



# **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.2 (AS)**

**Physical & Chemical Properties**

Great Britain

February 2025

## Version History

<b>When</b>	<b>What</b>
<b>June 2024</b>	Initial DAR
<b>February 2025</b>	Updates made after ECP
<b>February 2025</b>	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]

# Contents

<b>B.2. PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE .....</b>	<b>4</b>
<b>B.2.1. MELTING POINT AND BOILING POINT .....</b>	<b>4</b>
<b>B.2.2. VAPOUR PRESSURE, VOLATILITY .....</b>	<b>5</b>
<b>B.2.3. APPEARANCE (PHYSICAL STATE, COLOUR) .....</b>	<b>5</b>
<b>B.2.4. SPECTRA (UV/VIS, IR, NMR, MS), MOLAR EXTINCTION AT RELEVANT WAVELENGTHS, OPTICAL PURITY</b>	<b>6</b>
<b>B.2.5. SOLUBILITY IN WATER .....</b>	<b>7</b>
<b>B.2.6. SOLUBILITY IN ORGANIC SOLVENTS .....</b>	<b>7</b>
<b>B.2.7. PARTITION COEFFICIENT N-OCTANOL/WATER.....</b>	<b>8</b>
<b>B.2.8. DISSOCIATION IN WATER .....</b>	<b>8</b>
<b>B.2.9. FLAMMABILITY AND SHELF-HEATING .....</b>	<b>8</b>
<b>B.2.10. FLASH POINT .....</b>	<b>9</b>
<b>B.2.11. EXPLOSIVE PROPERTIES .....</b>	<b>9</b>
<b>B.2.12. SURFACE TENSION.....</b>	<b>10</b>
<b>B.2.13. OXIDISING PROPERTIES.....</b>	<b>10</b>
<b>B.2.14. OTHER STUDIES.....</b>	<b>11</b>
<b>B.2.15. REFERENCES RELIED ON.....</b>	<b>13</b>

## B.2. Physical and chemical properties of the active substance

Aqueous extract from the germinated seeds of sweet *Lupinus albus* has the same composition as the end-use product PROBLAD PLUS (intended to be marketed as 'PROBLAD'), therefore some of the reports refer to PROBLAD PLUS. The lead component is the BLAD protein, and where the analysis of mixture was not possible, the applicant provided data for the lead component. It is worth noting that during the manufacturing process the BLAD protein is not isolated.

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
<b>B.2.1. Melting point and boiling point</b>						
<b>Melting, freezing or solidification point</b> B.2.1/01	-	-	-	<b>Not required</b>  Melting point not applicable – the formulation active substance is liquid Considering the extract contains significant amount of water, freezing point is expected to be close to the freezing point of water.	-	-
<b>Boiling point</b> B.2.1/02	OPPTS 830.7220 OECD TG 103	PROBLAD PLUS Lot #: 27022012 20% BLAD	100°C	<b>Acceptable</b>  Correct test guideline OECD TG 103 was used, which is equivalent to EEC A2. Considering the extract contains significant amount of water, boiling point is close to that of water.	Y	C. Wo, 2012 (34852)
<b>Decomposition / Sublimation temperature</b> B.2.1/03	-	-	-	<b>Not required</b>	-	-

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
				The determination of melting point and boiling point was not constraint by decomposition or sublimation.		
<b>B.2.2. Vapour pressure, volatility</b>						
Vapour pressure B.2.2/01	-	-	-	<b>Not required</b>	-	-
Volatility (Henry's Law constant) B.2.2/02	-	-	-	Not applicable PROBLAD PLUS is a protein based aqueous solution therefore it is not technically feasible to conduct vapour pressure on this substance.  Theoretically it is possible to determine the vapour pressure of purified BLAD, but it is a large molecule (> 200 kDa), thus the vapour pressure is expected to be very low. Vapour pressure study for the purified BLAD is not required. Due to no data on vapour pressure, the Henry's law constant cannot be calculated.	-	-
<b>B.2.3. Appearance (physical state, colour)</b>						
Physical state and colour B.2.3/01	Visual assessment OPPTS 830.6302 OPPTS 830.6303 OPPTS 830.6304	PROBLAD PLUS Lot #: 16102012 20.17% BLAD	Dark brown coloured, viscous liquid with a pungent odour	<b>Acceptable</b>	Y	W. D. Gravelle, 2014 (35988)
	Visual assessment	PROBLAD PLUS Lot #: 16102012 20.17% BLAD	Dark brown colored, viscous liquid with a pungent odour	<b>Acceptable</b>	Y	W. D. Gravelle, 2015 (35987)
	Visual assessment OPPTS 830.6302 OPPTS 830.6303 OPPTS 830.6304	PROBLAD PLUS Lot #: 040511 20% BLAD	Dark brown colour, very viscous liquid, sweet-like odor	<b>Acceptable</b>	Y	C. Wo, 2012 (32388)

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference																				
B.2.4. Spectra (uv/vis, ir, nmr, ms), molar extinction at relevant wavelengths, optical purity																										
Ultraviolet/visible (UV/VIS) B.2.4/01	OPPTS 830.7050 OECD TG 101	BLAD standard Lot #: 03.2017 95% BLAD	<div>UV-vis absorption spectra, neutral, 1.857 x 10<sup>-6</sup> mol/L</div> <table><tr><td>Wavelength of maximum (nm)</td><td>Molar absorption coefficient (ε) value calculated (L/(mol·cm))</td></tr><tr><td>204.944</td><td>1.862 x 10<sup>6</sup></td></tr><tr><td>276.233</td><td>1.335 x 10<sup>5</sup></td></tr><tr><td>289.97*</td><td>6.139 x 10<sup>4</sup></td></tr></table> <div>UV-vis absorption spectra, acidic, 2.114 x 10<sup>-7</sup> mol/L</div> <table><tr><td>Wavelength of maximum (nm)</td><td>Molar absorption coefficient (ε) value calculated (L/(mol·cm))</td></tr><tr><td>201.124</td><td>3.903 x 10<sup>6</sup></td></tr><tr><td>276.009</td><td>6.149 x 10<sup>4</sup></td></tr><tr><td>289.97*</td><td>9.461 x 10<sup>3</sup></td></tr></table> <div>*there were no maxima at this wavelength, but the applicant calculated the molar absorption coefficients in neutral and acidic conditions. The data is presented for completeness</div> <div>UV-vis absorption spectra, alkaline, 4.229 x 10<sup>-7</sup> mol/L</div> <table><tr><td>Wavelength of maximum (nm)</td><td>Molar absorption coefficient (ε) value calculated (L/(mol·cm))</td></tr><tr><td>208.415</td><td>2.979 x 10<sup>6</sup></td></tr></table>	Wavelength of maximum (nm)	Molar absorption coefficient (ε) value calculated (L/(mol·cm))	204.944	1.862 x 10 <sup>6</sup>	276.233	1.335 x 10 <sup>5</sup>	289.97*	6.139 x 10 <sup>4</sup>	Wavelength of maximum (nm)	Molar absorption coefficient (ε) value calculated (L/(mol·cm))	201.124	3.903 x 10 <sup>6</sup>	276.009	6.149 x 10 <sup>4</sup>	289.97*	9.461 x 10 <sup>3</sup>	Wavelength of maximum (nm)	Molar absorption coefficient (ε) value calculated (L/(mol·cm))	208.415	2.979 x 10 <sup>6</sup>	<div>Acceptable</div> <div>The applicant indicated that PROBLAD PLUS is an aqueous solution containing many components (a UVCB substance) including 20% of the lead component BLAD which is a protein molecule therefore it is not technically feasible to conduct spectra on this substance</div> <div>Instead, the applicant provided UV spectra of a BLAD standard, wavelength of maximum and molar absorption coefficient values were reported at neutral, acidic, and basic conditions.</div>	Y	C. Wo, 2019 (48288)
Wavelength of maximum (nm)	Molar absorption coefficient (ε) value calculated (L/(mol·cm))																									
204.944	1.862 x 10 <sup>6</sup>																									
276.233	1.335 x 10 <sup>5</sup>																									
289.97*	6.139 x 10 <sup>4</sup>																									
Wavelength of maximum (nm)	Molar absorption coefficient (ε) value calculated (L/(mol·cm))																									
201.124	3.903 x 10 <sup>6</sup>																									
276.009	6.149 x 10 <sup>4</sup>																									
289.97*	9.461 x 10 <sup>3</sup>																									
Wavelength of maximum (nm)	Molar absorption coefficient (ε) value calculated (L/(mol·cm))																									
208.415	2.979 x 10 <sup>6</sup>																									

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

Volume 3 – B.2 (AS)

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results			Comments (Acceptable / Non acceptable)	GLP	Reference
			290.391	1.655 x 10 <sup>5</sup>				
Infrared (IR) B.2.4/02	-	-	Not applicable PROBLAD PLUS is an aqueous solution containing many components (a UVCB substance) including 20% of the lead component BLAD which is a protein molecule therefore it is not technically feasible to conduct spectra on this substance			Not applicable  Considering the size and type of molecule, the IR, NMR and MS data is not required.	-	-
Nuclear magnetic resonance (NMR) B.2.4/03	-	-					-	-
Mass spectra (MS) B.2.4/04	-	-					-	-
Spectra for impurities B.2.4/05	-	-	See Volume 3 CA B5.			No impurities identified as relevant.  Quinolizidine alkaloids (QAs) are found in the legume plant family and are listed as relevant impurities. Mass spectra have been provided as part of the GC-MS/MS analytical method described in Volume 3 CA B5. Additionally, these components are naturally occurring substances with known structures. Further data is not required.	-	-
B.2.5. Solubility in water								
Solubility in water B.2.5/01	-	-	-			Not applicable  PROBLAD PLUS is an aqueous solution containing 20% of the lead component BLAD. PROBLAD PLUS is a UVCB substance that is in water, therefore the testing of water solubility is not technically feasible or necessary.	-	-
B.2.6. Solubility in organic solvents								
Solubility in organic solvents	-	-	-			Not applicable	-	-

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
B.2.6/01				PROBLAD PLUS is an aqueous solution containing 20% of the lead component BLAD. PROBLAD PLUS is a UVCB substance that is in water, therefore the testing of solvent solubility is not technically feasible or necessary.		
<b>B.2.7. Partition coefficient n-octanol/water</b>						
Partition coefficient n-octanol/water B.2.7/01	-	-	-	<b>Not applicable</b>  PROBLAD PLUS is an aqueous solution containing 20% of the lead component BLAD. PROBLAD PLUS is a UVCB substance that is in water, therefore the testing of the partition coefficient is not technically feasible or necessary.	-	-
<b>B.2.8. Dissociation in water</b>						
Dissociation constant B.2.8/01	-	-	-	<b>Not applicable</b>  PROBLAD PLUS is an aqueous solution containing 20% of the lead component BLAD protein that cannot dissociate.	-	-
<b>B.2.9. Flammability and self-heating</b>						
Flammability B.2.9/01	-	-	-	<b>Not applicable</b>  PROBLAD PLUS is not a solid or a gas and is an aqueous solution containing 20% of the lead component BLAD protein.	-	-
Self heating B.2.9/02	-	-	The active substance (as manufactured) is a very viscous liquid that is an aqueous solution and as such the self-heating test (UN N.4) is not applicable to liquids.	<b>Acceptable</b>	-	-



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

**Volume 3 – B.2 (AS)**

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
			<p>If the auto-ignition temperature (in accordance with EC A.15) was performed, this would essentially provide a result for an aqueous solution rather than the lead component.</p> <p>In addition, the flash point test was performed and a value of &gt; 100°C was obtained, which is consistent with an aqueous solution and therefore the test substance can be classified as non-flammable.</p>	<p>Please refer to information provided in support of this data requirement for the active substance:</p> <p>A theoretical estimation based on structure meeting the criteria set out in Appendix 6 of the United Nations' Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria can be made. In accordance with Appendix 6, there are no chemical groups present in the molecule associated with explosive or self reactive properties. Additionally, there is sufficient evidence that the substance does not ignite spontaneously on coming into contact with air at normal temperatures and forms a stable mixture in water.</p>		
<b>B.2.10. Flash point</b>						
<b>Flash point B.2.10/01</b>	ASTM D93 OPPTS 830.6315	PROBLAD PLUS Lot #: 040511 20% BLAD	Flash point above 100°C, not flammable	<p><b>Acceptable</b></p> <p>Method A9 was recommended for this test, but the applicant used ASTM D93, which is a closed cup method and can be considered acceptable.</p> <p>No classification required.</p>	Y	C. Wo, 2012 (32388)
<b>B.2.11. Explosive properties</b>						
<b>Explosive properties B.2.11/01</b>	EEC A14	PROBLAD PLUS Batch: 16102012 20% w/w BLAD	Thermal test: no explosion occurred before 300s, yellow/orange flame was observed after about 20s	<p><b>Acceptable</b></p> <p>Correct method A14 was used. The formulation was not sensitive to the effect of flame and shock. PROBLAD PLUS is not explosive</p>	Y	S. Cage. 2013 (MIB0036)

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
			Shock: no evidence of ignition, explosion or decomposition  PROBLAD PLUS is not explosive	No classification required.		
<b>B.2.12. Surface tension</b>						
Surface tension B.2.12/01	EC A.5 OECD 115	PROBLAD PLUS Batch: 16102012 20% w/w BLAD	29.3 mN/m at 1.00 g/L aqueous dilution at 20.2°C	<b>Acceptable</b>  Correct method was used, the data was provided for 1 g/L dilution.  <b>Surface active product</b>	Y	T. P. Lien, 2013 (S13-00831)
	EC A5	PROBLAD PLUS Batch #: C191804-002 20% BLAD	22.4 mN/m at 1.6% w/v at 20°C	<b>Acceptable</b>  Correct method A5 was used. The applicant used 1.6% w/v concentration.  <b>Surface active product</b>	Y	C. Wo, 2018 (48183)
<b>B.2.13. Oxidising properties</b>						
Oxidizing properties B.2.13/01	EEC A21	PROBLAD PLUS Batch: 16102012 20% w/w BLAD	Mean pressure rise time for test substance was significantly higher than for the reference substance.  PROBLAD PLUS is not oxidising	<b>Acceptable</b>  Correct method A21 was used. PROBLAD PLUS is not oxidising  No classification required.	Y	S. Cage. 2013 (MIB0036)
	OPPTS 830.6314	PROBLAD PLUS Lot #: 040511; Batch 1 20% BLAD	Compatible with water, 10% monoammonium phosphate solution, iron powder, kerosene.	<b>This test is not required, but no adverse data noted.</b>  The data supports previous finding that PROBLAD PLUS is not oxidising.	Y	C. Wo, 2012 (32388)

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

### Volume 3 – B.2 (AS)

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
			Incompatible with 10% potassium permanganate solution, heat was produced within 2 minutes			
			PROBLAD PLUS is an aqueous solution containing the lead component BLAD. BLAD is a 20 kDa polypeptide of $\beta$ -conglutin, or characterized as a fragment of the amino acid sequence of $\beta$ -conglutin. As such it is not expected to be oxidizing. No other components of PROBLAD PLUS contain groups that would imply oxidising properties such as nitrates, metal oxides, hypofluorites, difluoroaminopolynitroaryls, perchlorates, bromates and iodites. Therefore PROBLAD PLUS will not be oxidising.	<b>Acceptable</b>  The applicant provided a case based on functional groups in the mixture (aqueous extract from the germinated seeds of sweet <i>Lupinus albus</i> ) which supports previous findings that PROBLAD PLUS is not oxidising.	N	-
<b>B.2.14. Other studies</b>						
	-	-	None submitted	-	-	-

## Summary and Conclusions

Aqueous extract from the germinated seeds of sweet *Lupinus albus* is an aqueous solution containing the lead component BLAD protein at 20% w/w. The lead component is not separated during manufacturing process and the proposed representative product PROBLAD PLUS (intended to be marketed as PROBLAD) has the same composition as the aqueous extract from the germinated seeds of sweet *Lupinus albus*. It is a viscous, dark brown liquid with a pungent odour and is not classified as explosive, flammable or oxidising. Due to low surface tension, it is a surface active compound.

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

The applicant provided UV-Vis spectra of purified BLAD standard. No other analytical techniques were used to characterise the substance. Considering the UVCB nature of the aqueous extract from the germinated seeds of sweet Lupinus albus and the nature of the lead component BLAD (large molecule, protein composition), it is not feasible to conduct any other spectra on the substance; no further data are required.

## B.2.15. References relied on

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
<b>CA 2.1/01</b>	Wo, C.	2012a	PROBLAD PLUS: Physical and Chemical Characteristics: Boiling Point Company Report No. 34852 Eurofins PSL, USA GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of assimilated Regulation No 1107/2009	CEV	None
<b>CA 2.3/01</b>	Wo, C.	2012b	PROBLAD PLUS Physical and Chemical Characteristics: Color, Physical State, Odor, Oxidation/Reduction, Flammability, pH,	N	Y	Data protection is claimed in accordance with Article 59 of assimilated Regulation No 1107/2009	CEV	None

**Aqueous extract from the germinated seeds of sweet *Lupinus albus***  
**Volume 3 – B.2 (AS)**

			Viscosity, and Density/Relative Density (amended) Company Report No. 32388 Eurofins PSL, USA GLP, Unpublished					
<b>CA 2.4/01</b>	Wo, C.	2019	BLAD standard Physical and Chemical Characteristics: UV/Visible absorption Company Report No. 48288, amendment no 1 Eurofins PSL, USA GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of assimilated Regulation No 1107/2009	CEV	None
<b>CA 2.10/01</b>	Wo, C.	2012b	PROBLAD PLUS Physical and Chemical Characteristics: Color, Physical State, Odor, Oxidation/Reduction, Flammability, pH,	N	Y	Data protection is claimed in accordance with Article 59 of assimilated Regulation No 1107/2009	CEV	None

**Aqueous extract from the germinated seeds of sweet *Lupinus albus***  
**Volume 3 – B.2 (AS)**

			Viscosity, and Density/Relative Density (amended) Company Report No. 32388 Eurofins PSL, USA GLP, Unpublished					
<b>CA 2.11/01</b>	Cage, S.	2013	PROBLAD PLUS: Explosive properties and oxidising properties Company Report No. MIB0036 Huntingdon Life Sciences, Eye, UK GLP, Unpublished Study submitted to meet data requirements	N	Y	Data protection is claimed in accordance with Article 59 of assimilated Regulation No 1107/2009	CEV	None
<b>CA 2.12/01</b>	Lien, T.P.	2013	Surface tension of PROBLAD PLUS Company Report No. S13-00831 Eurofins, Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of assimilated Regulation No 1107/2009	CEV	None

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

**Volume 3 – B.2 (AS)**

<b>CA 2.13/01</b>	Wo, C.	2012b	PROBLAD PLUS Physical and Chemical Characteristics: Color, Physical State, Odor, Oxidation/Reduction, Flammability, pH, Viscosity, and Density/Relative Density (amended) Company Report No. 32388 Eurofins PSL, USA GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of assimilated Regulation No 1107/2009	CEV	None
<b>CA 2.13/02</b>	Cage, S.	2013	PROBLAD PLUS: Explosive properties and oxidising properties Company Report No. MIB0036 Huntingdon Life Sciences, Eye, UK GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of assimilated Regulation No 1107/2009	CEV	None