Banda de *Lupinus albus* doce (BLAD)
PC Code 030006

Preliminary Work Plan
Case Number 6318

Approved by: Le, Madison Digitally signed by Le, Madison Date: 2024.05.29 18:03:10 -04'00'

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I. Introduction

This document is the Environmental Protection Agency's (EPA or the Agency) Preliminary Work Plan (PWP) for Banda de *Lupinus albus* doce (hereafter referred to as BLAD) (Case 6318) and is being issued pursuant to 40 CFR § 155.50. This document explains what EPA's Office of Pesticide Programs (OPP) knows about BLAD, highlights anticipated data and assessment needs, identifies types of information that would be especially useful to the Agency in conducting the review, and provides an anticipated timeline for completing the registration review process for BLAD. As stated in 40 CFR § 155.50 the opening of this docket initiates the current cycle of registration review for BLAD.

A registration review decision is the Agency's determination of whether a pesticide meets, or does not meet, the standard for registration in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, which mandates the continuous review of existing pesticides. All pesticides distributed or sold in the United States generally must be registered by the Agency based on scientific data showing that they will not cause unreasonable adverse effects to human health or to the environment when used as directed on product labeling. The registration review program is intended to ensure that, as the ability to assess and reduce risk evolves and as policies and practices change, all registered pesticides continue to meet the statutory standard of no unreasonable adverse effects. Changes in science, public policy, and pesticide use practices will occur over time. Through the registration review program, the Agency periodically re-evaluates pesticides to ensure that as these changes occur, products in the marketplace can continue to be used safely. Information on this program is provided at www.epa.gov/pesticide-reevaluation.

In 2006, the Agency implemented the registration review program pursuant to FIFRA § 3(g). The Agency will review each registered pesticide every 15 years to determine whether it continues to meet the FIFRA standard for registration. The regulations governing registration review are provided in 40 CFR part 155, subpart C. The public phase of registration review begins when the initial docket is opened for the case. The docket is the Agency's opportunity to inform the public what it knows about BLAD and what additional risk analyses and data or information it believes are needed to make a registration review decision on BLAD.

The Agency encourages all interested stakeholders to review the PWP and to provide comments and additional information that will help the Agency's decision-making process for BLAD. Interested stakeholders could include the following: environmental nonprofit or interest groups; pesticide manufacturers; agricultural labor or commodity groups; commercial, institutional, residential, and other users of pesticides; or the general public. In addition to general areas on which persons may wish to comment, there are some areas identified in the PWP about which the Agency specifically seeks comments and information.

After reviewing and responding to comments and data received in the docket during this initial comment period, the Agency will develop and commit to a Final Work Plan (FWP) and anticipated schedule for the registration review of the BLAD case. Additional information on BLAD can be found in the Agency's public docket (EPA-HQ-OPP-2023-0357) at www.regulations.gov.

This document is organized into five sections: the *Introduction*, which includes this summary and BLAD case overview; *Use Information*, which describes how and why BLAD is used and summarizes data on its use, and associated pesticide products; *Scientific Assessments*, which summarizes the Agency's risk assessments, any revisions, risk conclusions, and any anticipated data needs that will help the Agency's decision-making process for BLAD; *Guidance for Commentors*, which highlights topics of special interest, additional information and data the Agency should consider prior to issuing a FWP; and,

lastly, the *Next Steps* and *Timeline* which provides an anticipated timeline for the registration review process for BLAD.

Banda de *Lupinus albus* doce (BLAD) Registration Review Case Overview

Pursuant to 40 CFR § 155.50, the Agency will initiate a pesticide's registration review by establishing a docket for registration review of BLAD (Case 6318) and opening it for public review.

This PWP marks the beginning of the current cycle of registration review for BLAD, with the opening of public docket EPA-HQ-OPP-2023-0357 available at www.regulations.gov. The following list highlights significant events that have occurred during the current cycle of registration review for this case:

- June 2024 – The Agency is now publishing the *Banda de Lupinus albus* doce (BLAD) *Preliminary Work Plan* for a 60-day public comment period.

II. Use Information

The first pesticide product containing BLAD as an active ingredient was registered by the Agency in 2013. Currently, there are two end-use products containing BLAD registered under FIFRA section 3 and one end-use product registered under FIFRA section 24(c), each with 20% active ingredient.

BLAD is a seed storage protein derived from sweet lupines. It acts against fungal crop diseases by binding to chitin and destroying the fungal cell wall. BLAD is a biochemical pesticide active ingredient intended for use as a fungicide for the control and suppression of powdery mildew, *Botrytis*, anthracnose, and other diseases in various crops such as almonds, broccoli, peanuts, and ornamentals.

Table 1. Banda de *Lupinus albus* doce (BLAD) Use Information

Ingredient Name	Banda de *Lupinus albus* doce (BLAD)
PC Code	030006
Pesticide Classification	Fungicide
Use Site Locations	Agricultural (Outdoor): Crop
Application Types	Broadcast, Spray Drench
No. of Registrations	2 FIFRA Section 3 products[1] 1 FIFRA Section 24(c) product
Physical Forms	Solution

III. Scientific Assessments

A summary of the Agency's human health and ecological risk assessments for BLAD is presented below. Refer to the Appendices for a detailed listing of product analysis, human health assessment, and nontarget organism data that support the scientific assessments for this registration review. For further information on the human health and environmental risk assessments, including a summary of data and literature search findings, please see Appendices B and C.

A. Human Health Assessment

Summary of Hazard Characterization

The toxicological database is considered complete for characterizing hazard from the active ingredient in terms of acute toxicity. BLAD can be classified as Toxicity Category IV for acute oral and acute inhalation; Toxicity Category III for eye irritation and acute dermal, and Toxicity Category IV for

[1] FIFRA labels can be obtained from the Pesticide Product Label System (ordspub.epa.gov/ords/pesticides/f?p=PPLS:1)

primary dermal irritation (U.S. EPA, 2012). Uncertainty has been identified regarding the product identity/analysis of impurities warranting additional review regarding the allergenicity of this product. These uncertainties and resulting data needs have been outlined in the ***Anticipated Data Needs*** section and more information can be found in the **Summary of Registration Review Human Health Data Needs** section. These data are required to fully evaluate hazard in support of an updated assessment. See Appendix B for more details.

Summary of Dietary Exposure and Risk Characterization

Various uncertainties were identified regarding residue studies and the product identity/analysis of impurities related to the allergenicity of the ingredient that warrant additional review of dietary exposure to BLAD. The Agency is seeking data listed in the ***Anticipated Data Needs*** section to determine if people would be exposed to BLAD via dietary exposure. See Appendix B for more details.

Food Tolerances

Following the initial registration of products containing BLAD and the establishment of an exemption from the requirement of a tolerance for food uses of BLAD on March 22, 2013, the Agency proposed to revoke the tolerance exemption for BLAD due to concerns regarding potential allergenicity of BLAD and to instead establish tolerances for residues of BLAD in or on certain commodities (80 FR 30640, May 29, 2015, and 85 FR 7698, Feb. 11, 2020). The rulemaking is pending and, if finalized as proposed, would result in the revocation of the tolerance exemption and establishment of tolerances for certain commodities. At this time, the tolerance exemption established in 2013 remains in place. The tolerance exemption is stated as follows:

> **40 CFR § 180.1319 Banda de *Lupinus albus* doce (BLAD); exemption from the requirement of a tolerance.** An exemption from the requirement of a tolerance is established for the residues of Banda de *Lupinus albus* doce (BLAD), a naturally occurring polypeptide from the catabolism of a seed storage protein (β-conglutin) of sweet lupines (*Lupinus albus*), in or on all food commodities when applied as a fungicide and used in accordance with label directions and good agricultural practices. [78 FR 17600, Mar. 22, 2013]

Uncertainties regarding the residue data and product identity/analysis of impurities for BLAD warrant additional review so that the Agency can update the human health risk assessment. Failure to adequately address the uncertainties described in detail within the ***Anticipated Data Needs*** section below may result in the revocation of the exemption and establishment of tolerances for certain commodities. See Appendix B for more details.

Summary of Residential and Non-Occupational Exposure and Risk Characterization

The Agency does not expect any risks to children or adults in residential, school, or day care areas because the products containing this active ingredient are not registered for these uses. The label does not include droplet size restrictions for aerial application, which would further mitigate potential for non-occupational exposure via spray drift. A comprehensive assessment of these potential risks related to residential and non-occupational exposure will be addressed in the updated human health risk assessment for BLAD. See Appendix B for more details.

Summary of Occupational Exposure and Risk Characterization

Occupational exposure to BLAD should be mitigated by personal protective equipment (PPE) requirements included on the labels for applicators and handlers. However, due to uncertainties regarding the residue data and product identity/analysis of impurities for BLAD, additional data are

needed to allow the Agency to assess if there is a potential for risk concerns related to occupational exposure and allergenicity. This will be performed in the updated human health risk assessment for this active ingredient upon receipt and review of the requested confirmatory data. See Appendix B for more details.

Human Incidents

A search of the Office of Pesticide Programs' (OPP) Incident Data System conducted on April 23, 2024, revealed two reported incidents associated with BLAD. This database contains information dating back to the 1970s and is continuously updated as incidents are reported. One incident was reported to EPA Region 9 in June 2017 where workers were allegedly exposed to pesticides applied to a neighboring field. The second was reported to EPA Region 9 in 2018 involving workers at a county animal facility that were exposed to a pesticide application to strawberries. Both incidents involved the simultaneous application of the conventional pesticides spinetoram, novaluron, and bifenthrin. No details describing adverse reactions were reported, but if adverse reactions did occur it would be expected to be the result of the exposure to the conventional pesticides and not BLAD.

B. Environmental Risk Assessment

The available ecological toxicity data/rationales for BLAD were considered acceptable at the time of the original registration, completed in 2013. The submitted rationales relied heavily on limited exposure due to rapid biodegradability. However, in the process of this registration review the Agency revisited the underlying rapid biodegradability of BLAD and found the data/results were not applicable to outdoor settings. Those rationales are, therefore, not adequate to support the environmental risk assessment because the persistence of BLAD in the environment is uncertain. To help address this uncertainty and support the rationale for low exposure, environmental fate data on aerobic soil and aquatic metabolism are needed. These data will support both the human health and ecological risk assessments as they will help determine if BLAD rapidly degrades in the environment under more realistic conditions. If BLAD's rapid degradation in natural settings can be confirmed with new data, this supports previous conclusions concerning rationales for nontarget organism toxicity. If the requested environmental fate data suggest that there will be longer durations of exposure than originally assumed, data will be needed for avian acute oral toxicity, fish acute toxicity, aquatic invertebrate acute toxicity, and terrestrial plant toxicity (seedling emergence and seedling growth, and vegetative vigor). See Appendix C for more details.

Ecological Incidents

A search of OPP's Environmental Incident Information System conducted on April 23, 2024, revealed no reported ecological incidents associated with BLAD. This database contains information dating back to the 1970s and is continuously updated as incidents are reported.

Endangered Species Assessment

This section provides general background about the Agency's assessment of the effects of pesticides on listed species and designated critical habitats under the Endangered Species Act (ESA).

Developing Approaches for ESA Assessments and Consultation for FIFRA Actions

In 2015, EPA, along with the Services—the U.S. Fish and Wildlife Service (FWS) and the National Marine Fisheries Service (NMFS)—and the United States Department of Agriculture (USDA) (referred

to as "the agencies") released their joint Interim Approaches[2] for assessing the effects of pesticides to listed species. The agencies jointly developed these Interim Approaches in response to the 2013 National Academy of Sciences' recommendations that discussed specific scientific and technical issues related to the development of assessments of pesticides' effects to listed species. Since that time, the agencies have been continuing to work to improve the approaches for assessing effects to listed species. After receiving input from the Services and USDA on proposed revisions to the interim method and after consideration of public comments received, EPA released an updated *Revised Method for National Level Listed Species Biological Evaluations of Conventional Pesticides* ("Revised Method") in March 2020.[3]

The agencies also continue to work collaboratively through a FIFRA Interagency Working Group (IWG). The IWG was created under the 2018 Farm Bill to recommend improvements to the ESA section 7 consultation process for FIFRA actions and to increase opportunities for stakeholder input. This group is led by EPA and includes representatives from NMFS, FWS, USDA, and the Council on Environmental Quality (CEQ). The IWG outlines its recommendations and progress on implementing those recommendations in reports to Congress.[4]

Consultation on Chemicals in Registration Review

EPA initially conducted biological evaluations (BEs) using the interim method on three pilot chemicals representing the first nationwide pesticide consultations (final pilot BEs for chlorpyrifos, malathion, and diazinon were completed in January 2017). These initial pilot consultations were envisioned as the start of an iterative process. Later that year, NMFS issued a final biological opinion for these three pesticides. In 2019, EPA requested to reinitiate formal consultation with NMFS on malathion, chlorpyrifos and diazinon to consider new information that was not available when NMFS issued its 2017 biological opinion. EPA received a final malathion biological opinion[5] from FWS in February 2022 and a final biological opinion from NMFS on malathion, chlorpyrifos and diazinon in June 2022.[6] The Agency plans to implement both biological opinions according to the 18-month timeframes specified in the biological opinions.

In 2020, EPA released draft BEs for the first two chemicals conducted using the 2020 Revised Method—carbaryl and methomyl. Subsequently, EPA has used the Revised Method to complete final BEs for carbaryl, methomyl, atrazine, simazine, glyphosate, clothianidin, imidacloprid, and thiamethoxam. EPA is currently in consultation with the Services on these active ingredients.

EPA's New Actives Policy and the 2022 Workplan

In January 2022, EPA announced a policy[7] to evaluate potential effects of new conventional pesticide active ingredients to listed species and their designated critical habitat and initiate consultation with the Services, as appropriate, before registering these new pesticides. Before the Agency registers new uses of pesticides for use on pesticide-tolerant crops, EPA will also continue to make effects determinations. If these determinations are likely to adversely affect determinations, the Agency will not register the use unless it can predict that registering the new use would not have a likelihood of jeopardizing listed

[2] https://www.epa.gov/endangered-species/interim-approaches-pesticide-endangered-species-act-assessments-based-nas-report.
[3] https://www.epa.gov/endangered-species/revised-method-national-level-listed-species-biological-evaluations-conventional.
[4] https://www.epa.gov/endangered-species/reports-congress-improving-consultation-process-under-endangered-species-act.
[5] https://www.epa.gov/endangered-species/biological-opinions-available-public-comment-and-links-final-opinions.
[6] https://www.epa.gov/endangered-species/biological-opinions-available-public-comment-and-links-final-opinions.
[7] https://www.epa.gov/newsreleases/epa-announces-endangered-species-act-protection-policy-new-pesticides.

species or adversely modifying their designated critical habitats. EPA will also initiate consultation with the Services as appropriate.

In April 2022, EPA released a comprehensive, long-term approach to meeting its ESA obligations, which is outlined in *Balancing Wildlife Protections and Responsible Pesticide Use*.[8] This workplan reflects the Agency's most comprehensive thinking to date on how to create a sustainable ESA-FIFRA program that focuses on meeting EPA's ESA obligations and improving protection for listed species while minimizing regulatory impacts to pesticide users and collaborating with other agencies and stakeholders on implementing the plan.

On November 16, 2022, EPA released the *ESA Workplan Update: Nontarget Species Mitigation for Registration Review and Other FIFRA Actions*.[9] As part of this update, EPA announced its plan to consider and include, as appropriate, a menu of FIFRA Interim Ecological Risk Mitigation intended to reduce off-target movement of pesticides through spray drift and runoff in its registration review and other FIFRA actions. These measures are intended to reduce risks to nontarget organisms efficiently and consistently across pesticides with similar levels of risks and benefits. EPA expects that these mitigation measures may also reduce pesticide exposures to listed species.

C. Anticipated Data Needs

Additional data and information are required for updated risk assessments, as described below. Specifically, there are data needs regarding the environmental degradation rate/fate, residue data, sample handling, and validation of the Enzyme Linked Immunosorbent Assay (ELISA) used to measure the residues. There is also uncertainty regarding product identity/analysis of impurities. These data need to be provided to make an updated safety finding, given the potential allergenicity of BLAD to lupine- and/or peanut-sensitive individuals. Please see the appendices for more detailed information regarding BLAD's product chemistry, human health, and environmental risk assessments. The Agency will conduct an ecological risk assessment of BLAD, which will include an endangered species assessment, once new data regarding the persistence of BLAD in the environment have been submitted. For further information on the human health and environmental risk assessments, including a summary of data and literature search findings, please see Appendices B and C.

Table 2. Anticipated Product Chemistry, Human Health, Nontarget Organism Toxicity, and Environmental Fate Studies for the Registration Review of BLAD

OCSPP Guideline No.	Data Requirement	Active Ingredient	Test Substance	Time Needed to complete (months)	Use Site(s) Triggering Data Requirement	Applicable Exposure Scenario
880.1400	Discussion of Formation of Impurities[1]	BLAD	Other Proteins	12	All	All
830.1700	Preliminary Analysis[1]	BLAD	Other Proteins	8	All	All
860.1380	(Residue) Storage Stability[2]	BLAD	BLAD	24	All	Dietary (Tolerance Assessment), Occupational Handler, Occupational Applicator

[8] https://www.epa.gov/endangered-species.
[9] https://www.epa.gov/system/files/documents/2022-11/esa-workplan-update.pdf.

Table 2. Anticipated Product Chemistry, Human Health, Nontarget Organism Toxicity, and Environmental Fate Studies for the Registration Review of BLAD						
OCSPP Guideline No.	**Data Requirement**	**Active Ingredient**	**Test Substance**	**Time Needed to complete (months)**	**Use Site(s) Triggering Data Requirement**	**Applicable Exposure Scenario**
860.1000 860.1500	Residue Data[3] (Existing studies)	BLAD	BLAD	24	All	Dietary (Tolerance Assessment), Occupational Handler, Occupational Applicator
860.1000 860.1500	Residue Data[6]	BLAD	BLAD	24	All	Dietary (Tolerance Assessment), Occupational Handler, Occupational Applicator
Non-Guideline	Method Validation for ELISA[8]	BLAD	BLAD	9	All	Dietary (Tolerance Assessment), Occupational Handler, Occupational Applicator
Non-Guideline	*in Silico* Bioinformatics based Allergenicity Assessment[7]	BLAD	Other Proteins	12	All	Dietary
870.3700	Prenatal Developmental Toxicity[4]	BLAD	BLAD	24	All	Dietary (Tolerance Assessment), Occupational Handler, Occupational Applicator
870.5100	Bacterial Reverse Mutation Test[4]	BLAD	BLAD	9	All	Dietary (Tolerance Assessment), Occupational Handler, Occupational Applicator
870.5300	*In vitro* Mammalian cell Gene Mutation Test[4]	BLAD	BLAD	9	All	Dietary (Tolerance Assessment), Occupational Handler, Occupational Applicator
870.5375	*In vitro* Mammalian Chromosome Aberration Test[4]	BLAD	BLAD	9	All	Dietary (Tolerance Assessment), Occupational Handler, Occupational Applicator
850.2100	Avian Acute Oral Toxicity[5]	BLAD	BLAD	12	All	Avian acute oral
850.1075	Fish Acute Toxicity, Freshwater[5]	BLAD	BLAD	18	All	Fish acute

Table 2. Anticipated Product Chemistry, Human Health, Nontarget Organism Toxicity, and Environmental Fate Studies for the Registration Review of BLAD						
OCSPP Guideline No.	**Data Requirement**	**Active Ingredient**	**Test Substance**	**Time Needed to complete (months)**	**Use Site(s) Triggering Data Requirement**	**Applicable Exposure Scenario**
850.1010	Aquatic Invertebrate Acute Toxicity, Freshwater[5]	BLAD	BLAD	24	All	Aquatic invertebrate acute
850.4100	Terrestrial Plant Toxicity, Seedling Emergence and Seedling Growth[5]	BLAD	BLAD	12	All	Terrestrial plants
850.4150	Terrestrial Plant Toxicity, Vegetative Vigor[5]	BLAD	BLAD	12	All	Terrestrial plants
835.4100	Aerobic Soil Metabolism	BLAD	BLAD	24	All	All
835.4300	Aerobic Aquatic Metabolism	BLAD	BLAD	24	All	All

1: Provide the full protein sequences for all "other proteins" present in the product.
2: New residue storage stability data is needed unless the validity of the ELISA methodology can be adequately demonstrated.
3: New residue studies on one representative crop from each crop group with labelled uses are needed unless (1) validation of cold-chain maintenance within MRIDs 49319401, 49198301, 50307501 and 50149801 can be provided (i.e., sample chain of custody with temperature logs), (2) the ELISA methodology can be validated as prescribed in Table 5 and (3) a scientifically valid explanation for the low recovery rate in the residue studies can be provided along with clarification regarding whether the residue values reflect a calculation to correct for recovery. In addition, addressing how the low recovery rates affect the precision of the quantitative ELISA and the impact on the residue data values. Note that if residue data are not provided, worst case assumptions for exposure may be made (i.e., cannot rule out exposure).
4: The submitted rationales rely in part upon a lack of exposure due to "rapid biodegradation". Neither the peer reviewed literature nor the environmental fate data submitted support this conclusion. Furthermore, the Agency no longer bases safety findings for subchronic toxicity solely based upon acute studies when exposure cannot be ruled out. Updated rationales or studies must be submitted to adequately address these data requirements.
5: If BLAD's rapid degradation in natural settings can be confirmed with aerobic soil and aquatic metabolism (835.4100 and 835.4300), these studies are not required.
6: Residue field trial data on head lettuce, leaf lettuce, spinach, and mustard greens, which are the representative crops for crop subgroups 4-16A and B. An acceptable bridging rationale is needed to support the use of available residue data for fresh and dried basil, fresh and dried mint, peanuts, pecans, and coffee crops. The rationale must consider similarities and differences in plant morphology, applications and their impact on residue levels.
7: Follow the Codex Alimentarius Commission (of FAO/WHO) guidance for proteins derived from an allergic source.
8: In the description of specificity, describe whether or not the assay distinguishes between BLAD and other proteins in the product. Does the assay detect BLAD alone or all proteins in the product?

IV. Guidance for Commentors

Preliminary Work Plan

During the comment period, anyone may submit relevant data or information for the Agency's consideration. The public is invited to comment on the Agency's PWP. The areas below highlight topics of special interest to the Agency where comments, information and data, or reference to sources of additional information could be of particular use. The Agency will carefully consider all comments, as well as any additional information or data provided in a timely manner, prior to issuing a FWP for this case.

Additional Information

Stakeholders are also specifically asked to provide information and data that will assist the Agency in refining the risk assessments, including the ESA assessment. The Agency is interested in obtaining the following information regarding BLAD:

i. Confirmation of the following label information:
   - *Sites of application*
   - *Formulations*
   - *Application methods and equipment*
   - *Maximum application rates*
   - *Frequency of application, application intervals, and maximum number of applications*
   - *Geographic limitations on use*

ii. Use or potential use distribution (e.g., acreage and geographical distribution of relevant use sites)

iii. Median and 90th percentile reported use rates from usage data – national, state, and county

iv. Application timing (date of first application and application intervals) – national, state, and county

v. Usage/use information for agricultural and nonagricultural uses

vi. Typical application interval (days)

vii. State or local use restrictions

viii. Monitoring data

ix. Foreign technical registrants not listed above who supply pesticide products containing BLAD to the U.S. market

x. The Agency welcomes any information on the effects of BLAD that would help refine the ESA assessment

Environmental Justice

EPA seeks to achieve environmental justice, the fair treatment and meaningful involvement of all people, regardless of race, color, national origin, or income, in the development, implementation, and enforcement of environmental laws, regulations, and policies. To help address potential environmental justice issues related to registration review decisions, the Agency seeks information on any groups or segments of the population who, as a result of their location, cultural practices, or other factors, may have atypical, unusually high exposure to BLAD compared to the general population or who may otherwise be disproportionately affected by the use of BLAD as a pesticide. Please comment if you are aware of any such issues and can provide information to help the Agency to more fully consider and address potential environmental justice issues.

V. Next Steps and Timeline

A Federal Register Notice will announce the docket opening for the current cycle of registration review for BLAD and a 60-day comment period for this *Preliminary Work Plan* to provide comments and additional information that will help the Agency's decision-making process for BLAD. After the 60-day comment period closes, the Agency will review and respond to any comments received in a timely manner, then issue a Final Work Plan for BLAD. The Agency's final decision on the BLAD registration review case will include a determination on the Endocrine Disruptor Screening Program (EDSP) obligations under FFDCA § 408(p) and completion of an endangered species determination and any necessary consultation with the Services.

Table 3. Anticipated Registration Review Schedule for Banda de *Lupinus albus* doce (BLAD)	
Anticipated Activity	**Estimated Month/ Year**
Opening the Docket	
Open Docket and 60-Day Public Comment Period for Preliminary Work Plan	June 2024
Close Public Comment Period	August 2024
Case Development	
Final Work Plan	December 2024
Issue Data Call-In	March 2025
Data Submission	March 2027
Open 60-Day Public Comment Period for Draft Risk Assessments	TBD
Close Public Comment Period	TBD
Registration Review Decision and Implementation	
Open 60-Day Public Comment Period for Proposed Registration Review Decision	TBD
Close Public Comment Period	TBD
Final Decision*	TBD

*The anticipated schedule will be revised as necessary (e.g., need arising under the Endocrine Disruptor Screening Program with respect to the active ingredients in this case).

Appendix A – Summary of Existing Product Analysis Data

During the registration of BLAD, certain product analysis data were accepted that, upon further review, are now considered unacceptable, but upgradable. Table 4 summarizes the current product analysis data requirements and results supporting registration review of BLAD. Since the original registration, some uncertainty has been raised regarding the precise identity of the proteins/polypeptides included in the active ingredient and end-use products (see the the **Summary of Registration Review Human Health Data Needs** section below for further detail). Further identification and characterization of the "Other Proteins" presented in the formulations and their toxicological significance or allergenicity are warranted, and additional data and information on OCSPP data requirements 880.1400 (Discussion of Formation of impurities) and 830.1700 (Preliminary Analysis) must be submitted. In the absence of these data, allergenicity may be assumed for the active ingredient and end-use products.

Table 4. Summary of Product Analysis Data (40 CFR § 158.2030)

Data Requirement	Guideline No.	Results / Findings	MRIDs
Product identity and composition	880.1100	Confidential Business Information (CBI) Acceptable	48587901
Description of Starting Materials, Production and Formulation Process	880.1200	CBI Acceptable	48587901
Discussion of Formation of Impurities	880.1400	CBI Upgradable Further identification and characterization of the "Other Proteins" presented in the formulations and their toxicological significance or allergenicity are warranted.	48587901
Preliminary Analysis	830.1700	CBI Upgradable Further identification and characterization of the "Other Proteins" presented in the formulations and their toxicological significance or allergenicity are warranted.	48587903
Certified Limits	830.1750	CBI Acceptable	48587901
Enforcement Analytical Method	830.1800	CBI Acceptable	48587903
Color	830.6302	Dark brown	48587902
Physical State	830.6303	Viscous liquid	48587902
Odor	830.6304	Sweet-like odor	48587902
Stability to Normal and Elevated Temperatures, Metals, and Metal Ions	830.6313	BLAD is resistant to high temperatures and denaturing agents. No contact with metal or metal ions during storage. EP (20% BLAD) is stable for one year in high density polyethylene (HDPE) bottles at ambient temperature.	(dos Ramos, 1997), 48587901, 49474501
pH	830.7000	6.3 at 22.8°C (1% aqueous solution)	48587902
UV/Visible Light Adsorption	830.7050	Acidic solution (pH = 1.71): mean wavelength maxima = 206.2 and 260.0 nm, absorbance = 0.433 and 0.167 Neutral solution (pH = 5.97): mean wavelength maxima = 201.5 nm, absorbance = 0.613 Basic solution (pH = 12.38): mean wavelength maxima = 223.1 nm, absorbance = 0.689	48901201
Melting Point/Melting Range	830.7200	N/A, because the substance is a liquid.	--
Boiling Point/Boiling Range	830.7220	100°C	48901201
Density	830.7300	1.255 g/ml at 20°C	48587902

Table 4. Summary of Product Analysis Data (40 CFR § 158.2030)			
Data Requirement	**Guideline No.**	**Results / Findings**	**MRIDs**
Particle Size, Fiber Length, and Diameter Distribution	830.7520	N/A, because the substance is nonfibrous	--
Partition Coefficient	830.7550-.7570	N/A, because the substance is soluble in water.	--
Water Solubility	830.7840	Soluble in water (no value was provided)	48587902
Vapor Pressure	830.7950	< 23.8 torr at 25°C (based on Raoult's Law)	--

Appendix B – Summary of Mammalian Toxicology Data

Acceptable acute toxicology data are available to support the continued registration of BLAD. However, the scientific rationales for the subchronic and genotoxicity studies are considered unacceptable based upon the uncertainties identified during registration review regarding the degradation of BLAD and within the residue studies. In addition, there is uncertainty regarding the quantification of BLAD due to the lack of validation data submitted for the Enzyme Linked Immunosorbent Assay (ELISA).

Table 5 summarizes the current mammalian toxicology data requirements and results supporting registration review of BLAD.

Table 5. Summary of Toxicology Data (40 CFR § 158.2050)

Data Requirement	Guideline No.	Results / Findings	MRIDs
Acute Oral Toxicity - Rat	870.1100	LD_{50} > 5,000 mg/kg EP[1], Toxicity Category IV Acceptable	48587904
Acute Dermal Toxicity	870.1200	LD_{50} > 2,000 mg/kg EP[1] (the highest dose tested), Toxicity Category III Acceptable	48587905
Acute Inhalation Toxicity	870.1300	LC_{50} > 5.34 mg/L EP[1], Toxicity Category IV Acceptable	48587906
Primary Eye Irritation – Rabbit	870.2400	Slight eye irritation EP[1], Toxicity Category III Acceptable	48587907
Primary Dermal Irritation	870.2500	Mild to slight dermal irritation[1], Toxicity Category IV Acceptable	48587908
Dermal Sensitization	870.2600	Not a contact dermal sensitizer[1] Acceptable	48587909
Hypersensitivity Incidents	N/A	—	—
90-Day Oral (One Species)	870.3100	Rationale submitted based upon low acute oral toxicity, history of use as a food item (sweet lupine), non-toxic mechanism of action, rapid biodegradation, and all inerts have tolerance exemptions. Unacceptable due to uncertainties identified below.	48587910
90-Day Dermal – Rat	870.3250	Rationale submitted based upon low acute dermal toxicity, history of use as a food item (sweet lupine), non-toxic mechanism of action, rapid biodegradation, and all inerts have tolerance exemptions. Unacceptable due to uncertainties identified below.	48587911
90-Day Inhalation – Rat	870.3465	Rationale submitted based upon low acute inhalation toxicity, history of use as a food item (sweet lupine), non-toxic mechanism of action, rapid biodegradation, and all inerts have tolerance exemptions. Unacceptable due to uncertainties identified below.	48587912
Prenatal Developmental	870.3700	Rationale submitted based upon low acute toxicity, history of use as a food item (sweet lupine), non-toxic mechanism of action, rapid biodegradation, and all inerts have tolerance exemptions. Unacceptable due to uncertainties identified below.	48587913
Bacterial Reverse Mutation Test	870.5100	Rationale submitted based upon low acute toxicity, history of use as a food item (sweet lupine), non-toxic mechanism of action, rapid biodegradation, and all inerts have tolerance exemptions. Unacceptable due to uncertainties identified below.	48587914
In vitro Mammalian cell Gene Mutation Test	870.5300		
In vitro Mammalian Chromosome Abberation Test	870.5375		
Crop Field Trials	860.1000 860.1500	Magnitude of the Residue of BLAD in Strawberries following ProBLAD Plus applications.	49319401 49198301

		Unacceptable due to uncertainties identified in the data needs section	
Crop Field Trials	860.1000 860.1500	Magnitude and Decline of BLAD Residues Following 1X and 5X Applications of ProBLAD Plus to Cherries and Cucurbits. Unacceptable due to uncertainties identified in the data needs section	50149801
Crop Field Trials	860.1000 860.1500	Magnitude and Decline of BLAD Residues Following 1X and 5X Applications of ProBLAD Plus Fungicide to Apples. Unacceptable due to uncertainties identified in the data needs section	50307501
(Residue) Storage Stability Data	860.1380	Storage Stability of BLAD in 3 Crops under Deep Frozen Conditions. Supplemental but upgradable due to the lack of validation of the ELISA method	50567801

[1] Studies were conducted using the EP. Request to bridge data from the EP to BLAD (TGAI) is acceptable.

Human Health Risk Assessment

Acceptable acute toxicology data are available for BLAD; however, there are uncertainties regarding the degradation of BLAD within the residue studies and due to the lack of validation of the ELISA. In addition, there is uncertainty surrounding the allergenicity and purity of the active ingredient. An updated risk assessment will be required in order to evaluate a safety finding.

Following the initial registration and establishment of a tolerance exemption on March 22, 2013 (40 CFR 180.1319) (78 FR 17600) (FRL-9380-6) for BLAD, the Food and Drug Administration (FDA) raised concerns regarding the potential allergenicity of BLAD to lupine- and/or peanut-sensitive individuals. BLAD is a 173-amino acid fragment polypeptide derived from a larger seed storage protein, β-conglutin, found only in 4-to-14-day-old germinated seedlings of sweet white lupine (*Lupinus alba*). Amino acid similarities between β-conglutin in sweet white lupine (the precursor to BLAD), and AraH1, an allergen of peanut, were described in the original submissions. Lupine sensitive individuals have been identified as well as lupine sensitivity in peanut sensitive individuals upon consumption of lupine containing foods. The European Food Safety Authority (EFSA) has also listed lupines (including β-conglutin Lup-an1 and Lup-1) as food allergens and requires labelling of lupine content in processed foods (EFSA, 2005).

Following further examination of additional data submitted by the registrant in 2014 (i.e., residue magnitude and decline studies, as well as serum testing), the Agency was unable to definitively conclude that BLAD was not a potential allergen. As a result, in 2015 the Agency proposed to revoke the tolerance exemption for BLAD residues and to establish pesticide tolerances for almonds, grapes, strawberries, and tomatoes (80 FR 30640, May 29, 2015). The proposed crop-specific tolerances were to be based on data indicating BLAD residues were likely to be at or below the level of detection, 0.005 ppm, after crop treatment as detected via quantitative ELISA. However, some commenters objected to the 2015 proposed rule due to concerns about EPA's approach to set tolerances at the Level of Detection (LOD).

Upon further review of the 2015 proposal and in consideration of commenters' scientific concerns, in 2018, the Agency reexamined the safety of the BLAD tolerance exemption and evaluated new residue data submitted by the registrant on its own initiative. EPA conducted a qualitative safety assessment based on the lack of exposure to BLAD and concluded there will be negligible to no detectable residues of BLAD on treated crops.

In 2020, the Agency issued a re-proposal in order to clarify the 2015 proposed rulemaking, as well as to propose to establish tolerances for additional commodities requested by the registrant (85 FR 7698, Feb. 11, 2020). The Agency concluded that the available residue data and food processing information supported establishing numerical tolerances at the level of quantitation, 0.02 ppm, for residues of BLAD in or on almond; almond, hulls; fruit, pome, group 11-10; fruit, stone, group 12-12; grape; hops, dried cones; strawberry; vegetable, cucurbit, group 9; and vegetable, fruiting, group 8-10 (Memorandum from J. Kough to M. Adams, June 26, 2019). The rulemaking is pending and, if finalized as proposed, would result in the revocation of the tolerance exemption and establishment of tolerances for certain commodities. At this time, however, the tolerance exemption established in 2013 remains in place.

New crops were approved in 2019 via label amendment (i.e., cucurbit vegetables, fruiting vegetables, hops, pome fruits, and stone fruits), based upon the existing tolerance exemption. No new residue data were submitted.

In 2021, the Agency received an application for several new uses [tree nuts (crop group 14-12); brassica leafy greens (crop subgroup 4-16B); cherry (crop subgroup 12-12A); peach (crop subgroup 12-12B); plum (crop subgroup 12-12C); herb fresh leaves (crop subgroup 25A); coffee; herb dried leaves (crop subgroup 25B); leafy greens (crop subgroup 4-16A); melon (crop subgroup 9A); squash/cucumber (crop subgroup 9B); peanuts; pepper/eggplant (crop subgroup 8-10B); non-bell pepper/eggplant (crop subgroup 8-10C); bushberry (crop subgroup 13-07B); fruit vine climbing (crop subgroup 13-07F); low growing berry (crop subgroup13-07G); and tomato (croup subgroup 8-10A)]. The new uses were approved under the existing tolerance exemption in June 2022, via label amendment.

A data package consisting of a quantitative risk assessment (no new residue data) which utilized allergenic testing using sera from individuals with a sensitivity to lupines and/or peanuts was also submitted (MRID 49276602). EPA's Human Studies Rule (40 CFR 26, subparts K-Q) establishes the ethical standards for research involving intentional exposure of human subjects to any substance, as well as the conditions that must be met for EPA to rely on such research. The Human Studies Rule prohibits EPA from relying on research involving intentional exposure of human subjects to any substance for decisions under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) or the Federal Food, Drug, and Cosmetic Act (FFDCA) unless EPA and an independent advisory committee, the Human Studies Review Board, review the protocol for the research before the study begins. The skin prick component of the research involved exposing subjects to a substance to determine whether they were sensitive to lupines and/or peanuts. This constitutes research involving intentional exposure of human subjects under 40 CFR 26, Subpart K. The registrant did not submit the protocol to EPA for review as required by EPA's regulation. Therefore, under the Human Studies Rule, EPA is prohibited from relying on the results of the research submitted.

Section 408(c)(2)(A)(i) of FFDCA allows EPA to establish or leave in effect an exemption from the requirement of a tolerance for pesticide chemical residues (the legal limit for a pesticide chemical residue in or on a food) only if EPA determines that the exemption is "safe." Section 408(c)(2)(A)(ii) of FFDCA defines "safe" to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." In order for the Agency to continue to support a safety finding for BLAD, uncertainties regarding the residue data, quantitative ELISA and product identity/analysis of impurities must be addressed so that the Agency can update the FFDCA portion of the human health risk assessment. Failure to adequately address the uncertainties described in detail within the **Summary of Registration Review Human Health Data Needs** section below may result in

the revocation of the exemption and establishment of tolerances for certain commodities, in the event that the safety finding under FFDCA cannot be supported.

Hazard Characterization

The toxicological database is considered complete for characterizing hazard from the active ingredient in terms of acute toxicity. Banda de *Lupinus albus* doce (BLAD) can be classified as Toxicity Category IV for acute oral and acute inhalation; Toxicity Category III for eye irritation and acute dermal, and Toxicity Category IV for primary dermal irritation (U.S. EPA, 2012). A rationale for lack of toxicity was submitted for the subchronic 90-day oral (870.3100), the 90-day dermal (870.3250) and the 90-day inhalation (870.3465) data requirements in lieu of guideline data. At the time of the original registration in 2013, the Agency determined that the rationale was acceptable based on the EP toxicological profile, summarized as follows:

- BLAD has a history of use in human and animal nutrition as a food and feed item (sweet lupines).
- BLAD has a non-toxic mode of action and is rapidly biodegraded, thus minimizing potential for toxic risk.
- The results of the submitted acute toxicity studies demonstrate that the product has very low acute oral, inhalation, and contact toxicity.

The same rationale was submitted to satisfy the developmental toxicity (870.3700), the bacterial reverse mutation test (870.5100), the *in vitro* mammalian cell gene mutation test (870.5300), and the *in vitro* mammalian chromosome aberration test (870.5375). Further detail on the rationales submitted to satisfy these subchronic data requirements can be found in Table 5. The rationales for the subchronic and genotoxicity studies are now considered unacceptable based upon the uncertainties identified during registration review regarding the degradation of BLAD and within the residue studies (see dietary exposure section below for details). In addition, there is uncertainty regarding the accuracy of the quantification of BLAD due to the lack of validation data submitted for the ELISA. Furthermore, uncertainty has been identified regarding the product identity/analysis of impurities warranting additional review regarding the allergenicity of this product. These uncertainties and resulting data needs have been outlined in the **Summary of Registration Review Human Health Data Needs** section below. These data are required to fully evaluate hazard in support of an updated FFDCA assessment.

Dietary Exposure and Risk Characterization

Uncertainties regarding the residue data, ELISA method validation, product identity/analysis of impurities, and environmental fate (e.g., biodegradation) for BLAD warrant additional review so that the Agency can update the human health risk assessment (see the **Summary of Registration Review Human Health Data Needs** section below). In order to address allergenicity concerns for dietary exposure, the Agency must be able to conclude that the residue samples tested to support the tolerance exemption were not already degraded prior to analysis due to improper sample handling (i.e., lack of cold chain, improper storage, repeat freeze-thaw events) and that the quantitative ELISA is sufficiently sensitive to detect BLAD at the limit of quantitation. For example, if the samples were degraded upon analysis and/or the method of detection is not sufficiently sensitive, residues may have been under quantified, thus underestimating actual exposure.

In the process of this registration review, the Agency also revisited the data submitted to demonstrate BLAD's biodegradability and determined that there is some uncertainty regarding BLAD's degradation in soils because the study submitted to determine biodegradability is not directly applicable to

degradation in the natural environment. In a ready biodegradability bottle study BLAD was easily degraded by environmental microbes (65.9% degradation within 4 days; MRID 48587917). However, this study is not equivalent to a soil degradation study and should not be utilized to satisfy the aerobic soil (or aquatic) metabolism study requirement due to the disparity between the study conditions. For example, the inoculum in a ready biodegradability study (bottle study) is activated sludge, whereas the aerobic soil metabolism study uses soil (ideally 4 different soil types). Activated sludge has much higher organic carbon (OC) content (~30-40%; Giovannini *et al*., 2008) compared to soil, which typically has 1-6% OC. Other properties of sludge can also differ from soil including pH, clay particle size, sorption behavior, etc. Thus, the biodegradation behavior of an active ingredient can differ in a ready biodegradability test compared to soil metabolism studies.

Studies conducted to examine the catabolism of seed storage proteins from *Lupinus albus* demonstrate that β-conglutin is subject to degradation by endopeptidases and exopeptidases in the lupine during germination and seedling growth in a stepwise manner (Ferreira *et al*., 1995). The first step occurs at the onset of germination and involves the *de novo* synthesis of an endopeptidase (proteinase A) which catalyzes limited proteolysis of the insoluble storage proteins, converting them to soluble peptides. In this way, the modified globulins become susceptible to the action of proteinase B, carboxypeptidases, aminopeptidases and dipeptidases, which are unable to attack the native proteins of ungerminated seeds but readily convert the soluble peptides into amino acids (Miintz *et al*. 1985; Shutov and Vaintraub, 1987). This is not considered equivalent to demonstrating BLAD's susceptibility to gastric proteases, and no proteolytic activity was detected in the study at pH 3.5 over the period of time studied. However, considerable proteolytic activity in the extracts prepared from dry seeds was observed at both at pH 5.5 and 7.5 (Ferreira *et al*., 1995). Therefore, there remains uncertainty regarding the susceptibility of BLAD to gastric proteases.

Due to uncertainties identified in the process of registration review, additional studies are needed to evaluate potential risks due to dietary exposure. Failure to adequately address the uncertainties described in detail within the Anticipated Data Needs section below may result in the revocation of the tolerance exemption and establishment of tolerances for certain commodities.

Residential and Non-Occupational Exposure and Risk Characterization

Exposure to BLAD should be minimal in residential and non-occupational settings when used according to the label instructions and because the products containing this active ingredient are not registered for residential use, only for commercial ornamentals and agricultural crops. The labels restrict spray application when windspeeds favor drift beyond the area intended for treatment. The label does not include droplet size restrictions for aerial application, which would further mitigate potential for non-occupational exposure via spray drift. Residential and non-occupational exposure and risk characterization will be updated when the uncertainties identified in the data needs sections are addressed.

Occupational Exposure and Risk Characterization

Occupational exposure to BLAD should be mitigated by PPE requirements included on the labels for applicators and handlers. Hazard due to toxicity of BLAD is not expected due to its low toxicity for the acute endpoints measured, therefore risk due to the toxicity resulting from occupational exposure to BLAD is not expected. However, due to uncertainties regarding the residue data, product identity/analysis of impurities, ELISA method validation, and environmental fate (e.g., biodegradation), additional data is needed to allow the Agency to assess if there is a potential for risk concerns related to

occupational exposure and allergenicity. Therefore, risk due to occupational exposure is currently uncertain based upon currently available data.

Overall Human Health Risk Characterization

Uncertainties have been identified during registration review regarding the degradation of BLAD and within the residue studies. In addition, there is uncertainty regarding the accuracy of the quantification of BLAD due to the lack of validation data submitted for the ELISA. Furthermore, uncertainty has been identified regarding the product identity/analysis of impurities warranting additional concern regarding the allergenicity of this product. These uncertainties and resulting data needs have been outlined in the ***Anticipated Data Needs*** section. These data are required in order to fully evaluate hazard in support of an updated FFDCA assessment. Failure to adequately address the uncertainties identified in this review may preclude the Agency from continuing to support a safety finding for BLAD under FFDCA and may therefore result in the revocation of the tolerance exemption and establishment of tolerances for certain commodities.

Summary of Registration Review Human Health Data Needs

Additional data and information are required for an updated human health risk assessment, as described below. Specifically, there are data needs regarding the environmental degradation rate/fate, residue data, sample handling, and validation of the Enzyme Linked Immunosorbent Assay (ELISA) used to measure the residues. There is also uncertainty regarding product identity/analysis of impurities. These data need to be addressed in order to make an updated safety finding, given the potential allergenicity of BLAD to lupine- and/or peanut-sensitive individuals.

Deficiencies Identified with Existing Residue Data:

A re-examination of the residue data on file revealed several uncertainties regarding the integrity of the residue samples upon analysis. For example, it appears that freeze-thaw events may have affected the integrity of the protein itself and/or the matrix, resulting in reduced extraction efficiency and/or signal interference, thus underestimating the actual residue concentrations that occurred at sampling. The various uncertainties regarding sample handling and storage stability of BLAD residues are as follows:

Agency concerns regarding MRIDs 49319401 and 49198301 (Grapes, tomatoes, strawberries)

p.19 of MRID 49319401: Samples and Handling

Grape, strawberry, and tomato residue samples were stored frozen at the field laboratory facilities after collection. All grape and tomato samples were shipped by freezer truck to the Eurofins processing laboratory facility in Forsyth, GA; and then onto Residue Analysis Laboratory in New Brunswick, NJ. All strawberry samples were shipped on dry ice and samples were received frozen at the laboratory within 29 days of shipment, and then stored in a freezer (<-18°C) when not in use.

- The Agency is unable to validate that cold chain was maintained during this 29-day transit period as no temperature logs were provided with the study, which is a deviation from OPPTS 860.1000. Cold chain documentation is required to use these residue studies for extrapolation and freeze thaw events must be addressed in a rationale that describes the likelihood that these cycles affected the integrity of the protein and its subsequent measurements in the ELISA.
- The average recovery, as shown in Table 2 (MRID 49319401) was around 50% for the crops tested, however the reason for this low recovery rate was not addressed. The recoveries for the spiked samples (Table 5, MRID 49319401) were similarly low suggesting a potential matrix

impact on the extraction efficiency. The Agency has concern that this may have resulted from the fact that the samples were in transit for over 3 weeks without documentation of a cold chain. It is unclear whether or not the samples were corrected for percent of recovery. The registrant will need to clarify and adjust the calculations as appropriate, such that the analytical value is optimally reflective of the residues as collected from the field.

Agency concerns regarding MRID 50149801 (Cherries and cucumbers) and MRID 50307501 (Apples)

Samples were homogenized in Lancaster, PA and then shipped to the United Kingdom three months later and were in transit for two days. There is no record of the cold chain maintenance. Samples were in storage for 143-146 days prior to extraction. The author cites a pending storage stability study to support the storage time, however the study spiked samples with BLAD prior to storage so this does not reflect potential sample degradation that may have occurred prior to storage during transit and repeat freeze-thaw events during processing. In addition, Stability to Normal and Elevated Temperatures, Metals, and Metal Ions Guideline 860.6313 is not an appropriate study to evaluate Residue Storage Stability as per Guideline 860.1380. Cold chain documentation is required to use these residue studies for extrapolation and freeze-thaw events must be addressed in a rationale that describes the likelihood that these cycles affected the integrity of the protein and its subsequent measurements in the ELISA.

ELISA Validation:

While MRID 49319401 includes a brief overview of the analytical method, it utilizes OPPTS 860.1000 which was written for analytical chemistry methods and so it does not cover all that is needed to validate a quantitative ELISA. The table below lists the experiments the Agency needs in order to assess the validity of the ELISA method used by the registrant to quantify BLAD. The specificity description should address whether or not the assay distinguishes between BLAD and the other proteins present in the product.

Table 6. Summary of BLAD ELISA Method Validation Missing Criterion

Example Experiment	Example Acceptability Measurement	Comment
Bioinformatics analysis to determine homology with other sequences on β-conglutin protein	NA	Can be used to inform which proteins to target in the specificity-cross reactivity assessment
Specificity-Cross reactivity assessment for applicable proteins	Purified test protein at 1 ug/ml mean optical density (OD)<2 times the assay background	Assess binding of the target to other portions of the β-conglutin protein
Specificity-Interference for applicable proteins in presence or absence of BLAD protein	Interpolate the result of the tested protein at 1 ug/ml combined with BLAD protein is < 20% relative to BLAD protein alone	—
Dilution agreement assessment using multiple samples of positive samples	%CV is ≤ 25% for positive sample results across analysts and days	Measures inter-assay variability
Extraction efficiency measuring BLAD protein by ELISA serially extracted positive samples.	>70% protein is extracted by method.	Extraction efficiency should be used to correct for final concentrations obtained.

NA- not applicable

Product Identity Human Health Concerns:

The active substance, aqueous extract (BLAD Technical) from the germinated seeds of sweet *Lupinus albus*, is considered a UVCB substance (Substance of Unknown or Variable composition, Complex reaction product or Biological material, EFSA 2020). The technical material is only a hypothetical stage

in the continuous production process of the end-use product (EFSA, 2020). BLAD is formed during germination of the sweet lupine seed as ß-conglutin undergoes a cycle of limited proteolysis in which many of its constituent subunits are processed into this 20 kDa polypeptide (Monteiro et al; 2010). BLAD is the predominant, but not sole peptide resulting from this process. ß-conglutin is a known food allergen and shares high sequence homology with (<35%) with soy, peanut, and pecan allergens. Based upon available information, the Agency has concerns that other potential allergenic proteins may be present in the product given that BLAD is derived from the full length ß-conglutin which is known to bind to IgE in allergic individuals. Therefore, the Agency is requesting that the company sequence proteins in the product that are not BLAD and perform an *in silico* bioinformatics analysis using these proteins to address allergenicity.

Literature Search Findings

To support registration review, the Biopesticides and Pollution Prevention Division (BPPD) conducts searches of the literature and incident databases to determine if there are any reports of adverse effects that might change risk conclusions or change knowledge of the state of the science for BLAD. To this end a literature search was conducted for BLAD utilizing the search engines Google Scholar and PubMed. Searches conducted for BLAD are described below.

Human Health Results:

A literature search was conducted with Google Scholar and the PubMed search engines using the terms "Banda de *Lupinus albus* doce" and "health." These terms yielded a total of 24 results, none of which were related to adverse impacts upon humans or mammals. A search was also performed using the term "Banda de *Lupinus albus* doce" and the search terms "toxicity," "toxin," "allergen", "allergenicity" and "adverse" which, returned 10, 5, 2, 0, and 6 results, respectively. None of the documents were related to adverse impacts to humans or mammals.

A literature search was conducted with Google Scholar and the PubMed search engines using the terms "Banda de *Lupinus albus* doce" and "endocrine disruption." These terms yielded one result titled "Peer review of the pesticide risk assessment of the active substance aqueous extract from the germinated seeds of sweet Lupinus albus" (EFSA, 2020). The authors concluded that "The aqueous extract from the germinated seeds of sweet *Lupinus albus* does not meet the criteria for endocrine disruption for humans and non-target organisms according to points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605." No additional information was gained from these searches that would alter the BPPD's understanding of the current state of the science for any potential effects of BLAD on humans. The literature that came up in the searches primarily related BLAD's lack of toxicity and benefits as being a biopesticide. The results which returned for BLAD and the search term "allergen" did not specifically refer to BLAD but the larger family of vicilin seed storage proteins.

Appendix C – Summary of Nontarget Organism Data

The available ecological toxicity data and rationales for BLAD were considered acceptable at the time of the original registration and are summarized in Table 7 below. The submitted rationales relied heavily on limited exposure due to "rapid biodegradability". However, in the process of this registration review the Agency revisited the study (MRID 48587917) underlying rapid biodegradability of BLAD and found the data/results were not applicable to outdoor settings (refer to **Appendix B- Dietary Exposure and Risk Characterization** section for details). The submitted rationales are therefore no longer adequate to support avian, freshwater fish, aquatic invertebrate, or terrestrial plant toxicity data requirements because the persistence of BLAD in the environment is uncertain. To help address this uncertainty and support the rationale for low exposure and risk to birds, freshwater fish, aquatic invertebrates, and terrestrial plants, environmental fate data on aerobic soil and aquatic metabolism (835.4100 and 835.4300) are needed. These data will support both the human health and ecological risk assessments as they will help determine if BLAD rapidly degrades in environmental compartments under more realistic conditions. If BLAD's rapid degradation in natural settings can be confirmed with new data, this supports previous conclusions concerning rationales for nontarget organism toxicity. If the requested fate data suggest longer durations of exposure than originally assumed, data will be needed for avian acute oral toxicity, fish acute toxicity, aquatic invertebrate acute toxicity, and terrestrial plant toxicity (seedling emergence and seedling growth, and vegetative vigor). In the absence of the environmental fate and contingent nontarget organism toxicity data, highest toxicity, maximum residue levels, and allergenicity may be assumed for the active ingredient and end-use products.

Table 7 Summary of Nontarget Organism Data (40 CFR § 158.2060)

Data Requirement	Guideline No.	Results / Findings	MRIDs
Honeybee Acute Contact Toxicity Test	850.3020	Oral: LD_{50} (48 h) > 109.42 µg active ingredient (a.i.)/bee (practically non-toxic) Contact toxicity: LD_{50} (48 h) > 100 µg a.i./bee (practically non-toxic) ACCEPTABLE/GUIDELINE	48587916
Assessment of Side Effects on the Activity of the Soil Microflora	OECD 216 and 217 (2000)	No adverse effect on short-term respiration and nitrogen turnover after 28 days exposure to a concentration of 10.4 mg a.i./kg soil dry weight ACCEPTABLE/GUIDELINE	48587918
Toxicity to the Aphid Parasitoid, *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae), in the Laboratory - Rate Response Test	N/A	No effects on mortality or reproduction at application rates up to 2.36 lbs a.i./acre, the highest rate tested. ACCEPTABLE/NON-GUIDELINE	48587919
Acute Toxicity on Earthworms, *Eisenia fetida*, using an Artificial Soil Test	OECD 207 (1984)	LC_{50} > 200 mg a.i./kg soil dry weight ACCEPTABLE/GUIDELINE	48587920
Acute Toxicity on Earthworms, *Eisenia fetida*, using an Artificial Soil Test	OECD 207 (1984)	LC_{50} > 200 mg a.i./kg soil dry weight ACCEPTABLE/GUIDELINE	48587920
Avian Acute Oral Toxicity	850.2100	Addressed with rationales based on BLAD's natural occurrence, being a protein fragment in food and feed item (sweet lupines), and low acute oral toxicity in rats (LD50 > 5,000 mg/kg, Toxicity Category IV).	48587921

Table 7 Summary of Nontarget Organism Data (40 CFR § 158.2060)			
Data Requirement	**Guideline No.**	**Results / Findings**	**MRIDs**
		UNACCEPTABLE based on lack of supporting data for rapid degradation	
Avian Dietary Toxicity	850.2200	Addressed with rationales based on BLAD's natural occurrence, being a protein fragment in food and feed item (sweet lupines), and low acute oral toxicity in rats (LD50 > 5,000 mg/kg, Toxicity Category IV). UNACCEPTABLE based on lack of supporting data for rapid degradation	48587921
Fish Acute Toxicity, Freshwater	850.1075	Addressed with rationales based on BLAD's natural occurrence, being a protein fragment in food and feed item (sweet lupines), and lack of observed adverse effects on the soil microflora, aphid parasitoids (*Aphidius rhopalosiphi*), earthworms (*Eisenia fetida*), and honeybees (*Apis mellifera*). UNACCEPTABLE based on lack of supporting data for rapid degradation	48587922
Aquatic Invertebrate Acute Toxicity, Freshwater	850.1010	Addressed with rationales based on BLAD's natural occurrence, being a protein fragment in food and feed item (sweet lupines), and lack of observed adverse effects on the soil microflora, aphid parasitoids (*Aphidius rhopalosiphi*), earthworms (*Eisenia fetida*), and honeybees (*Apis mellifera*). UNACCEPTABLE based on lack of supporting data for rapid degradation	48587922
Terrestrial Plant Toxicity, Seedling Emergence and Seedling Growth	850.4100	Addressed with rationales based on BLAD's natural occurrence. UNACCEPTABLE based on lack of supporting data for rapid degradation	48587923
Terrestrial Plant Toxicity, Vegetative Vigor	850.4150	Addressed with rationales based on BLAD's natural occurrence. UNACCEPTABLE based on lack of supporting data for rapid degradation	48587923

LD_{50} = median lethal dose.

Environmental Risk Assessment

Nontarget organism toxicity and environmental fate data and rationales necessary to meet the standard for BLAD were found acceptable at the time of the original registration. BLAD was practically nontoxic to honeybees (*Apis mellifera*) with an oral LD_{50} (48 h) > 109.42 µg a.i./bee and a contact LD_{50} (48 h) > 100 µg a.i./bee (MRID 48587916). The rest of the Tier I nontarget organism data requirements were addressed with scientific rationales (MRIDs 48587921 – 48587923), which were based on BLAD's natural occurrence, being a protein fragment in food and feed items (sweet lupines), low acute oral toxicity in rats (LD_{50} > 5,000 mg/kg, Toxicity Category IV), rapid biodegradability and therefore limited exposure, and lack of observed adverse effects on the soil microflora (no adverse effect on short-term respiration and nitrogen turnover after 28 days exposure to a concentration of 10.4 mg a.i./kg soil dry wight, MRID 48587918), aphid parasitoids (*Aphidius rhopalosiphi*, no adverse effects on mortality or

reproduction at application rates up to 2.36 lbs a.i./acre, the highest rate tested, MRID 48587919), earthworms (*Eisenia fetida*, LC_{50} > 200 mg a.i./kg soil dry weight, MRID 48587920), and honeybees (*Apis mellifera*, oral LD_{50} (48 h) > 109.42 µg a.i./bee, MRID 48587916).

The submitted rationales at the time of the original registration relied heavily on limited exposure due to "rapid biodegradability". However, in the process of this registration review the Agency revisited the study (MRID 48587917) underlying rapid biodegradability of BLAD and found the data/results were not applicable to outdoor settings (refer to **Appendix B-Dietary Exposure and Risk Characterization** section for details). The submitted rationales are therefore no longer adequate to support avian, freshwater fish, aquatic invertebrate, or terrestrial plant toxicity data requirements because the persistence of BLAD in the environment is uncertain.

To help address this uncertainty and support the rationale for low exposure and risk to birds, freshwater fish, aquatic invertebrates, and terrestrial plants, environmental fate data on aerobic soil and aquatic metabolism (835.4100 and 835.4300) are needed. These data will support both the human health and ecological risk assessments as they will help determine if BLAD rapidly degrades in environmental compartments under more realistic conditions. If BLAD's rapid degradation in natural settings can be confirmed with new data, this supports previous conclusions concerning rationales for nontarget organism toxicity. If the requested fate data suggest longer durations of exposure than originally assumed, data will be needed for avian acute oral toxicity, fish acute toxicity, aquatic invertebrate acute toxicity, and terrestrial plant toxicity (seedling emergence and seedling growth, and vegetative vigor). Additional details on the environmental fate and contingent nontarget organism studies requested for this registration review are listed in Table 2.

The Agency will conduct an ecological risk assessment of BLAD, which will include an endangered species assessment, once new data regarding the persistence of BLAD in the environment have been submitted.

Summary of Registration Review Environmental Data Needs

Additional environmental fate and ecotoxicity data are needed for an updated ecological risk assessment, as described below.

Environmental Fate and Ecotoxicity Data:

Upon re-examination of the environmental fate data submitted to support BLAD's biodegradability, the Agency has determined that there is uncertainty regarding the applicability of the ready biodegradability bottle study (MRID 48587917) to degradation in the natural environment. Due to differences in study condition and the inoculum used (activated sludge vs. soil), the results of the ready biodegradability study should not be utilized to satisfy the aerobic soil (or aquatic) metabolism study requirement. Therefore, the submitted rationales are no longer adequate to support avian, freshwater fish, aquatic invertebrate, or terrestrial plant toxicity data requirements because the persistence of BLAD in the environment is uncertain. To help address this uncertainty and support the rationale for low exposure and risk to birds, freshwater fish, aquatic invertebrates, and terrestrial plants, environmental fate data on aerobic soil and aquatic metabolism (835.4100 and 835.4300) are needed. These data will support both the human health and ecological risk assessments as they will help determine if BLAD rapidly degrades in the environment under more realistic conditions. If BLAD's rapid degradation in natural settings can be confirmed with new data, this supports previous conclusions concerning rationales for nontarget organism toxicity. If the requested fate data suggest longer durations of exposure than originally assumed, data will be needed for avian acute oral toxicity, fish acute toxicity, aquatic invertebrate acute

toxicity, and terrestrial plant toxicity (seedling emergence and seedling growth, and vegetative vigor) in order to assess risk to these taxa.

Literature Search Findings

To support registration review, BPPD conducts searches of the literature and incident databases to determine if there are any reports of ecological adverse effects that might change risk conclusions or change knowledge of the state of the science for BLAD. Searches conducted for BLAD are described below.

Ecological results:

Searches were conducted using PubMed and Google Scholar databases with terms "Banda de *Lupinus albus* doce"/ "BLAD" and "toxicity", "Banda de *Lupinus albus* doce"/ "BLAD" and "ecotoxicity", or "Banda de *Lupinus albus* doce"/ "BLAD" and "non-target" and "toxicity". These searches returned 0 relevant results.

No additional conclusive information was gained from these searches that would alter the BPPD's understanding of the current state of the science for any potential effects of BLAD on nontarget organisms.

Appendix D – Endocrine Disruptor Screening Program (EDSP)

The Federal Food Drug and Cosmetic Act (FFDCA) §408(p) requires EPA to develop a screening program to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." (21 U.S.C. 346a(p)). In carrying out the Endocrine Disruptor Screening Program (EDSP), FFDCA section 408(p)(3) requires that EPA "provide for the testing of all pesticide chemicals," which includes "any substance that is a pesticide within the meaning of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), including all active and pesticide inert ingredients of such pesticide." (21 U.S.C. 231(q)(1) and 346a(p)(3)). However, FFDCA section 408(p)(4) authorizes EPA to, by order, exempt a substance from the EDSP if the EPA "determines that the substance is anticipated not to produce any effect in humans similar to an effect produced by a naturally occurring estrogen." (21 U.S.C. 346a(p)(4)).

The EDSP initiatives developed by EPA in 1998 includes human and wildlife testing for estrogen, androgen, and thyroid pathway activity and employs a two-tiered approach. Tier 1 consists of a battery of 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid pathways. Tier 2 testing is designed to identify any adverse endocrine-related effects caused by the substance and establish a dose-response relationship for any adverse estrogen, androgen, or thyroid effect. If EPA finds, based on that data, that the pesticide has an adverse endocrine-related effect on humans, FFDCA § 408(p)(6) also requires EPA, "… as appropriate, [to] take action under such statutory authority as is available to the Administrator … as is necessary to ensure the protection of public health." (21 U.S.C. 346a(p)(6))[10].

Between October 2009 and February 2010, EPA issued Tier 1 test orders/data call-ins (DCIs) for its first list of chemicals ("List 1 chemicals") for EDSP screening and subsequently required submission of EDSP Tier 1 data for a refined list of these chemicals. EPA received data for 52 List 1 chemicals (50 pesticide active ingredients and 2 inert ingredients). EPA scientists performed weight-of-evidence (WoE) analyses of the submitted EDSP Tier 1 data and other scientifically relevant information (OSRI) for potential interaction with the estrogen, androgen, and/or thyroid signaling pathways for humans and wildlife.[11]

In addition, for FIFRA registration, registration review, and tolerance-related purposes, EPA collects and reviews numerous studies to assess potential adverse outcomes, including potential outcomes to endocrine systems, from exposure to pesticide active ingredients. Although EPA has been collecting and reviewing such data, EPA has not been explicit about how its review of required and submitted data for these purposes also informs EPA's obligations and commitments under FFDCA section 408(p). Consequently, on October 27, 2023, EPA issued a Federal Register Notice (FRN) providing clarity on the applicability of these data to FFDCA section 408(p) requirements and near-term strategies for EPA to further its compliance with FFDCA section 408(p). This FRN, entitled *Endocrine Disruptor Screening Program (EDSP): Near-Term Strategies for Implementation' Notice of Availability and Request for Comment* (88 FR 73841) is referred to here as EPA's EDSP Strategies Notice. EPA also published three documents supporting the strategies described in the Notice:

[10] For additional details of the EDSP, please visit https://www.epa.gov/endocrine-disruption.
[11] Summarized in *Status of Endocrine Disruptor Screening Program (EDSP) List 1 Screening Conclusions*; EPA-HQ-OPP-2023-0474-0001; https://www.regulations.gov/document/EPA-HQ-OPP-2023-0474-0001

- *Use of Existing Mammalian Data to Address Data Needs and Decisions for Endocrine Disruptor Screening Program (EDSP) for Humans under FFDCA Section 408(p)*;
- *List of Conventional Registration Review Chemicals for Which an FFDCA Section 408(p)(6) Determination is Needed;* and,
- *Status of Endocrine Disruptor Screening Program (EDSP) List 1 Screening Conclusions* (referred to here as List 1 Screening Conclusions).

The EDSP Strategies Notice and the support documents are available on www.regulations.gov in docket number EPA-HQ-OPP-2023-0474. As explained in these documents, EPA is prioritizing its screening for potential impacts to the estrogen, androgen, and thyroid systems in humans, focusing first on conventional active ingredients. Although EPA voluntarily expanded the scope of the EDSP to screening for potential impacts to the estrogen, androgen, and thyroid systems in wildlife, EPA announced that it is not addressing this discretionary component of the EDSP at this time, considering its current focus on developing a comprehensive, long-term approach to meeting its Endangered Species Act obligations (See EPA's April 2022 ESA Workplan[12] and November 2022 ESA Workplan Update[13]). However, EPA notes that for 35 of the List 1 chemicals (33 active ingredients and 2 inert ingredients), Tier 1 WoE memoranda[14] indicate that available data were sufficient for FFDCA section 408(p) assessment and review for potential adverse effects to the estrogen, androgen, or thyroid pathways for wildlife. For the remaining 17 List 1 chemicals, Tier 1 WoE memoranda made recommendations for additional testing. EPA expects to further address these issues taking into account additional work being done in concert with researchers within the EPA's Office of Research and Development (ORD).

As discussed in EPA's EDSP Strategies Notice and supporting documents, EPA will be using all available data to determine whether additional data are needed to meet EPA's obligations and discretionary commitments under FFDCA section 408(p). For some conventional pesticide active ingredients, the toxicological databases may already provide sufficient evaluation of the chemical's potential to interact with estrogen, androgen, and/or thyroid pathways and EPA will generally not need to obtain any additional data to reevaluate those pathways, if in registration review, or to provide an initial evaluation for new active ingredient applications. For instance, EPA has endocrine-related data for numerous conventional pesticide active ingredients through either a two-generation reproduction toxicity study performed in accordance with the current guideline (referred to here as the updated two-generation reproduction toxicity study; OCSPP 870.3800 - Reproduction and Fertility Effects) or an extended one-generation reproductive toxicity (EOGRT) study (OECD Test Guideline 443 - Extended One-Generation Reproductive Toxicity Study). In these cases, EPA expects to make FFDCA 408(p)(6) decisions for humans without seeking further estrogen or androgen data. However, as also explained in the EPA's EDSP Strategies Notice, where these data do not exist, EPA will reevaluate the available data for the conventional active ingredient during registration review to determine what additional data, if any, might be needed to confirm EPA's assessment of the potential for impacts to estrogen, androgen, and/or thyroid pathways in humans. For more details on EPA's approach for assessing these endpoints, see EPA's EDSP Strategies Notice and related support documents.

[12] https://www.epa.gov/system/files/documents/2022-04/balancing-wildlife-protection-and-responsible-pesticide-use_final.pdf
[13] https://www.epa.gov/system/files/documents/2022-11/esa-workplan-update.pdf
[14] https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-screening-determinations-and

Also described in the EPA's EDSP Strategies Notice is a framework that represents an initial approach by EPA to organize and prioritize the large number of conventional pesticides in registration review. For conventional pesticides with a two-generation reproduction toxicity study performed under a previous guideline (i.e., an updated two-generation reproduction toxicity study or an EOGRT is not available), EPA has used data from the Estrogen Receptor Pathway and/or Androgen Receptor Pathway Models to identify a group of chemicals with the highest priority for potential data collection (described in EPA's EDSP Strategies Notice as Group 1 active ingredients). For these cases, although EPA has not reevaluated the existing endocrine-related data, EPA has sought additional data and information in response to the issuance of EPA's EDSP Strategies Notice to better understand the positive findings in the ToxCast™ data for the Pathway Models and committed to issuing DCIs to require additional EDSP Tier 1 data to confirm the sufficiency of data to support EPA's assessment of potential adverse effects to the estrogen, androgen, and/or thyroid pathways in humans and to inform FFDCA 408(p) data decisions. For the remaining conventional pesticides (described in EPA's EDSP Strategies Notice as Group 2 and 3 conventional active ingredients), EPA committed to reevaluating the available data to determine what additional studies, if any, might be needed to confirm EPA's assessment of the potential for impacts to endocrine pathways in humans.

Although EPA has prioritized conventional active ingredients as presented in EPA's EDSP Strategies Notice, EPA is planning to develop similar strategies for biopesticide and antimicrobial pesticide (*i.e.*, nonconventional) active ingredients and will provide public updates on these strategies, when appropriate. At this time, EPA is making no findings associated with the implementation of EDSP screening of BLAD. Such issues will be addressed in future updates by EPA on its strategies for implementing FFDCA section 408(p).

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