2.6/01
Report Title:	PROBLAD PLUS: dermal sensitization study in guinea pigs (Buehler Method)
Author(s) & Year:	██████ (2012f)
Document No, Authority registration No	Unpublished report No.: 31004
Substance used:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as 'PROBLAD PLUS' Material no.: 201009, batch: 7 20% BLAD (the lead component; 80% other ingredients)
Method of analysis:	Not required for skin sensitisation studies
Guideline(s):	Yes, OECD 406 (1992)
Deviations:	None compared to the latest test guideline (dated 2022)

GLP or GEP:	Yes, GLP
Acceptability:	Yes
Study relied upon:	Yes

Method

Preliminary irritation test: In order to identify the highest non-irritant concentration (HNIC) of aqueous extract from the germinated seeds of sweet *Lupinus albus*, one group of 4 male Hartley albino guinea pigs were each exposed to single dermal applications (0.4 mL) of the test item (also known as PROBLAD PLUS) at 25, 50, 75 and 100% w/w i.e. 4 test sites per animal. All dilutions were prepared in distilled water. Occlusive conditions were maintained for 6 h, after which time the exposed area was washed. Dermal reactions were assessed approximately 24 h post-application.

Main Buehler test: The main Buehler test was conducted using groups of 20 and 10 guinea pigs for the test article and negative control treatments, respectively.

All inductions of the test article exposure group (n=20) were performed with the undiluted test article PROBLAD PLUS. Inductions were performed once a week for three weeks, with dermal reactions determined at 24 and 48 h after each application. The challenge exposure at the previously unexposed flank was performed 27 days after the first induction. Dermal reactions were assessed approximately 24 and 48 h post-application and bodyweights were recorded before induction and after challenge.

The negative control group animals (n = 10) were exposed on a single flank to undiluted PROBLAD PLUS at the challenge phase only, and dermal reactions and bodyweights were assessed in parallel with the exposed group.

Positive control: A concurrent positive control with a known sensitiser was not performed in this study. However, a separate positive control study (reliability check) had been performed at the laboratory within 6 months prior to the current study's experimental phase. The positive control study with alpha-hexylcinnamaldehyde 75% w/w demonstrated that the laboratory's test system was able to detect a known sensitising compound.

Results

Preliminary irritation test: At concentrations of 25 and 50% w/w, no skin reactions were observed. At concentrations of 75 and 100% w/w, 2/4 animals exhibited very faint erythema (score of 0.5). From these results, the HNIC was determined to be 100% and appropriate for use during both the induction and challenge phases in the main Buehler test.

Main Buehler test: In the induction phase of the test article exposed group, very faint to faint erythema (score 0.5 to 1) was noted for all test sites following each of the three applications. In the challenge phase, very faint erythema (0.5) was noted at 12/20 test sites at 24 h, with all reactions clearing by 48 h. The test criteria for a positive result were not met. In the naïve, negative control animals, very faint erythema (0.5) was noted for 3/10 sites at 24 h after challenge, with irritation clearing from all affected test sites by 48 h.

Conclusion

Under the conditions of this GLP and OECD test guideline compliant Buehler test, aqueous extract from the germinated seeds of sweet *Lupinus albus* was not shown to have the potential to induce skin sensitisation. The results of this study do not meet the criteria for classification in accordance with assimilated Regulation No 1272/2008.

Additional information

Reference:	Data Point 5.2.6
Report Title:	Expert opinion on the dermal penetration of BLAD
Author(s) & Year:	██████ (2019)
Document No, Authority registration No	Report No. 0387776-Tox3
Substance assessed:	BLAD
Method of analysis:	N/A
Guideline(s):	N/A
Deviations:	N/A

GLP or GEP:	N/A
Acceptability:	Yes
Study relied upon:	Yes

The Applicant has submitted their assessment of the dermal absorption of BLAD, the principal component in aqueous extract from the germinated seeds of sweet *Lupinus albus*. As this would be the first stage in the development of any skin sensitisation reaction, HSE considers it useful to summarise the Applicant's evaluation of dermal penetration under the current datapoint, noting that the same considerations are applicable to the assessment of dermal absorption of the representative product (see Volume 3 CP B6).

The Applicant's case rests primarily on the relatively poor dermal absorption of proteins such as BLAD, and other macromolecules, in comparison to agrochemicals and pharmaceutical chemicals. The Applicant discusses the relatively large molecular weight of BLAD, a 173 amino acid polypeptide, which is 210 kDa and its predicted poor dermal absorption, in comparison to insulin which is a 51 amino acid, 6 kDa polypeptide. It is known that human skin is an effective barrier against the dermal penetration of polypeptides such as insulin, which due to its small size can be considered to be a worse-case scenario compared to the dermal penetration of BLAD. It is known that significant chemical or physical interventions are needed in order to ensure the dermal penetration of insulin e.g. electric, sonic, encapsulation and microneedle methods. Therefore, it is unlikely that relevant components of the active substance will penetrate the human dermis or epidermis. This leads to the conclusion that aqueous extract from the germinated seeds of sweet *Lupinus albus* is unlikely to present a skin sensitisation hazard.

B.6.2.7. Phototoxicity

No phototoxicity data have been generated and none are considered necessary. The aqueous extract from the germinated seeds of sweet *Lupinus albus* is of 'unknown or variable composition, complex reaction products or biological materials' (UVCB) containing a protein mixture. ~~Such test articles are not considered suitable for testing in the in vitro 3T3 NRU phototoxicity assay due to interference during irradiation (resulting in diffraction of UV light, shielding of cells leading to uneven dosing of UV irradiation), nor is~~ It is not technically feasible to establish the molar extinction coefficient of such protein-based UVCB substances. For this reason, it was considered unnecessary to conduct a phototoxicity study. ~~However, it is unlikely that the active substance has a phototoxic potential.~~

B.6.2.8. Summary of acute toxicity

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

These data confirm that aqueous extract from the germinated seeds of sweet *Lupinus albus* is of extremely low oral, dermal and inhalation toxicity and is not a dermal or eye irritant, nor is it a skin sensitiser. The table below provides an overview of the acute toxicity, irritation and skin sensitisation potential of aqueous extract from the germinated seeds of sweet *Lupinus albus* (Table B.6.2-2).

Table B.6.2-2: Summary of acute toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus*

Guideline, reference	Species	Result	Classification
Acute Oral toxicity			
OECD 425 [REDACTED] (2012a)	Rat	LD ₅₀ > 5000 mg/kg bw	None
Acute Dermal toxicity			
OECD 402 [REDACTED] (2012b)	Rat	LD ₅₀ > 2000 mg/kg bw	None
Acute Inhalation toxicity			
OECD 403 [REDACTED] (2012c)	Rat	LC ₅₀ 4h > 5.34 mg/L nose-only	None
Skin Irritation			
OECD 404 [REDACTED] (2012d)	Rabbit	Mild signs of initial irritation which were reversible	None

Guideline, reference	Species	Result	Classification
Eye Irritation			
OECD 405 [REDACTED] (2012e)	Rabbit	Mild signs of initial irritation which were reversible	None
Skin Sensitisation			
OECD 406 [REDACTED] (2012f)	Guinea Pig	Not sensitising (Buehler)	None

B.6.3. Short-term toxicity

The Applicant submitted a 90-day oral repeat dose toxicity study and a 22-day dermal toxicity study. Both studies were performed in rats and in accordance with GLP and OECD guidelines. A validated method of analysis was available for the oral repeat dose study (Volume 3 CA B5.1.1).

No other short-term toxicity studies in either mice or dogs were conducted, and considering the following aspects, no further vertebrate testing is considered necessary under assimilated Regulation No 1107/2009:

- the available repeat dose toxicity studies in the rat indicate a very low systemic toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus* and no toxic effects need confirming in a second and/or non-rodent species.
- the active substance is a well-defined botanical plant extract, as described in the confidential section (Volume 4 of this Assessment Report).
- As the active substance contains the naturally occurring polypeptide component BLAD (the lead component), upon absorption, the protein will be broken down, enter the amino acid pool and be consumed into normal catabolic processes irrespective of the species of animal used. It is therefore reasonable to assume that metabolism and toxicokinetics between species will not differ. Refer to Volume 4 for consideration of other components within the active substance.

B.6.3.1. Oral 28-day study

A 28-day study was not submitted and is not considered necessary.

B.6.3.2. Oral 90- day study

Reference:	CA 5.3.2/01
Report Title:	PROBLAD PLUS: 13 week oral (gavage) administration toxicity study in the rat
Author(s) & Year:	██████ (2016)
Document No, Authority registration No	Report No.: 8325453
Substance assessed:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as 'PROBLAD PLUS' Batch D3133.0615 20% BLAD (the lead component; 80% other ingredients)
Method of analysis:	Yes, see Volume 3 CA B5.1.1
Guideline(s):	Yes, OECD 408 (1998). This is not the current (2018) test guideline
Deviations:	No significant deviations in relation to the guideline applicable at the time of study conduct. The latest (2018) version of this TG includes measurements of endocrine-sensitive parameters e.g. thyroid hormones and sperm pathology. Considering the expected metabolic fate of the active substance and the acceptable waivers re. non-submission of chronic/carcinogenicity and reproductive studies, the lack of these ED-sensitive endpoints from the current study does not impact on the validity of the study.
GLP or GEP:	Yes, GLP
Acceptability:	Yes
Study relied upon:	Yes

Method

In a GLP and OECD test guideline compliant study, aqueous extract from the germinated seeds of sweet *Lupinus albus* was administered via gavage to groups of 10 male and 10 female [REDACTED]:WI (Han) rats per test group, at doses of 0, 250, 500 and 1000 mg/kg bw/d, over a period of 13 weeks. The test article was dosed in an undiluted form, with the dose volume adjusted to achieve the required dose level. Control animals received purified water.

Results

There were no adverse effects on clinical observations, mortality, body weight or body weight gain, food consumption, clinical chemistry, haematology, organ weights or gross pathology. No notable findings were reported in the functional observation battery (FOB) or in motor activity.

Histopathology: A single female rat at the limit dose (1000 mg/kg bw/d, Group 4 animal ID no. 0140) was found with spinal and brain vacuolation. In the spine, the vacuolation was detected in sections of the cervical and lumbar regions, and was described as being either minimal or slight in grade. In the brain, the lesions were detected in slides prepared from grey matter (cerebral cortex) of the mid-brain and medulla oblongata, and were described as being slight in grade. None of the vacuolation was associated with localised inflammation and no other animals of either sex in any test group presented with similar histopathological alterations. Noting that there were no alterations in the individual rat's clinical signs nor behavioural changes, and the employment of a sub-optimal histopathology fixing procedure, it was likely that the finding was artefactual in nature. However, due to the following rationale, a genuine pathological response could not be excluded:

- A lack of association with other artefactual changes (e.g. dark neurons);
- A bilateral symmetrical and unusual distribution;
- This effect was not seen in control animals from previous Wistar rat studies run at the Test Facility.

As BLAD is unlikely to be absorbed by the oral route to any significant extent (see ADME section), if this effect is real, it is most likely caused by the other components of sweet Lupin.

Conclusion

To summarise, in this reliable and well-performed repeat dose gavage study in the rat, oral exposure to aqueous extract from the germinated seeds of sweet *Lupinus albus* for 13 weeks, had no effect on a wide range of parameters, up to the limit dose

of 1000 mg/kg bw/d. There were no indications of either immuno- or neurotoxicity in this study. However, there were some indications of low-grade vacuolation in the brain and spine in a single female at 1000 mg/kg bw/d, which although were suspected to be an artefact of tissue processing, the lesion could not be dismissed with certainty. The absence of many other potentially significant findings at such a high dose demonstrated a very low systemic toxicity profile of the active substance. In the absence of further evidence, the vacuolation in the spine and brain in a single animal was considered sufficient to establish a precautionary LOAEL at 1000 mg/kg bw/d. This leads to a conservative NOAEL of 500 mg/kg bw/d.

B.6.3.3. Other routes

Reference:	CA 5.3.3/01
Report Title:	PROBLAD PLUS: 21 day dermal administration toxicity study in the rat
Author(s) & Year:	██████████ (2015)
Document No, Authority registration No	Report No.: 8297704
Substance assessed:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as 'PROBLAD PLUS' Batch D31-012014 20% BLAD (the lead component; 79.7% other ingredients)
Method of analysis:	None required
Guideline(s):	Yes, OECD 410 (1981)
Deviations:	None
GLP or GEP:	Yes, GLP
Acceptability:	Yes. HSEs note that this 22-day dermal toxicity was generated in support of registration in a non-GB regulatory regime. However, relevant toxicity was observed, supporting its regulatory acceptance in GB

Study relied upon:	Yes
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Method

In a GLP and OECD test guideline compliant study, aqueous extract from the germinated seeds of sweet *Lupinus albus* was applied under semi-occlusive conditions for 6 h/d to the clipped dorsal skin of groups of ■:WI (Han) rats (5/sex/per test group), at doses of 0, 100, 300 and 1000 mg/kg bw/d, over a period of 22 consecutive days. The test article was dosed in an undiluted form, with the dose volume adjusted to achieve the required dose level. Control animals received purified water.

Results

There were no adverse effects on clinical observations, mortality, body weight or body weight gain, food and water consumption, clinical chemistry, haematology or gross pathology.

Clinical observations: Signs of dermal irritation comprising very slight to well-defined erythema and scabbing were noted in all test groups. Although there was no dose-response in the severity of the irritation, these findings were more frequently observed across multiple days at the top dose in 9/10 animals (both sexes combined). At the top dose in males, two individuals also presented with peeling skin and/or shiny skin. There were no other clinical signs of toxicity.

Organ weights:

Adrenal gland: Group mean adrenal weights, adjusted for the overall group mean terminal body weight, were increased by 16%, 18% and 38% in males at 100, 300 or 1000 mg/kg bw/d (respectively) when compared with concurrent controls. At the top dose of 1000 mg/kg bw/d, this was statistically significant ($p \leq 0.05$) compared to the control group. However, there was no statistical significance in relative adrenal organ weight when data were adjusted to the individual animal's terminal bodyweight. The HSE notes that the statistical power of this study is low, due to the limited animal numbers. There were no similar finding in females, nor was the elevated organ weight accompanied by any pathological changes (all male dose groups were analysed). Overall, there were no biologically meaningful changes in any organ weights.

Histopathology (see Table B.6.3-1):

Skin: In the treated skin, minimal hyperkeratosis was recorded in males and females at 1000 mg/kg bw/d, characterised by a minor increase in thickness of epidermis with increased keratohyaline granules.

Kidney: In males at the top dose of 1000 mg/kg bw/d, there was a marginal increase in the grading scores of hyaline droplets, characterised by eosinophilic cytoplasmic inclusions in the proximal tubular epithelial cells. Hyaline droplets were also observed in all males in the concurrent control, at a marginally lower grading score. There were no equivalent findings in females of any group. Hyaline droplets generally represent accumulations of alpha-2-microglobulin, a naturally occurring male rat protein. Chemicals which bind to alpha-2-microglobulin form a complex which is more resistant to catabolism and will result in accumulations of hyaline droplets. The minor increase in hyaline droplets in high dose males in this study may have been associated with renal metabolism or excretion of the test article, however, the Applicant did not confirm the presence of alpha-2-microglobulin by immunohistochemical staining. Overall, considering the prevalence in concurrent controls and the lack of similar renal findings in the oral 90 d repeat dose study where systemic exposure would be expected to be significantly higher, the very minor increase in the severity of the hyaline droplets is unlikely to be an adverse finding.

Table B.6.3-1: Selected histopathology findings¹ in the 22-day dermal toxicity study in the rat.

Parameter	Males mg/kg bw/d		Females mg/kg bw/d	
	0	1000	0	1000
Skin; hyperkeratosis	5 (5,0,0,0)	5 (0,5,0,0)	5 (5,0,0,0)	5 (1,4,0,0)
Kidney; hyaline droplets	5 (0,2,2,1)	5 (0,1,1,3)	5 (0,0,0,0)	5 (0,0,0,0)

¹ Number of animals with finding at grade (no finding, minimal, slight, moderate)

Conclusion

Following 22 days of dermal exposure to the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus*, some marginally adverse findings at the exposed areas were observed at the top dose of 1000 mg/kg bw/d in both males and females. During the in-life phase, mild erythema and scabbing was noted at an increased frequency, and this coincided with the appearance of hyperkeratosis in all affected individuals at this dose level. Overall, a local effects LOAEL can be established at the top dose of 1000 mg/kg bw/d, leading to a local effects NOAEL at the mid-dose of 300 mg/kg bw/d.

There was no significant systemic toxicity induced in the ■■■:WI (Han) rat up to the limit dose of 1000 mg/kg bw/d. There were some isolated findings in the adrenal gland (increased relative organ weight) and kidney (hyaline droplets) in males only, but these were marginal effects and overall, in the absence of other corroborating findings, were not clear signs of test-substance driven toxicity. Therefore, the dose level of 1000 mg/kg bw/d is considered to the systemic NOAEL.

B.6.3.4. Summary of short-term toxicity

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

considered predictive of the systemic toxicity via other routes. The active substance demonstrated very low toxicity by both the oral and dermal routes of exposure, however, some marginal effects were noted in both studies.

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

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

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

Classification for STOT-RE is not required, (HSE, 2023)¹.

Table B.6.3-2: Summary of repeated-dose toxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus*

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

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

B.6.4. Genotoxicity

The genotoxicity of the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus* was investigated in a battery of in vitro and in vivo studies.

- in vitro bacterial reverse mutation assay (Ames test, OECD 471)
- in vitro mammalian cell gene mutation assay (mouse lymphoma assay, OECD 490)
- in vitro micronucleus assay (human primary lymphocytes, OECD 487)
- in vivo comet assay (OECD 489)

All studies were performed to GLP and well conducted, with no major deficiencies. The database is sufficiently complete and reliable to conclude that there are no indications of mutagenic potential for the aqueous extract from the germinated seeds of sweet *Lupinus albus*.

B.6.4.1. In vitro studies

Three in vitro studies are available, investigating the mutagenicity, clastogenicity and aneugenicity potential of the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus*, also referred to as 'PROBLAD PLUS' in the original study reports.

Ames Test

Reference:	CA 5.4.1/01
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Report Title:	PROBLAD PLUS: Bacterial reverse mutation assay using a treat and plate modification.
Author(s) & Year:	M. Ballantyne (2016)
Document No, Authority registration No	Unpublished report No.: 8325399
Substance used:	<p>Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as PROBLAD PLUS in the study report.</p> <p>Lot D3133.0615 Active substance as supplied.</p> <p>20% BLAD (the lead component; 80% other ingredients)</p>
Method of analysis:	Not required
Guideline(s):	OECD TG No. 471 (1997)
Deviations:	YesNo. The 'treat and plate' methodology was used in preference to the standard preincubation test as the test article is a highly proteinaceous plant extract, which may cause artefacts due to growth stimulation when incorporated in a standard overlay agar.
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Method

The potential for the active substance to induce gene mutations in bacteria was investigated in two experiments, using a modified method known as the 'treat and plate' method, and with *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537, and a strain of *E. coli* WP2 uvrA. The assay was performed, both in the

presence and absence of metabolic activation (\pm S9). The activity of the commercial S9 mix was confirmed by the manufacturer using B[a]P and 2AA. Vehicle (water) and strain-specific positive controls were included in each experiment. The active substance was tested at 6 concentrations, ranging from 16 to 5000 $\mu\text{g}/\text{plate}$, confirming that the maximum concentration recommended by the test guideline had been achieved. Treatments of all the test strains were performed \pm S9, using final concentrations of 'PROBLAD PLUS' at 16, 50, 160, 500, 1600 and 5000 $\mu\text{g}/\text{mL}$ (experiment 1) and at 0, 51.2, 128, 320, 800, 2000, 5000 $\mu\text{g}/\text{mL}$ (experiment 2). Each concentration and positive control was tested in triplicate and vehicle controls were tested in quintuplicate. Cytotoxicity was assessed as thinning or loss of the bacterial background lawn, with or without a concurrent marked reduction in revertant numbers. A validated method of analysis for in vitro genotoxicity studies is not required.

Results

Mutagenicity: Treatment with PROBLAD PLUS did not cause an increase in the number of revertants in either experiment under any tested conditions (Tables B.6.4-1 and B.6.4-2). There were no increases in revertant numbers that were ≥ 2 -fold (in strains TA98, TA100 and WP2uvrA pKM101) or ≥ 3 -fold (in strains TA1535 and TA1537) above the concurrent vehicle control. Consequently, there was no evidence of a mutagenic effect of the aqueous extract from the germinated seeds of sweet *Lupinus albus* in this study.

Solubility and Cytotoxicity: The test article was soluble in the aqueous solvent (water) at all concentrations treated, in each of the experiments performed. In experiment 1, there was evidence of toxicity on the mutation plates treated at 1600 $\mu\text{g}/\text{mL}$ and above in strain TA1537 \pm S9, and at 5000 $\mu\text{g}/\text{mL}$ in strains TA98 and TA100 (-S9) and in strain TA1535 (\pm S9). In experiment 2, evidence of toxicity in the form of a slight thinning of the bacterial background lawn, with or without a concurrent marked reduction in revertant numbers was observed at 2000 and/or 5000 $\mu\text{g}/\text{mL}$ in strain TA1537 (\pm S9) and in strains TA98, TA100 and TA1535 (-S9). Therefore, it can be concluded that the test was performed up to the limit of cytotoxicity and at concentration level recommended by the test guideline.

Validity

The positive controls induced the appropriate response in the corresponding strains - a clear, treatment-related increase in the number of revertants, within the range of the historical positive control data (Table B.6.4-3), thus demonstrating the sensitivity of the test system. The vehicle control induced number of revertants was within the range of the historical control data for each strain. On this basis, the performance of the test was shown to be acceptable.

Table B.6.4-1: Bacterial (reverse) gene mutation treat and plate data – Experiment 1

Conc (µg/mL)	TA98		TA100		TA1535		TA1537		WP2uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0	24.6	21.6	98.6	78.8	15.2	16.6	5.8	4.4	131.4	161.6
16	21.7	26.3	91.0	99.3	12.3	15.3	4.0	5.3	131.0	150.0
50	11.7	28.0	91.0	90.3	14.0	13.3	5.0	3.3	--- ¹	--- ¹
160	15.0	22.3	94.3	81.0	13.3	12.7	3.3	4.7	132.7	145.3
500	23.0	21.3	98.3	98.0	15.0	15.0	7.3	3.3	197.7	150.3
1600	15.7	23.0	79.7	81.3	8.7	15.7	3.0	1.7	128.3	148.3
5000	12.0 ^V	23.0	65.7	103.0	8.7 ^S	7.3 ^S	^T	2.0 ^V	117.3	152.0
+ve	1066	782.3	931.7	537	1066	133.0	807.3	24.0	514.3	357.7

1 No bacteria present. Likely lost following washing procedure. Strain however acceptable as 5 scorable doses are present

S Slight thinning of bacterial lawn

T Toxic, no revertant colonies

V Very thin background bacterial lawn

+ve controls:

-S9 (absence of metabolic activation):

TA98: 2-nitrofluorene

TA100: 4-nitroquinoline 1-oxide

TA1535: N-methyl-N'-nitro-N-nitrosoguanidine

TA1537: ICR-191 mutagen

WP2uvrA: N-methyl-N'-nitro-N-nitrosoguanidine

+S9 (presence of metabolic activation):

All strains: 2-aminoanthracene

Table B.6.4-2: Bacterial (reverse) gene mutation treat and plate data – Experiment 2

Conc (µg/mL)	TA98		TA100		TA1535		TA1537		WP2uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0	21.2	22.8	100.2	104.4	16.4	21.4	8.0	7.2	150.4	165.8
51.2	25.7	25.7	95.7	102.3	18.0	25.7	11.0	6.0	166.3	162.3
128	21.7	32.0	108.3	110.3	16.3	20.3	9.3	9.3	164.3	158.3
320	24.7	29.0	105.0	110.0	15.3	25.3	7.7	6.7	161.0	155.0
800	21.0	29.7	111.3	141.0	15.3	22.3	8.0	5.0	181.3	163.3
2000	14.0 ^S	25.7	96.0	111.7	22.0	14.7	4.0 ^S	5.7	159.7	160.0
5000	14.0 ^S	24.7	79.0 ^S	106.0	8.7 ^S	29.3	4.3 ^S	2.0 ^S	153.3	156.7
+ve	1280	443	734	427	1113	119	1012	28	877	289

S Slight thinning of bacterial lawn

+ve controls:

T Toxic, no revertant colonies

-S9 (absence of metabolic activation):

V Very thin background bacterial lawn

TA98: 2-nitrofluorene

TA100: 4-nitroquinoline 1-oxide

TA1535: N-methyl-N'-nitro-N-nitrosoguanidine

TA1537: ICR-191 mutagen

WP2uvrA: N-methyl-N'-nitro-N-nitrosoguanidine

+S9 (presence of metabolic activation):

All strains: 2-aminoanthracene

Table B.6.4-3: Bacterial (reverse) gene mutation treat and plate data – Laboratory historical control data

	TA98		TA100		TA1535		TA1537		WP2uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Historical vehicle control strain values										
No. of studies	19								17	
Mean	22	22	94	102	13	13	10	7	176	190
Median	21	21	92	102	13	13	9	6	177	187
95% r.r	5-39	11-53	52-159	62-155	4-25	2.0-33	2-28	1-21	108-252	93-297
Historical positive control strain values										
No. of studies	19								17	
Mean	1190	397	667	322	1280	54	1388	39	512	520
Median	1082	408	639	318	1320	50	1422	37	517	239
95% r.r	610-2499	47-609	119-1500	134-493	588-2128	29-130	99-2550	11-131	225-904	117-404

Ranges calculated in February 2011 by Covance Statistics, using data selected without bias, from 19 studies started during the periods given below:

S. typhimurium strains TA98, TA100, TA1535, TA1537- January 2006 to March 2009

E. coli strain WP2uvrA pKM101 - January 2006 to August 2008

+ve controls:

-S9 (absence of metabolic activation):

TA98: 2-nitrofluorene

TA100: 4-nitroquinoline 1-oxide

TA1535: N-methyl-N'-nitro-N-nitrosoguanidine

TA1537: ICR-191 mutagen

WP2uvrA: N-methyl-N'-nitro-N-nitrosoguanidine

+S9 (presence of metabolic activation):

All strains: 2-aminoanthracene

95% r.r.: 95% reference range - calculated from percentiles of the observed distributions

Conclusion

Under the conditions of this GLP and OECD test guideline compliant bacterial reverse mutation (Ames) test, the aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS, batch number D3133.0615) was not mutagenic, either in the presence or absence of metabolic activation, up to the limit concentration for this test.

Mammalian Cell Gene Mutation (Mouse lymphoma)

Reference:	CA 5.4.1/02
Report Title:	PROBLAD PLUS: In vitro L5178Y gene mutation assay at the tk locus
Author(s) & Year:	Z. Keig-Shevlin (2015a)
Document No, Authority registration No	Unpublished report No.: 8325403
Substance used:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as PROBLAD PLUS in the study report. Lot D3133.0615 Active substance as supplied. 20% BLAD (the lead component; 80% other ingredients)
Method of analysis:	Not required
Guideline(s):	OECD TG No. 476 (1997) and also compliant with OECD 490 (2016)
Deviations:	No
GLP or GEP:	Yes, GLP
Acceptability:	Yes
Study relied upon:	Yes

Method

The potential for the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus* to induce gene mutations in mammalian cells cultured in vitro was investigated in a GLP and OECD test guideline-compliant study, at the TK locus in mouse lymphoma cells. Preliminary cytotoxicity, solubility, precipitation and pH tests were conducted to determine the concentrations used in the two experiments of the main study. The preliminary study was performed at 6 concentrations, ranging from 156.3 to 5000 µg/mL (single cultures per concentration); the main study was performed at 8 to 12 concentrations across two independent experiments, ranging from 150 to 5000 µg/mL (duplicate cultures concentration). All exposures to the test article were for 3 h, before cells were washed and cultured for a further 2 days to allow for gene expression. Cells were then plated on 96-well plates in selective or complete medium (mutant selection). The selection agent was trifluorothymidine (TFT). After another 10-14 days incubation, cells were counted and the cloning efficiency, mutation frequency (MF) and relative total growth (RTG) were calculated. Microplate wells with growth indicated evidence of TFT-resistant mutants. Colony sizing was also performed to determine the number of small and large colonies. Concurrent negative (vehicle; water) and positive (methyl methanesulphonate MMS (-S9) or benzo[a]pyrene B[a]P (+S9) were included.

The test article was considered mutagenic in this assay if:

- The MF of any test concentration exceeded the sum of the vehicle control mutant frequency plus Global Evaluation Factor (GEF). For microwell assays, the GEF is defined as 126 mutants per 10⁶ viable cells.
- The linear trend test was statistically significant.

Results (Tables B.6.4-4 and B.6.4-5)

Cytotoxicity and precipitation: In experiment 1 (+S9), the maximum concentration applied was 2500 µg/mL. However post-exposure precipitate beginning at 1500 µg/mL and above was observed, therefore this concentration was retained as the upper limit of the subsequent incubations. After 2 days' expression time, the test article range was further limited to ≤ 1200 µg/mL as the study director concluded that sufficient test concentrations would be available. Following the period of mutant selection, the cultures at 1200 µg/mL were not included in the data analysis due to excessive cytotoxicity (< 10% RTG), resulting in 4 analysable concentrations from 150 to 900 µg/mL. In the absence of S9 (-S9), the maximum concentration of active substance applied was 5000 µg/mL, with post-exposure precipitate noted at ≥ 2000 µg/mL. This concentration was the upper limit of 4 analysable concentrations.

In experiment 2 (\pm S9), there was no evidence of cytotoxicity or precipitation, therefore all test concentrations were available for analysis.

Mutagenicity:

In both main test experiments with metabolic activation (+S9), treatment with the aqueous extract from the germinated seeds of sweet *Lupinus albus* resulted in relevant increases in mutation frequency i.e. an increase in mutation frequency (MF) which exceeded the 'GEF + vehicle control MF' (Tables B.6.4-4 and B.6.4-5). In the presence of S9, in experiment 1, there was relevant increase in mutant frequency at the highest concentration analysed (900 $\mu\text{g/mL}$; 14% RTG) which gave rise to a highly significant linear trend ($p \leq 0.001$). At 900 $\mu\text{g/mL}$, increases in both small and large colony mutant frequencies were observed. This result was reproduced in experiment 2 (+S9), where there was a comparable increase in mutant frequency which exceeded sum of the GEF + vehicle control MF at concentrations of 1000 to 1200 $\mu\text{g/mL}$ (30% to 19% RTG). These increases in MF were unaffected by excessive cytotoxicity and not obscured by precipitation.

In both main test experiments in the absence of metabolic activation (-S9), no increases in MF which exceeded sum of the GEF + vehicle control MF were observed at any concentration, and no statistically significant linear trend was noted.

Table B.6.4-4: Mouse lymphoma toxicity and mutant frequency data - experiment 1

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

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

PP: precipitate observed at the end of treatment

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

Table B.6.4-5: Mouse lymphoma toxicity and mutant frequency data - experiment 2

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

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

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

Validity:

Negative (vehicle) and positive controls produced acceptable responses within the acceptability criteria with the exception of the MF in the vehicle control in experiment 2. In experiment 2, the vehicle control MF was just below the normal range (46.88×10^6 vs range of 50 to 170×10^6 viable cells). However, as experiment 2 confirmed the positive test substance response which had been observed in experiment 1, and the positive control response was acceptable, the data from experiment 2 were considered acceptable and valid.

The pH and osmolality of the post-exposure medium remained within the physiological range and at least 4 analysable concentrations (\pm S9) were included in each experiment.

In each experiment (\pm S9), the maximum acceptable test substance concentration analysed was $\geq 2000 \mu\text{g/mL}$ (the limit concentration according to OECD TG 490) or was selected based on precipitation or cytotoxicity.

Overall, the study met all acceptance criteria according to the laboratory protocol, and OECD TGs 476 and 490.

Conclusion

Under the conditions of this reliably performed in vitro assay, the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus*, referred to as PROBALD PLUS, induced gene mutation at the tk locus of L5178Y mouse lymphoma cells when tested up to toxic concentrations in the presence of S9 metabolic activation. Under the same test system, in the absence of metabolic activation, the active substance did not induce mutations when tested up to either a precipitating or toxic concentration.

In vitro micronucleus assay

Reference:	CA 5.4.1/03
Report Title:	PROBLAD PLUS: In vitro human lymphocyte micronucleus assay.
Author(s) & Year:	M. Lloyd (2015)
Document No, Authority registration No	Unpublished report No.: 8325400
Substance used:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as PROBLAD PLUS in the study report. Lot D3133.0615 Active substance as supplied. 20% BLAD (the lead component; 80% other ingredients)
Method of analysis:	Not required
Guideline(s):	OECD TG No. 487 (2014)
Deviations:	No deviations from most recent version published 2016
GLP or GEP:	Yes, GLP
Acceptability:	Yes
Study relied upon:	Yes

Method

The potential for the active substance aqueous extract from the germinated seeds of sweet *Lupinus albus* to induce micronuclei in primary human lymphocytes was investigated in a GLP and OECD test guideline compliant in vitro micronucleus test. Cell cultures were established in the presence of the mitogen, phytohaemagglutinin. Preliminary cytotoxicity, solubility, precipitation and pH tests were conducted at up to 2000 µg/mL in single cultures, to determine the concentrations used in the main study. In the main study, the test article was incubated in duplicate with cells for 3

hours (\pm S9) mix and 24 hours (-S9) at concentrations of 100 to 2000 $\mu\text{g/mL}$. Concurrent vehicle (water) and positive controls (cyclophosphamide (+S9), MMC or vinblastine (-S9, 3 h and 24h respectively) were included. In both experiments of the main assay, duplicate human lymphocyte cultures prepared from the pooled blood of two male donors were exposed to the aqueous extract from the germinated seeds of sweet *Lupinus albus* at a minimum of 7 concentrations, up to 2000 $\mu\text{g/mL}$, the maximum recommended concentration according to OECD 487. A minimum of 3 concentrations per treatment were selected for data analysis. CytoB was added to post-exposure cultures to inhibit cytokinesis. At least 2000 cells per test group (1000 per culture) were scored and recorded as percent binucleate cells with micronuclei (MNBN). To investigate cytotoxicity, the replicative index (RI) was determined in at least 500 cells per culture. The RI in comparison to the vehicle control was then used to determine percent cytostasis, aiming for a maximum of 50-60%.

Results (Table B.6.4-6)

Cytotoxicity, precipitation and osmolality:

At the maximum concentration tested in the preliminary test (tabulated data not presented here), 2000 $\mu\text{g/mL}$, there were no post-treatment precipitation or changes in osmolality in the culture medium. No marked toxicity (as measured by reduction in RI greater than $45\pm 5\%$ vs. vehicle control) or cytostasis (1% and 18% in the absence and presence of S9, respectively) was observed in the 3 h treatments undertaken. In the 24 h (-S9) treatment, cytostasis was observed at 1200 and 2000 $\mu\text{g/mL}$ at 31 and 55%, respectively. Therefore, the highest concentration tested in the main test 24 h treatment was established at 1600 $\mu\text{g/mL}$.

In the main assay, there was no precipitation and no cytostasis was observed at the 3 h treatment (\pm S9), up to 2000 $\mu\text{g/mL}$. In the 24 h treatment, cytostasis $\geq 62\%$ was observed $\geq 1800 \mu\text{g/mL}$, therefore these concentrations were discarded and 1600 $\mu\text{g/mL}$ (cytostasis 58%) was chosen for data analysis as it was considered to be at the upper range of acceptability based on cytotoxicity.

Micronucleus assay:

Short treatment (3 h; \pm S9): Treatment of cells with the active substance either with, or without metabolic activation, resulted in frequencies of MNBN cells that were generally similar to, and not significantly higher than the concurrent vehicle controls at all concentrations analysed. No positive trend was observed for either treatment condition. The MNBN cell frequencies of all treated cultures were within the 95th percentile of the current historical control (normal) range (Table B.6.4-7) under both treatment conditions, with the exception of a single culture at 2000 $\mu\text{g/mL}$ for the 3 h (-S9) treatment in which the MNBN cell frequency (1.5%) marginally exceeded the normal range of 0.2 to 1.4% but fell within the observed range of 0.2 to 1.5%.

Longer treatment (24 h; no S9): A statistically-significant increase of micronucleated cells (MNBN 0.95%) was seen only at the highest concentration but this result was accompanied by cytotoxicity at the upper range of acceptability (1600 µg/mL; cytostasis 58%). This value was also well within the range of the historical control data for the solvent control (95% ctrl limit: 0.10-1.10%) and in the absence of any positive trend test, the isolated increase is considered to be within normal biological variation.

Table B.6.4-6: Summary of results of the in vitro micronucleus test in human lymphocyte micronucleus test with the aqueous extract from the germinated seeds of sweet *Lupinus albus*

3 h treatment						24 h treatment		
- S9			+S9			-S9		
Conc (µg/mL)	Cyto (%) ^a	MNBN freq. ^b	Conc (µg/mL)	Cyt o (%) ^a	MNBN freq. ^b	Conc (µg/mL)	Cyt o (%) ^a	MNBN freq. ^b
0	-	0.55	0	-	0.35	0	-	0.50
800	8	0.60	800	1	0.45	200	0	0.70
1600	22	0.65	1600	9	0.35	400	18	0.65
2000	19	0.95 NS	2000	11	0.35	1200	36	0.75
MMC 0.2	22	3.55 ^{***}	CP 2.0	57	1.65 ^{***}	1600	58	0.95 ^{**}
						VIN 0.04	59	3.60 ^{***}

Table B.6.4-7: in vitro Micronucleus assay – Laboratory historical control data

Historical vehicle control ranges (Calculated in February 2014 by CLEH Statistics, for studies started between May 2012 and October 2013)			
Frequency of MNBN cells/cells scored (%):			
Mean: 0.60	Mean: 0.60	Mean: 0.40	
Median: 0.64	Median: 0.62	Median: 0.44	
SD: 0.318	SD: 0.281	SD: 0.230	
95% reference range: 0.20-1.40	95% reference range: 0.20-1.20	95% reference range: 0.10-1.10	
[21 studies (63 cultures)]	[22 studies (64 cultures)]	[11 studies (54 cultures)]	

** $p \leq 0.05$; *** $p \leq 0.001$;+ve controls: MMC: Mitomycin C;
CP: Cyclophosphamide; VIN:
vinblastine

a Cytostasis based on replication index

b mean micronucleated binucleate
frequency (%)**Validity:**

All solvent control values were within the range of the laboratory historical negative control data. In both the 3 and 24 h treatments, positive controls showed distinct increases in cells with micronuclei, thereby demonstrating the sensitivity of the test system. An appropriate number of cells per culture/concentration were scored under each treatment condition and the active substance was tested to either the maximum concentration recommended in the test guideline, or to the limits of cytotoxicity. Overall, the study was considered valid.

Conclusion

Under the conditions of this GLP and OECD test guideline-compliant study, the aqueous extract from the germinated seeds of sweet *Lupinus albus* (referred to as PROBALD PLUS) did not induce micronuclei, with or without metabolic activation up to concentrations causing cytotoxicity or the limit concentration of this test, as

determined by the in vitro micronucleus test in human lymphocytes. Therefore, the aqueous extract from the germinated seeds of sweet *Lupinus albus* is considered to be non-mutagenic in this in vitro micronucleus test.

B.6.4.2. In vivo studies in somatic cells

The Applicant has submitted a single in vivo study – the comet assay (OECD 489). From the available in vitro data, a positive result was obtained from the mammalian gene mutation assay (indicative of gene mutations and clastogenicity) in the presence of metabolic activation. Several expert groups, including the UK Committee on Mutagenicity (CoM guidance, 2021) considers that the in vivo comet assay has appropriate sensitivity to detect chemicals which induce both gene mutations and/or clastogenicity. HSE accepts that target organ exposure of a systemic organ would have been difficult to conclusively prove due to the nature of the active substance, thus the submitted study investigating comets in the stomach, an organ of direct contact with the test material is considered to be acceptable.

In vivo comet assay

Reference:	CA 5.4.2/01
Report Title:	PROBLAD PLUS: Rat alkaline comet assay
Author(s) & Year:	██████████ (2015b)
Document No, Authority registration No	Unpublished report No.: 8325402
Substance used:	Aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> referred to as PROBLAD PLUS in the study report. Active substance as supplied. 20% BLAD (the lead component; 80% other ingredients), batch no D3133.0615
Method of analysis:	Due to the complex and variable nature of the active substance, no bioanalytical method is available to detect target organ exposure. The lead protein – BLAD - will be broken down, enter the amino acid pool and be consumed into normal catabolic processes.
Guideline(s):	OECD TG No. 489 (2014)

Deviations:	No
GLP or GEP:	Yes, GLP
Acceptability:	Yes
Study relied upon:	Yes

Method

In a GLP and guideline comet assay, Han Wistar male rats (6 animals / group) were treated by gavage at dose levels of 500, 1000 and 2000 mg/kg bw on two consecutive days. The dose range selected was based on a range-finder test, where the maximum dose (2000 mg/kg bw/day) recommended by the test guideline was administered to males and females (3 animals/group) without any adverse effects on bodyweight or clinical signs. The second dose was administered approximately 21 hours after the first dose. No tissues were isolated in the range-finder test. In the comet assay, termination and tissue (stomach) sampling occurred approximately 3 hours after the second dose. Blood was collected via the abdominal aorta for clinical chemistry analysis. Cell suspensions were prepared within an hour of necropsy.

The concurrent vehicle control group (6 animals/group) received water and the positive control group (3 animals/group) received a single dose of 200 mg/kg bw ethyl methanesulphonate (EMS) approximately 3 h prior to necropsy.

As the test item was administered orally (by gavage), and in the absence of appropriate radiolabelled methods of analysis, the stomach, being a site of direct contact, was selected for sampling.

Three slides/animal were analysed for comets according to standard laboratory alkaline gel electrophoresis methods. Percent tail intensity (TI) and percent tail moment (TM; product of tail length and %tail intensity) were obtained from 150 cells/animal. The slides were also examined for any overt toxicity, e.g., an increase in background debris and/or an increase in the incidence of excessively damaged cells ('hedgehog' cells). To avoid the risk of false positive results, 'hedgehogs' were not used for comet analysis and have been reported separately.

Results (Table B.6.4-8)

Preliminary range-finder: There were no clinical signs of toxicity observed following dosing at 2000 mg/kg bw/day in either males or females, with all animals gaining weight during the dosing/observation period. As there were no sex-related differences in toxicity, the comet assay was conducted in male animals only.

Comet assay: There were no increases in comet TI, nor TM in the stomach of any group dosed with the aqueous extract from the germinated seeds of sweet *Lupinus albus*. There was also no dose-related effect on % hedgehog cells, which confirms the test system was unaffected by excessive or misleading DNA damage. At all dose levels, the % hedgehog cells were within 95% limits of the HCD (Table B.6.4-9). Vehicle and positive control groups met all criteria for acceptability, confirming the sensitivity of the study.

There were no effects on bodyweight gain, clinical chemistry parameters or clinical signs of toxicity in any dose group. No notable gross or histopathological findings were reported.

Table B.6.4-8: in vivo Comet assay group mean and individual animal stomach data

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

Table B.6.4-9: in vivo Comet assay – Laboratory historical control data

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

95% reference range:	16.92-39.73	2.06-6.49	9.00-25.00
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+ve control: EMS – ethyl methylsulphonate

SEM = standard error mean

a median values of each slide calculated. The mean of the slide medians were calculated to give the individual mean animal value. The individual mean animal values were averaged to provide group mean

b no cells present on the slides examined, however data available from 5 animals within the group

* t test $p \leq 0.001$

Validity: A sufficient number of animals (samples) were available, with an acceptable number of cells scored per animal. The concurrent vehicle negative control TI was within range of the HCD. The concurrent positive control produced a statistically significant increase in TI when compared to the concurrent vehicle control and was within 95% ctrl limits of the HCD. The maximum dose recommended by the TG was well tolerated in this study. Overall, the study is considered reliable.

Conclusion

Under the conditions of this GLP and OECD test guideline-compliant in vivo rat comet assay (in stomach epithelial tissue), the aqueous extract from the germinated seeds of sweet *Lupinus albus* (PROBLAD PLUS, batch number D3133.0615) was not mutagenic up to the limit dose of 2000 mg/kg bw.

B.6.4.3. In vivo studies in germ cells

No information for this data point has been submitted, nor is any required. The active substance aqueous extract from the germinated seeds of sweet *Lupinus albus* was not mutagenic in somatic cells and further testing in germ cells is unnecessary.

B.6.4.4. Photomutagenicity

No information on the molecular extinction coefficient of the aqueous extract from the germinated seeds of sweet *Lupinus albus* is available, nor is it possible to generate such data in accordance with OECD TG 101. As the lead component in the active substance is BLAD, an oligomeric protein, this would be expected to cause interference in any phototoxicity test (in vitro 3T3 NRU phototoxicity test guideline OECD 432), rendering any generated test results as void. Furthermore, no agreed photomutagenicity testing strategy for plant protection products is currently available. For these reasons it was considered unnecessary to conduct a photomutagenicity study.

B.6.4.5. Summary of genotoxicity

The genotoxic potential of the aqueous extract from the germinated seeds of sweet *Lupinus albus* was tested in an in vitro and in vivo battery of valid and reliable OECD TG-compliant tests. In vitro testing was performed via a bacterial reverse mutation assay (Ames test), an in vitro mammalian cell gene mutation test (mouse lymphoma assay) and an in vitro micronucleus assay in cultured human peripheral lymphocytes. Testing in vivo was performed as a comet assay in the rat (site of contact target tissue - stomach) (Table B.6.4-10).

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

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

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

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

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


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

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

In conclusion, the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not genotoxic in vivo and the data requirements of assimilated Regulation No 283/2013 have been met. Therefore, classification of the active substance for mutagenicity is not warranted (see also aligned HSE Technical Report, HSE (2023)).

Table B.6.4-10: Summary of genotoxicity of the aqueous extract from the germinated seeds of sweet *Lupinus albus*

Type of study	Test system	Dose range tested	Result	Reference
In vitro	Bacterial (5 strain, Ames) gene mutation (treat and plate methodology)	+/-S9: 16 to 5000 ^a µg/plate	\pm S9: negative	Ballantyne (2016)

In vitro	Mammalian (L5178Y tk ^{+/+}) gene mutation	Expt. 1: 3 h –S9: 500 to 2000 ^b µg/mL 3 h +S9: 150 to 900 ^c µg/mL Expt 2: 3 h –S9: 500 to 2500 ^c µg/mL 3 h +S9: 300 to 1200 ^c µg/mL	-S9: negative +S9: positive	Keig- Shevlin (2015a)
In vitro	Mammalian (cultured human lymphocytes) micronucleus	3 h +/-S9: 0 to 2000 ^d µg/mL 24 h –S9: 100 to 1600 ^c µg/mL	±S9: negative	Lloyd (2005)
In vivo	Rat stomach comet	0, 500, 1000, 2000 ^e mg/kg bw/d	negative	 (2015b)

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

b precipitate observed at the end of treatment

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

d maximum recommended concentration for the in vitro micronucleus assay

e maximum recommended dose in accordance with OECD 489

B.6.5. Long-term toxicity and carcinogenesis

No long term chronic toxicity and carcinogenicity studies have been conducted and none are considered necessary. The aqueous extract from the germinated seeds of sweet *Lupinus albus* contains 20% BLAD (the lead component). BLAD, is a naturally occurring polypeptide formed during the early days of the germination process of sweet lupin seeds (*Lupinus albus*). BLAD is used in human and animal nutrition, as a food and feed item, and has a pesticidal mode of action which is specific to fungi only (BLAD binds to chitin and chitosan which weakens the cell wall structure; this target is not found in mammalian biological systems) and it is rapidly biodegradable. It is known to be susceptible to proteolytic degradation and the protein will be broken down, enter the amino acid pool and be consumed into normal metabolic processes. A complete genotoxicity test battery confirmed a lack of genotoxic potential. Furthermore, the 90-day study did not raise any indication of potential non-genotoxic carcinogenicity. There is no evidence in the public domain to suggest that proteins similar to BLAD, which contains a segment of β -conglutin (which shares strong homology to other members of the vicilin family (globulin storage protein associated with leguminous seeds such as peas and lentils)) are associated with an increased incidence of cancer. Based on this, it can be concluded that neither the lead component, or the active substance as a whole, are likely to be considered a carcinogen. Refer to Volume 4 for consideration of other components within the substance.

B.6.6. Reproductive toxicity

No reproductive or fertility studies have been conducted with the active substance, and none are considered necessary. The aqueous extract from the germinated seeds of sweet *Lupinus albus* contains 20% BLAD (the lead component). BLAD is a naturally occurring polypeptide formed during the early days of the germination process of sweet lupins (*Lupinus albus*) and is used in human and animal nutrition, as a food and feed item. It has a pesticidal mode of action which is specific to fungi only (BLAD binds to chitin and chitosan which weakens the cell wall structure; this target is not found in mammalian biological systems) and it is rapidly biodegradable. It is known to be susceptible to proteolytic degradation and the protein will be broken down, enter the amino acid pool and be consumed into normal metabolic processes. There were no indications in the 90 day oral study in the rat, of adverse effects on reproductive organs. There is no evidence in the public domain to suggest that proteins similar to BLAD, which contains a segment of β -conglutin (which shares strong homology to other members of the vicilin family (globulin storage protein associated with leguminous seeds such as peas and lentils)) are associated with reproductive or developmental toxicity. Based on this, it can be concluded that neither the lead component, or the active substance as a whole, is likely to be

considered a reproductive or developmental toxicant. Refer to Volume 4 for consideration of other components within the substance.

B.6.7. Neurotoxicity

B.6.7.1. Neurotoxicity studies in rodents

The requirement for a specific neurotoxicity study in rodents is not triggered; BLAD (the lead component) does not belong to a class of chemicals known to be associated with neurotoxicity and there were no signs of neurotoxicity in acute dose studies. In the 90 day repeat dose toxicity study, there was slight to minimal vacuolation in the brain and spine in a single female at the top dose of 1000 mg/kg bw/d. However, this was not considered to be robust evidence of neurotoxicity since it was an isolated finding and the functional observational battery and locomotor assessment in this study did not reveal any adverse effects. Refer to Volume 4 for consideration of other components within the UVCB substance.

B.6.7.2. Delayed polyneuropathy studies

A specific study is not required; BLAD (the lead component) does not belong to a class of chemicals known to be associated with delayed neurotoxicity and there were no signs of neurotoxicity in acute dose studies and a 90-day oral study in rats. Refer to Volume 4 sections C.1.2.4 and C.1.3.4 for consideration of other components within the active substance.

B.6.8. Other toxicological studies

No studies are available and none are considered necessary, however the Applicant has submitted some additional information to inform on the potential for oral allergenicity of products derived from seeds of sweet *Lupinus albus* (see below).

B.6.8.1. Supplementary studies on the active substance

Oral allergenicity

Reasoned case

The Applicant has submitted a reasoned case in order to justify the lack of concern regarding the potential for oral allergenicity of aqueous extract from the germinated seeds of sweet *Lupinus albus*. The Applicant's case has focussed on the oral allergenicity of the BLAD protein (a Lupin protein of the β -conglutins family), the lead component within the active substance. It should be noted that the relevant impurities quinolidizine alkaloids (QAs), of which lupanine is the major component, are not allergenic. It is the Lupin proteins which have allergenic potential.

Reference:	CA 5.2.6/02
Report Title:	Potential allergenicity of lupine seeds (<i>Lupinus</i> sp.) with special emphasis on BLAD, an intermediate in the breakdown process of the major storage protein during germination of lupine seeds
Author(s) & Year:	Boavida Ferreria, R. (2011)
Document No, Authority registration No	Unpublished report No.: CEV110820
Acceptability:	Yes
Study relied upon:	Yes; data protection does not apply to reasoned cases

The initial concern for a potential for oral allergenicity of aqueous extract from the germinated seeds of sweet *Lupinus albus*, stems from the presence of BLAD and the observations that (i) BLAD comprises an internal segment of β -conglutin, (ii) β -conglutin exhibits a relatively strong homology to the other members of the vicilin family, including well known allergens contained in other legumes such as peanuts and soybeans, and (iii) there are a considerable number of studies concerning the allergenicity of lupine-derived products, reasonably triggering an assessment of BLAD potential oral allergenicity.

The Applicant's case addresses the dietary route of exposure, and the evaluation of BLAD as a food-borne allergen in comparison to a well-established legume allergen, peanut Ara h 1. It is noted that any food containing proteins may be capable of eliciting an allergic reaction in humans, and therefore the assessment of BLAD followed the criteria established by the Codex Alimentarius (2003²) and FAO/WHO (2001)³ which included several key elements:

² Codex Alimentarius Guidelines. Foods derived from modern biotechnology. 2nd ed. WHO/FAO (2009) p. 7-34

³ FAO/WHO (2001) Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 22-25 January 2001

-
- (1a) amino acid residue homology $\geq 35\%$, or
 - (1b) identity in one or more sets of ≥ 6 contiguous amino acid residues, or
 - (1c) cross-reactivity to known allergens;
 - (2) high resistance to proteolytic attack and
 - (3) ingestion of sufficient amounts

The Applicant's discussion of the first two criteria in relation to BLAD is given below:

“(1a): BLAD exhibits a high sequence homology (58%) when compared to a section of Ara h 1 and of other legume seed storage proteins, especially from the vicilin family. This homology comes as no surprise, since BLAD contains within itself the cupin-2 domain, identified as an allergenic domain in several vicilins and legumins.

(1b): Given the 58% sequence homology between BLAD and the corresponding section in Ara h 1, the presence of a single ≥ 6 amino acid residue sequence (when compared to other vicilins) suggests a more likely presence of IgE recognition epitopes on the vicilins rather than on BLAD.

(1c): The available literature suggests that the peanut-lupine cross-reactivity allergenic potential is high, but unrelated to lupine β -conglutin (BLAD precursor).

The overall weight of evidence provided under criteria 1a, 1b and 1c suggests that BLAD exhibits a much smaller potential of oral allergenicity than other legume vicilins.

(2): BLAD exhibits a very high sensitivity to proteases, with no proteolytic fragments leftover.

~~The evidence provided under criterion 2 makes it unlikely that BLAD may constitute a serious allergen when compared to other legume globulins, especially Ara h 1. Although no direct evidence of the proteolysis of BLAD has been provided, BLAD's assumed great susceptibility to proteolytic attack is in agreement with published data indicating that the allergenic potential from lupine seeds seems to be due to γ -conglutin, and less from α - and β -conglutins due to their susceptibility to proteolysis. BLAD belongs to the family of the β -conglutins.~~

(3): Although the Applicant produced an estimation of the amount of BLAD potentially ingested in order to address criterion 3, this argumentation was not considered necessary in support of the hazard assessment by HSE.

HSE Conclusion

Considering the criteria 1a, b, c and 2, it seems unlikely that BLAD presents with an allergenic potential via the oral route as it undergoes complete proteolysis and does not contain amino acid sequences with significant antigenic potential.

Clinical study on the potential allergenicity of the BLAD protein

Reference:	CA 5.2.6/03
Report Title:	Evaluation of the allergenic and cross-allergenic potential of the BLAD polypeptide
Author(s) & Year:	Todo-Bom, A. and Loureiro, C. (2013)
Document No, Authority registration No	Unpublished report No.: CHUC-021113
Acceptability:	Yes
Study relied upon:	Yes

In a clinical study (conducted by the Immuno-Allergology Department from Coimbra University Hospital, Portugal, and following written consent), the serum of 26 individuals allergic to lupine and/or peanut (confirmed by skin prick tests and the presence of specific serum IgE to lupine and/or peanut) was checked for reactivity towards the BLAD protein by immunoblot analysis. There was no evidence of binding of the BLAD protein to serum IgE from these allergic individuals. These data show that the BLAD protein is not allergenic in humans.

Overall conclusion

Overall, different strands of evidence demonstrate that the BLAD protein, the main component of aqueous extract of sweet Lupin, is not orally allergenic in humans.

B.6.8.2. Studies on endocrine disruption

No specific studies to investigate endocrine activity or adversity have been conducted with the active substance, and none are considered necessary. The aqueous extract from the germinated seeds of sweet *Lupinus albus* contains 20% BLAD (the lead component). BLAD is a naturally occurring polypeptide formed during the early days of the germination process of sweet lupins (*Lupinus albus*) and is used

in human and animal nutrition, as a food and feed item. It has a pesticidal mode of action which is specific to fungi only (BLAD binds to chitin and chitosan which weakens the cell wall structure; this target is not found in mammalian biological systems) and it is rapidly biodegradable. It is known to be susceptible to proteolytic degradation and the protein will be broken down, enter the amino acid pool and be consumed into normal metabolic processes. There were no indications in the 90 day oral study in the rat, of adverse effects on the endocrine system. There is no evidence in the public domain to suggest that either aqueous extract from the germinated seeds of sweet *Lupinus albus* or proteins similar to BLAD, are associated with reproductive or developmental toxicity. Based on this, it can be concluded that neither the lead component, or the active substance as a whole, are likely to be considered an endocrine disruptor.

B.6.8.3. Immunotoxicity

No specific studies on the immunotoxic potential of aqueous extract from the germinated seeds of sweet *Lupinus albus* were generated and none are considered necessary. In the available short term toxicity database in the rat (90 d oral and 22 d dermal studies), there were no adverse effects on haematology, biochemical parameters, or organ weight and pathology of the spleen, thymus and lymphoid tissue.

B.6.9. Medical data and information

The Applicant has provided a summary statement to inform on this data point (Barata, A. (2016)). The HSE notes that the active substance has been manufactured on an industrial scale (Refer to confidential information Volume 4), albeit not for commercial use.

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

The manufacture of the active substance involves a continuous non-stop process occurring at the manufacturing site.

During production periods, a total of ■ workers (age ■ to ■ years) are employed at the industrial manufacturing plant, of which, ■ (age ■ to ■ years) are allocated to the manufacturing production line. The Applicant reports that no incidents of poisoning or allergenic symptoms have been attributed to exposure associated with the handling or manufacturing of either the active substance or exposure to the raw material.

The plant operators follow precautionary measures in the manufacturing production line. These safety actions are justified by the fact that operators are dealing with industrial equipment and cleaning operations of the plant and equipment are

conducted daily. During those cleaning operations workers use disinfectants, detergents or other specific industrial cleaning products, following the adequate safety actions and PPE use which are required when dealing with these specific products.

Considering the above, the Applicant does not conduct routine annual staff medical examinations. Although no specific medical surveillance data or monitoring studies on manufacturing plant personnel are available, considering the toxicity profile and manufacturing site operating conditions, none are considered necessary.

B.6.9.2. Data collected on humans

No studies are available and none are considered necessary.

B.6.9.3. Direct observation

No reports of adverse health effects have been observed or received, therefore no direct observation data are available.

B.6.9.4. Epidemiological studies

The active substance has not yet been sold commercially, no studies are available and none are considered necessary.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

Given the low chronic and acute toxicity of the active substance, it would not be expected that accidental overexposure by any route of exposure would lead to serious illness or mortality.

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

No specific therapeutic regime is known for the active substance. First aid measures have been summarised from the safety data sheet for the active substance and follow symptomatic treatment:

Ingestion:

Call a poison control centre or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by a poison control centre or doctor. Do not give anything by mouth to an unconscious person

Skin

contact:

Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control centre or doctor for treatment advice

Eye contact:

Hold eyes open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control centre or doctor for further treatment advice

Inhalation:

Move to fresh air. If person is not breathing, call an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control centre or doctor for further treatment advice

B.6.9.7. Expected effects of poisoning

No poisoning cases have been reported therefore no specific signs of human poisoning are known. Based on data in laboratory animals, the active substance poses very little hazard to human health under either acute or chronic exposure.

B.6.10. References relied on**LITERATURE SEARCH**

A total of three literature searches were performed by the Applicant; all three were in accordance with the EFSA Guidance (EFSA journal 2011; 9(2):2092). The first two literature reviews are summarised in this document, with the third search (Data Point CA 9.0/03) located in Volume 4 of this Assessment Report. Each of the literature reviews encompassed all areas of the assessment: Toxicology; Ecotoxicology; Metabolism; Residues and Environmental Fate and Behaviour.

The first search was performed in 2016, followed by a further search in 2018. Both searches employed identical search strategies, with the only difference being in search terms. Therefore only one summary of the common search strategy is presented below.

A further literature search (CEV/02/01-LRR4) was performed by the applicant in Feb 2022 (6 months before submission of the dossier to HSE) to identify any potentially new publications that may have become available since 2018 and to specifically retrieve articles related to lupanine. The literature search did not identify any relevant publications.

Reference:	CA 9.0/01
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Report Title:	Sweet Lupin (seeds), <i>Lupinus albus</i> L., germ., ext. and PROBLAD PLUS Submission of Scientific Peer-Reviewed Open Literature under Regulation (EC) No 1107/2009
Author(s) & Year:	Tucker. K (2016)
Document No., Authority	CEV/02/01-LRR1
Substances:	BLAD, PROBLAD, PROBLAD PLUS, β -conglutin, lupinene, vicilin
Deviations	No
Study acceptable:	Yes
Study relied upon:	Yes; data protection does not apply to reasoned cases

Reference:	CA 9.0/02
Report Title:	Sweet Lupin (seeds), <i>Lupinus albus</i> L., germ., ext. and PROBLAD PLUS Submission of Scientific Peer-Reviewed Open Literature under Regulation (EC) No 1107/2009
Author(s) & Year:	Tucker. K and Cartwright (2018)
Document No., Authority	CEV/02/01-LRR2
Substances:	<i>Lupinus albus</i> , <i>Lupinus albus</i> (sweet lupin) seeds, sweet lupin(e), lupines and <i>Lupinus albus</i> seeds
Deviations	No
Study acceptable:	Yes
Study relied upon:	Yes

Search strategy: The literature search considered scientific peer-reviewed open literature published between 01/01/2006-05/05/2016 (Document no. CEV/02/01-LRR1) or 01/01/2006 – 04/06/2018 (Document no. CEV/02/01-LRR2) and included the following search terms:

Tox OR hazard OR adverse OR health OR NOAEL OR NOEL OR LOAEL OR LOEL OR BMD OR vivo OR vitro or storage stability OR storage OR stability OR metabolic OR metabolism OR degradation OR breakdown OR residues OR residue OR processing OR hydrolysis OR rotation OR plant OR crop OR feed OR animal OR livestock OR hen OR cattle OR ruminant OR goat OR cow OR pig OR risk assessment OR consume OR exposure or soil OR water OR air OR environment OR fate OR endocrine disrupt OR bioaccumulation OR biomagnification OR bioconcentration OR poison OR effect.

The search employed the STN platform, with the following chosen databases: Analytical abstracts, Biosis, Chemical abstracts plus, Embase, Medline, RTECS, Scisearch, Toxcenter. Three other databases were also included: Pubmed, Wiley online library and Science Direct.

Evaluation

Following the removal of duplicated hits and patents, all publications were assessed for their relevance and reliability in a two-step process:

Assessing the relevance of published literature:-

- 1) Rapid review: An initial rapid assessment of studies against the relevance criteria only was carried out by reference to their titles and if necessary, abstracts. Those studies that were considered to meet the relevance criteria or were of unclear relevance, following review of their abstracts, were obtained in full. Anything considered to have potential relevance was not rejected at this stage.
- 2) Detailed review: Publications that were not excluded for relevance were then assessed for reliability.

Assessing the reliability of potentially relevant published literature:-

The applicant has stated that the reliability assessment for all relevant publications was done according to the criteria outlined by Klimisch et al. (1997) and HSE considered this to be a widely accepted scoring system.

The criteria for both relevance and reliability are summarised below:

Criteria for relevance	
Toxicological and metabolism studies	<ol style="list-style-type: none"> 1. Well defined test material (including its purity and impurity profile) 2. Relevant test species (to the mammalian toxicological assessment - preferred species are rodents - rats and mice, the dog is the preferred non-rodent species) 3. Number of animals per group sufficient to establish a statistical significance 4. Several dose levels tested (at least 3), preferably including a negative control, to establish a dose-response 5. Relevant route of administration in terms of risk assessment (oral, dermal or by inhalation) 6. Description of the observations, examinations, analysis performed, or necropsy 7. In addition: studies which may be helpful for the interpretation of other studies present in the dossier, but do not fit under a specific toxicological endpoint
Criteria for reliability	
	<p>Reliability criteria: This aspect considers the extent to which a study or information, is free from bias and its finding are scientifically valid. Assignment of reliability for relevant studies was done according to Klimisch et al., (1997).</p>

Literature Search Results

The number of records retrieved for each stage of the process are shown below for the two searches.

Stage of study selection process	Lit. Search 2016	Lit. Search 2018
	01/01/2006-05/05/2016	01/01/2006-04/06/2018
Total number of summary records retrieved after de-	3859	4185
Number of summary records excluded from the search results after rapid assessment for relevance	3832	4171
Total number of full-text documents assessed in detail	24	14
Number of studies excluded from further consideration after detailed assessment for relevance and/or reliability	22	2
Number of Toxicology-related studies not excluded for relevance or reliability after detailed assessment	8	0
Number of studies not excluded for relevance or reliability after detailed assessment, that were relevant to the Toxicology	0	0
Number of relevant and reliable publications identified by the applicant's literature review which are included in the HSE evaluation of aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i>	0	0

HSE Conclusion

The Applicant's choices of search terms, filters and databases are acceptable and appropriate justifications for exclusion of non-relevant publications were presented within each literature review report. Following a full text relevance assessment of the 8 papers identified as meeting the Applicant's relevance criteria, HSE considers that

none are appropriate for inclusion in the toxicology evaluation of the aqueous extract from the germinated seeds of sweet *Lupinus albus*. The main reasons for excluding these publications from further integration into the dossier were as follows:

- No information on the toxicological profile of the active substance
- Focus on dietary exposure levels of lupin-based foods with no descriptions of toxicity
- Case studies of skin prick tests performed on pre-sensitised individuals, hence not clearly associated with potential exposure to the active substance
- Case studies of cross-reactivity with small numbers of patients suffering from peanut allergies.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
CA 5.2.1/01	[REDACTED]	2012a	PROBLAD PLUS: Acute oral toxicity up and down procedure in rats (amended report) Company Report No. 31002 [REDACTED] GLP, Unpublished	Y	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009	CEV	None
CA 5.2.2/01	[REDACTED]	2012b	PROBLAD PLUS: Acute dermal toxicity study in rats – limit test (amended report) Company Report No. 31003 [REDACTED] GLP, Unpublished	Y	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009	CEV	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
CA 5.2.3/01	[REDACTED]	2012c	PROBLAD PLUS: Acute inhalation toxicity study in rats – limit test (amended report) Company Report No. 30998 [REDACTED] GLP, Unpublished	Y	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009	CEV	None
CA 5.2.4/01	[REDACTED]	2012d	PROBLAD PLUS: Primary skin irritation study in rabbits (amended report) Company Report No. 31000 [REDACTED] GLP, Unpublished	Y	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009	CEV	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
CA 5.2.5/01	████████	2012e	PROBLAD PLUS: Primary eye irritation study in rabbits (amended report) Company Report No. 30999 ████████████████████ GLP, Unpublished	Y	Claimed but not granted	Breach on Art 62 therefore not granted	CEV	None
CA 5.2.6/01	████████	2012f	PROBLAD PLUS: Dermal sensitisation study in guinea pigs (Buehler method) (amended report) Company Report No. 31004 ████████████████████ GLP, Unpublished	Y	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009	CEV	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
CA 5.2.6/02	Boavida Ferreria, R.	2011	Potential allergenicity of lupine seeds (<i>Lupinus</i> sp.) with special emphasis on BLAD, an intermediate in the breakdown process of the major storage protein during germination of lupine seeds Company Report No. CEV110820 Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal Not GLP, Unpublished	N	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009	CEV	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
CA-5.2.6/03	Todo-Bom, A. and Loureiro, C.	2013	Evaluation of the allergenic and cross-allergenic potential of the BLAD polypeptide Company report CHUC-021113 Immuno-Allergology Department, Coimbra University Hospital, Portugal Not GLP, Unpublished	N	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009	CEV	None
CA 5.3.2/01		2015	PROBLAD PLUS: 13 week oral (gavage) administration toxicity study in the rat Company Report No.	Y	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009	CEV	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
			8325453 [REDACTED] GLP, Unpublished					
CA 5.3.3/01	[REDACTED]	2015	PROBLAD PLUS. 21 day dermal administration toxicity study in the rat (OECD 410) Company Report No. 8297704 [REDACTED] GLP, Unpublished	Y	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009	CEV	
CA 5.4.1.1/01	Ballantyne, M.	2016	PROBLAD PLUS: Bacterial reverse mutation assay using a treat and plate modification	N	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009		

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
			Company Report No. 8325399 Covance Laboratories Ltd, Harrogate, UK GLP, Unpublished					
CA 5.4.1.2/01	Keig-Shevlin, Z.	2015a	PROBLAD PLUS: In vitro L5178Y gene mutation assay at the tk locus Company Report No. 8325403 Covance Laboratories Ltd, Harrogate, UK GLP, Unpublished	N	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009		
CA 5.4.1.3/01	Lloyd, M.	2015	PROBLAD PLUS: In vitro human lymphocyte micronucleus assay Company Report No. 8325400	N	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009		

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
			Covance Laboratories Ltd, Harrogate, UK GLP, Unpublished					
CA 5.4.2.1/01	[REDACTED],	2015b	PROBLAD PLUS: Rat alkaline comet assay Company Report No. 8325402 [REDACTED] GLP, Unpublished	Y	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009		
CA 5.4.1.2/01	Keig-Shevlin, Z.	2015a	PROBLAD PLUS: In vitro L5178Y gene mutation assay at the tk locus Company Report No. 8325403 Covance Laboratories	N	Y	Article 59(1) & (2) of assimilated Regulation No 1107/2009		

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
			Ltd, Harrogate, UK GLP, Unpublished					
CA 5.9.1/01	Barata, A.	2016	Information to address data requirements 5.9 and 5.9.1 of the Commission Regulation (EU) No 283/2013, in accordance with Regulation (EC) No 1107/2009 Company Report No. n/a CEV SA, Portugal	N	N	Article 59(1) & (2) of assimilated Regulation No 1107/2009		