

The first RNAi-based biopesticide approved in the United States: regulatory implications for the European Union – the case of Ledprona (*Calantha*TM)

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Abstract

RNA interference (RNAi), a naturally occurring gene-silencing mechanism, has emerged as a promising platform for developing innovative, non-genetically modified (GM) plant protection products. In the context of climate change, biodiversity loss, and growing resistance to traditional pesticides, the need for highly specific, environmentally compatible alternatives for sustainable pest management is increasing. RNAi-based sprays are a novel approach in which double-stranded RNA (dsRNA) molecules silence essential genes in target pests without acting like toxic conventional compounds. A significant advancement in this technology occurred with the 2023 approval of *Calantha*TM (Ledprona) in the USA, marking the first authorization of an RNAi-based biopesticide for agricultural use. Ledprona targets the Colorado potato beetle by silencing specific genes following foliar dsRNA application. Here, we summarize the mechanism of action, efficacy, environmental behavior, and safety assessment of Ledprona as evaluated by the US Environmental Protection Agency. In addition, we discuss the broader regulatory implications of this approval and highlight key challenges and opportunities for assessing and implementing RNAi-based plant protection products within the European Union's regulatory framework.

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1 INTRODUCTION

Modern plant production faces a multitude of complex challenges: climate change and its consequences, biodiversity loss, and rapid population growth are placing increasing pressure on agricultural systems worldwide. At the same time, international and European sustainability strategies require that integrated pest management (IPM) contributes more to achieving environmental goals in the future. Society is also increasingly calling for alternative plant protection products that minimize risks to the environment and human health. Scientific studies further confirm the link between the use of conventional plant protection products and biodiversity loss, as well as the growing problem of resistance to active substances among target pests. These developments highlight the urgent need for innovative technologies that are capable of reducing overall pesticide input while maintaining effective pest control and limiting unintended environmental effects to complement sustainable IPM measures.

One promising alternative is RNA interference (RNAi) technology. RNAi is a naturally occurring biological mechanism in which small RNA molecules, known as small interfering RNAs, specifically inhibit the expression of target genes in the cell (video RNAi; www.plantrna.org). The first evidence of such gene-silencing

phenomena was reported in petunias as early as 1990 and described as 'co-suppression'.^{1,2} The molecular principle of RNAi was later elucidated in *Caenorhabditis elegans*, a discovery for which Fire *et al.*³ were awarded the Nobel Prize in 2006. Since then, RNAi has become firmly established in both biotechnology and medicine. A prominent example from medicine is the RNAi-based therapeutic Patisiran (Onpattro®), which received regulatory approval in 2018.⁴ These developments demonstrate that double-stranded RNA (dsRNA)-based interventions can be assessed within established regulatory frameworks when supported by appropriate mechanistic, pharmacokinetic, and toxicological data. RNAi technology is also opening new perspectives in plant protection.

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2 RNAi-BASED PLANT PROTECTION AS AN INNOVATIVE ALTERNATIVE

In agriculture, RNAi technology has emerged as an effective method for pest control, primarily because of its high degree of specificity and selectivity. RNAi-based agents bind specifically to selected messenger RNAs (mRNAs) of harmful organisms and thus prevent the synthesis of necessary proteins in a sequence-specific manner, either by cleaving the corresponding mRNA or by suppressing translation, thereby limiting pest survival. Initial regulatory milestones have already been achieved. For example, the European Food Safety Agency has evaluated RNAi-related applications, including the genetically modified maize SmartStax® PRO.⁵ However, given the limited public acceptance of genetically modified (GM) plants, especially in Germany and throughout Europe, the development of non-GM RNAi applications is therefore a priority. Research on RNAi has spanned more than two decades and has yielded notable advances, particularly in its application as an 'RNA spray'.⁵ For more than ten years, non-GM strategies have been developed in which RNA molecules are applied exogenously to plants, for example, via foliar sprays or stem injections. This method has already proven effective against a range of insect pests and plant pathogens.^{6–8}

3 PRODUCT DEVELOPMENT AND EFFECTIVENESS OF RNAi TECHNOLOGY AGAINST THE COLORADO POTATO BEETLE

RNAi is highly effective against beetles (Coleoptera), especially the Colorado potato beetle (*Leptinotarsa decemlineata*). As early as 2007, studies showed that dsRNA targeting the V-ATPase enzyme of the Colorado potato beetle resulted in up to 100% larval mortality.⁹ In greenhouse trials, dsRNA application provided plant protection for up to 4 weeks. Notably, larvae stopped feeding on dsRNA-treated plants within 2 days after treatment. Furthermore, the dsRNA exhibited high stability under ultraviolet (UV) radiation and irrigation conditions.¹⁰

In June 2021, GreenLight Biosciences submitted an application to the Environmental Protection Agency (EPA) in the USA seeking approval for the dsRNA-based biopesticide Ledprona, marketed under the brand name Calantha™. Ledprona's mode of action is based on the inhibition of the PSMB5 (proteasome subunit beta 5) protein in the Colorado potato beetle. Suppression of this protein leads to the accumulation of defective proteins, thus impairing pest viability. In greenhouse trials, Ledprona reduced leaf loss to <5% and showed efficacy comparable with that of the insecticide spinosad at an application rate of 0.8 g ai ha⁻¹.

Field trials conducted in the USA between 2019 and 2020 confirmed the high efficacy of Ledprona. With a maximum application rate of 9.9 g ai ha⁻¹, Ledprona required approximately ten times less application rate than spinosad (88 g ai ha⁻¹) and chlorantraniliprole (73 g ai ha⁻¹). With three applications (weekly), leaf loss was reduced to 6.81% ± 2.71%. Large larvae of the Colorado potato beetle were particularly sensitive, with mortality rates exceeding 99%, although this effect became apparent only after 7–8 days. However, the early onset of RNAi activity already reduced the pests' food intake, resulting in an immediate protective effect.

The promising results of these studies, coupled with significantly lower application rates than those of conventional insecticide preparations, underscore the potential of RNAi technology in plant protection. Since May 2023, additional field trials have

been conducted across 11 states in the USA to collect further data on the efficacy and duration of protection provided by Ledprona and to optimize the performance of the formulated active ingredient.

4 APPROVAL OF CALANTHA™ AS A BIOPESTICIDE IN DECEMBER 2023

Ledprona is a dsRNA specifically designed to knockout a vital gene in the Colorado potato beetle (*L. decemlineata*). Calantha™ is the formulated crop protection product containing Ledprona as its active ingredient. For clarification, Ledprona refers to the pure bioactive dsRNA component, whereas Calantha™ denotes the ready-to-use product that contains Ledprona in combination with formulation adjuvants and is used in practice for foliar application. In addition, an exemption from the maximum residue limit was requested for the active ingredient Ledprona. The EPA confirmed receipt of the application on 21 June 2021, through publication in the Federal Register and issued a Notice of Filing (NOF) for the tolerance exemption on 28 June 2021. During the public comment period, one comment was received in response to the Notice of Registration; however, none were received regarding the NOF. On 4 May 2023, the EPA granted an Experimental Use Permit (EUP 94614-EUP-1) to GreenLight Biosciences for field trials using Ledprona. This authorization, valid to 30 April 2025, permitted application on 318 acres and the use of 127.5 gallons of Calantha™ across several states in the USA to optimize application methods. In connection with this experimental application, a temporary tolerance exemption for residues in potatoes was also granted, expiring on 30 April 2025. On 18 July 2023, Ledprona received full EPA approval for a period of 5 years, superseding the temporary tolerance exemption.

In the USA, pesticides, including biopesticides, are registered by the EPA, which conducts comprehensive evaluations of safety and efficacy. Biopesticides are categorized into three main groups: microbial pesticides, biochemical pesticides, and plant-incorporated protectants. This classification is intended to ensure that such products meet established standards for the protection of human health and the environment. The testing process includes analysis of biochemical properties, environmental stability, and potential effects on non-target organisms. Biopesticides based on natural mechanisms, such as RNAi, are subject to lower requirements in areas such as long-term carcinogenicity studies, metabolite analyses, and broad-spectrum toxicity testing, thus reflecting their expected lower risk profile.

Approval of Ledprona in the USA raises the question of how comparable dsRNA-based products would be assessed under the European Union (EU) Regulatory Framework. Currently no specific authorization procedure exists for biopesticides in the EU. Instead, in the EU, plant protection products are regulated under Regulation (EC) No 1107/2009, which establishes a hazard- and risk-based authorization system for active substances and formulated products. Although dsRNA would formally fall within the definition of an active substance, the extent to which current guidance documents adequately address RNAi-specific characteristics remains a subject of scientific and regulatory debate.

Nevertheless, the EU Regulatory Framework includes specific procedures for the approval of low-risk active substances and the authorization of low-risk plant protection products, which may include certain biopesticides. These procedures apply equally to biologically derived and chemically synthesized

substances, provided they meet the criteria for classification as 'low-risk' active substances. By definition, biopesticides are generally viewed as offering advantages over conventional chemical pesticides. They are characterized by their lower toxicity to humans and non-target organisms, faster environmental degradation, and greater target specificity. Therefore, their use may help reduce reliance on conventional pesticides. Ledprona, the first dsRNA-based biopesticide to receive regulatory approval in the USA, shows high specificity toward the Colorado potato beetle and appears to act exclusively against this species and closely related beetles. According to EPA assessments, Ledprona is expected to offer substantial environmental benefits, including protecting pollinators and other beneficial insects, while effectively controlling resistant Colorado potato beetle populations.

5 RESULTS OF FIELD TRIALS IN THE EUP PROGRAM

An EUP program was granted to conduct field trials to evaluate the efficacy and risk of Calantha™ under field conditions. In the case of Calantha™, it should be noted that the product is a formulated preparation of a dsRNA active ingredient. This formulation may pose additional risks that were specifically evaluated during the approval process. Although the dsRNA active ingredient is considered safe, the formulations used must also be considered. These usually contain approved inert additives designed to improve usability, stability, and compatibility with conventional plant protection products, and they generally have a low risk profile. Nevertheless, consistent with the broader regulatory evaluation of plant protection products, environmental and safety aspects remain relevant. The field trials were conducted across ten states in the USA, with participation from universities, contract research organizations (CROs), and private agricultural enterprises. The aim of the EUP program was to assess the performance of Calantha™ under large-scale commercial conditions. In particular, the trials evaluated product efficacy, environmental compatibility, stability under varying climatic conditions, effects on non-target organisms, and the efficiency of application by different farmers. The aim was to understand how Calantha™ performs in real-world applications and across diverse agricultural contexts. Testing the new product on areas exceeding 10 ha was considered essential, because smaller plots often cannot reflect the complex conditions of commercial application. Therefore, GreenLight Biosciences established a minimum site size of 20 ha. However, securing suitable land for the trials proved challenging. Many farmers were reluctant to allocate untreated land, fearing that the application of new plant protection products, including dsRNA-based products, might affect the cropping system or crop rotation.

5.1 Objectives and implementation of the EUP program

5.1.1 Evaluation of Calantha™ performance under commercial conditions using different application methods

Trials were conducted on large areas, with farmers applying Calantha™ using their own equipment; for example, commercial ground sprayers or aircraft.

Trial design:

- Each participating farmer allocated 20 ha for the application of Calantha™ and an additional 30 ha for the application of a standard commercial insecticide.
- The application methods included ground spraying and aerial application.

- Calantha™ was applied according to established protocols: an initial application when 10–50% emergence of the first generation of beetle larvae was observed, followed by up to four repeat applications at intervals of 7–10 days.
- Farmers documented relevant agronomic and environmental variables, including fertilization, tillage, potato variety, planting date, planting density, weather conditions at the time of application, and tank mixtures used (e.g., fungicides, herbicides, biostimulants, and fertilizers).

5.1.2 Comparison of the effect of Calantha™ with standard commercial insecticides

In parallel with the large-block trials, GreenLight Biosciences conducted trials on smaller plots in collaboration with universities and CROs. Rodrigues *et al.*¹¹ previously showed that Calantha™ is comparable with products such as Blackhawk®, Entrust® (spinosad), Coragen® (chlorantraniliprole), and pyrethroids. The objective of the program was to confirm the performance of Calantha™ relative to these products under real-world conditions and to evaluate its efficacy in comparison with commercially available insecticides.

5.1.3 Evaluation of Calantha™ performance across various IPM programs

Based on the results of small-plot trials,¹¹ IPM programs were developed to assess the integration of Calantha™ into holistic pest management strategies. Farmers and university collaborators tested Calantha™ in combination with other pest control methods to investigate its role as a biological plant protection product. The aim was to determine how Calantha™ could serve as a supplement to or a replacement for synthetic chemical insecticides, thereby minimizing the impact on the ecosystem while optimizing pest control.

5.1.4 Determining the impact of Calantha™ on potato yield

Data on yield increases as a result of Colorado potato beetle control were collected in both large-block trials and smaller replicated plot trials. These data were essential to quantify the agronomic benefit of effective pest control under field conditions and to demonstrate the economic relevance of Calantha™ application beyond biological efficacy alone.

5.1.5 Investigating Calantha™ degradation under various environmental factors

In the northwestern USA, irrigation with surface water reduced the efficacy of Calantha™. GreenLight hypothesizes that this reduction is due to the rapid degradation of dsRNA by microbes and nucleases present in surface water. Tests on the stability of dsRNA under UV light, in the presence of microbes, nucleases, and various chelating agents (e.g., ammonium sulfate) were conducted under greenhouse and field conditions.

5.1.6 Evaluating the effect of Calantha™ on different larval stages of the Colorado potato beetle

Standardized small-plot trials were conducted at four locations to investigate the effect of Calantha™ on different larval stages of the Colorado potato beetle. Several application schedules were tested, ranging from early initial application (administered when 10% of eggs had hatched) to later applications (administered 7–28 days after larval emergence).

5.2 Key findings from the EUP program

- The results showed that large-block trials are essential for a realistic assessment of Calantha™ performance under commercial conditions. Although smaller-plot trials provided valuable information, they could not capture the complexity of commercial cultivation.
- The trials confirmed the high efficacy of Calantha™, especially against early larval stages of the Colorado potato beetle.
- The use of surface water for irrigation resulted in reduced performance, suggesting microbial degradation of dsRNA. Chelating agents such as ammonium sulfate could potentially mitigate this effect.
- Tank mixtures incorporating fungicides, herbicides, and fertilizers were tested to identify potential interactions and their influence on the efficacy of Calantha™.

6 ASSESSMENT OF EXPOSURE AND RISK TO HUMAN HEALTH

As part of the risk assessment for Ledprona, the active ingredient in Calantha™, various exposure routes, including oral, dermal, ocular, and inhalation, and their potential effects on human health were examined in detail. Both acute and chronic risks were assessed. The following sections provide a detailed overview of each exposure pathway and the associated health risks, based on regulatory testing protocols and scientific studies.

6.1 Oral and dermal exposure

Oral and dermal exposure to Ledprona is considered negligible because of limited exposure, physical and biochemical barriers in the body, and rapid degradation of Ledprona by gastric and intestinal fluids, which prevents absorption. Consequently, no substantial systemic uptake is expected.

6.2 Ocular exposure

Potential eye exposure to Ledprona may occur during mixing, filling, or application of the product. Because Ledprona is unmodified RNA, it is efficiently degraded by RNases present in mucosal secretions, including tear fluid. The likelihood of Ledprona-like transcripts being expressed in ocular tissues is minimal; therefore, the risk of harmful exposure is considered negligible. However, safety goggles are recommended when handling Calantha™ to further minimize the risk of eye exposure.

6.3 Dermal exposure and skin sensitization

Dermal exposure to Ledprona is considered very low because of its high molecular weight and the physicochemical barrier properties of the skin. Sensitization tests conducted in accordance with Office of Chemical Safety and Pollution Prevention guidelines (870.2600) show that Ledprona in its technical formulations (Ledprona Technical and GS2 Technical-Dry) has no sensitizing effect. By contrast, Calantha™ has been classified as a weak sensitizer, which is reflected on the product label: 'Prolonged or repeated skin contact may cause allergic reactions in some individuals.' Recent studies confirm that dermal exposure to Ledprona is considered negligible when Calantha™ is used as directed.¹²

6.4 Inhalation risk

The risk of inhaling Ledprona during application is considered negligible when appropriate protective equipment is used. This assessment is based on the low concentration of Ledprona in Calantha™, the low application rates, and the body's physiological

barriers. Although immune reactions and sequence-specific risks are considered highly unlikely, respiratory protective equipment is recommended when handling Calantha™ to minimize the risk of inhalation.

6.5 Toxicity studies

Ledprona has been classified in toxicity category IV based on assessments of acute oral, inhalation, and dermal toxicity as well as eye and skin irritation, which means that it is practically non-toxic and non-irritating.

6.6 Additional studies

Additional studies corroborate the low potential for skin sensitization and support reconsideration of respiratory protection requirements. Studies involving Calantha™ and four common tank-mix partners (Revus Top®, Warrior II, BRAVO® Weather Stik®, and Luna Experience®) show that Ledprona is completely degraded within 10 min in simulated gastric fluid, irrespective of the mixtures. None of the active ingredients tested affected the stability of Ledprona. These results confirm that Ledprona is degraded in Calantha™ in a manner comparable to plant RNA, without producing harmful effects.¹²

6.7 Bioinformatics and off-target predictions

Bioinformatic analysis of Ledprona identified two potential human off-target genes. The probability of Ledprona-associated 21-mers being generated *in vivo* is considered low. Three 21-mers showed low sequence similarity with human long non-coding RNAs (regulatory RNA molecules that are not translated into proteins but perform a variety of functions in gene regulation and cell biology; for example, in the control of transcription, chromatin structure or cell differentiation). One transcript (XR_001745322.1) is expressed exclusively in the testes, and the risk of unintended effects is considered negligible. For the second transcript (XR_007064582.1), no detailed information on tissue-specific expression is available, suggesting minimal risk.

6.8 Overall exposure and health assessment

The EPA has assessed the overall exposure to Ledprona, including potential exposure through food and drinking water. Given its low toxicity, rapid degradation in stomach and intestinal fluids, and the natural physiological barriers of the human body, the associated risk is considered negligible. Calantha™ is not approved for residential use, and occupational exposure is minimized through the use of protective equipment.

6.9 Summary assessment

The available data support the registration of Calantha™ under the Federal Insecticide, Fungicide, and Rodenticide¹³ Act Section 3 without the need for further toxicological testing. The EPA concludes that Ledprona residues do not pose a risk to the US population, including sensitive groups such as infants and children, when used in accordance with approved label instructions. No additional safety measures are required, as no adverse effects have been identified. Ledprona is classified as safe by the EPA and does not present an unacceptable risk to human health when used properly. Further information is available in the registration dossier (EPA-HQ-OPP-2021-0271).¹²

7 EFFECTS ON NON-TARGET ORGANISMS

Calantha™, which contains the RNA-based active ingredient Ledprona, was developed specifically for use in potato cultivation.

Its use is restricted to treated soil areas, and drift control measures are required to protect adjacent non-target areas. As part of the risk assessment, potential exposure and possible effects on various groups of non-target organisms were comprehensively investigated. Non-target organisms are primarily exposed to treated areas. Above-ground animals may be exposed through direct contact with the applied substance or via residues on plant material. Soil organisms are exposed through contact with treated plant material. The EPA used a multitiered approach to assess toxicity. In phase I, the potential effects on a broad range of non-target organisms, such as birds, freshwater fish, aquatic invertebrates, plants, and insects, were examined under realistic worst-case exposure scenarios. Additional long-term studies were conducted in phases II and III under greenhouse or field conditions only if risks were identified in phase I. The underlying data relied on reference studies and specific scientific analyses.

On this basis, the EPA concluded that Ledprona does not pose a relevant risk to non-target organisms outside the order Coleoptera. This assessment is supported by three principal findings: (i) the calculated risk quotients were consistently below the regulatory thresholds of concern; (ii) bioinformatic analyses showed no relevant sequence similarity with the genomes of non-target species; and (iii) toxicity studies detected potential effects only in two closely related beetle species.

Non-target plants are unlikely to be adversely affected by the application, because exposure is substantially limited by the targeted application method, low spray drift, and high binding capacity of dsRNA to plant surfaces. Toxicological and phytopathological studies found no evidence of phytotoxicity in representative companion plants, including pepper, aubergine, or tomato.

Birds and mammals could theoretically be exposed through direct contact with treated surfaces or through ingestion of treated plant material. However, model-based calculations estimated an exposure dose of only 0.02 mg per kilogram of body weight – a value well below toxicologically relevant thresholds. Toxicological studies confirmed this assessment, as no harmful effects were observed, and the oral Lethal Dose 50% (LD_{50}) for Ledprona exceeded 5000 mg/kg. Furthermore, biological barriers, such as digestive enzymes and rapid RNA degradation, suggest that notable effects on vertebrates are not expected.

Potential exposure of non-target insects through contact or ingestion was also investigated, with particular emphasis on pollinators such as honey bees. The EPA studies showed that all tested toxicity endpoints exceeded the concentrations expected under field conditions. Bioinformatic analyses revealed only minor sequence similarity in five Coleoptera species. No treatment-related effects were observed in ecologically relevant species such as earthworms and ladybirds. These data confirm the high specificity of Ledprona, which targets the Colorado potato beetle and closely related beetle species while leaving other non-target insects unaffected.

The potential risk to endangered beetle species in the USA was carefully assessed. A combination of bioinformatic analyses, biological testing, and ecological habitat assessment showed that Ledprona acts exclusively on Coleoptera and that effects were observed in only two species: the red flour beetle and the southern corn rootworm. The risk assessment found that 15 of the 19 endangered beetle species listed by the US Fish and Wildlife Service occur in regions that do not overlap with potato cultivation areas. Habitat type, exposure pathway, application rate, and drift behavior were incorporated into the assessment of the remaining four species, which included the American sand beetle. The resulting exposure level was classified as negligible, and the

EPA concluded that no direct or indirect adverse effects on these species or their habitats are expected. A formal consultation with the US Fish and Wildlife Service or the National Marine Fisheries Service under the Endangered Species Act was not required.

8 ENVIRONMENTAL BEHAVIOR OF LEDPRONA

In assessing the environmental behavior of Ledprona, the EPA considered both the chemical and biological stability of dsRNA and the influence of formulation adjuvants used to enhance stability during storage and application. The formulated product Calantha™ contains approved inert substances designed to delay dsRNA degradation in the storage and application container. However, studies submitted in support of the EUP application show that Calantha™ degrades rapidly under aerobic and aquatic conditions, indicating a low likelihood of long-term environmental exposure.

Ledprona was tested under aerobic conditions in various soils representative of agricultural conditions. The results showed that more than 90% of the applied dsRNA degraded within 4 days. The time to 50% degradation (DT_{50}) ranged from 0.5 to 2.92 days, depending on the soil type. Although residues present in the first 12 days after application could potentially result in short-term exposure of non-target organisms, long-term persistence appears unlikely. Notably, the concentrations used in these studies were approximately 1650 times higher than the expected environmental concentration. Additional microbial degradation studies showed that degradation occurs more rapidly at lower doses. Rapid degradation of the formulated dsRNA was observed even at extremely high doses, supporting the assumption of minimal environmental persistence.

The potential for exposure in aquatic systems is limited to runoff or drainage following application. Because dsRNA sprayed onto foliage largely remains on the leaf surface, its entry into water bodies is expected to be minimal. Studies investigated the degradation of Ledprona in the water column and sediment of two representative aquatic systems. The results showed a rapid reduction in the initial concentration, with 90% of the active substance degraded within 6.18 days in the water column and within 4.2 days in sediment. The DT_{50} was 1.87 and 1.27 days, respectively. Because these studies were conducted using test doses substantially higher than expected environmental levels, the reported degradation rates are considered conservative. Furthermore, an independent study by Fischer *et al.* (2017)¹⁴ confirmed that even unformulated dsRNA degrades rapidly in aquatic environments. Therefore, these findings indicate that exposure to aquatic organisms is likely negligible.

The influence of formulation excipients on dsRNA stability was also investigated. A microbial stability study conducted by Green-Light Biosciences showed that the formulation has a stabilizing effect in the storage container, but that this effect decreases notably when diluted to field application concentrations. Although Calantha™ exhibits some stability at higher concentrations, this effect diminishes rapidly under environmental conditions. Thus, the formulation components are not expected to contribute substantially to increased environmental persistence.

In addition to degradation behavior, the potential for cumulative effects was investigated in accordance with Section 408(b) (2)(D)(v) of the Federal Food, Drug, and Cosmetic Act¹⁵. Under this provision, the EPA is required to consider possible interactions with other substances that share a common mechanism of toxicity when establishing, modifying, or revoking tolerance levels for

plant protection products. No toxic effects were found for Ledprona, either as a single active ingredient or in combination with other substances. Therefore, the EPA concluded that cumulative toxicity and cumulative effects posed no risk. This assessment is particularly relevant given that modern biopesticides are often more difficult to evaluate because of their novel modes of action and limited long-term data. However, the EPA emphasized that, in the case of Ledprona, the available data set is sufficiently robust and comprehensive to support a sound risk assessment without raising additional safety concerns.

9 PUBLIC COMMENTS AND DECISION-MAKING

The EPA provided three opportunities for public comment on the registration of Calantha™ and the application for a tolerance exemption. Information was published in the Federal Register and made available via www.regulations.gov. On 22 June 2021, the application for registration of the GS2 formulation (Calantha™) and GS2 technology (Ledprona technology), containing the new active ingredient Ledprona, was published in the Federal Register. During the comment period for the registration application, a supportive comment was received from the US Department of Agriculture.

On 28 June 2021, the EPA published an NOF in the Federal Register (86 FR 33890) concerning the application for exemption from a maximum residue level for Ledprona. No comments were received during the comment period for this notice.

Because Ledprona is a new active ingredient being used under field conditions for the first time, the EPA initiated an additional public comment period on the proposed registration decision for Calantha™. This action is in line with the agency's policy, introduced in October 2009, to increase public participation in registration procedures. The proposal to register Calantha™ was published on 29 September 2023, with a 15-day period for comments. This deadline was extended by an additional 15 days to 30 October 2023, upon request. During the comment period, the European Chemicals Agency received a total of 31 268 comments, comprising 182 individual comments and 31 086 contributions from mass campaigns. The majority of comments, particularly those from the campaigns, strongly criticized the approval, citing deficiencies in the database, the risk–benefit assessment, and the legal evaluation under Federal Insecticide, Fungicide, and Rodenticide Act. The individual technical comments were predominantly similar in their critical assessments, although some supportive comments were also received. The EPA responded to all comments. The responses are available at <https://www.regulations.gov/docket/EPA-HQ-OPP-2021-0271>. Altogether, EPA stated that the submitted data, together with existing scientific knowledge on dsRNA biology, were considered adequate for the purpose of regulatory decision-making and the submitted public comments did not ultimately alter the agency's final regulatory decision.

10 THE IMPORTANCE OF BIOINFORMATICS FOR PREDICTING OFF-TARGET EFFECTS IN THE RISK ASSESSMENT OF RNAi-BASED PLANT PROTECTION PRODUCTS

Bioinformatics plays a key role in the risk assessment of RNAi-based plant protection products, particularly in predicting off-

target effects. The application of bioinformatic tools can identify potential undesirable effects on non-target genes, which is crucial for ensuring the safe use of RNAi plant protection products.

A major challenge in applying bioinformatic methods to RNAi-based plant protection products lies in the incomplete or inaccurate annotation of genomes. Comprehensive and well-curated genome sequences are often unavailable, leading to gaps in genome analysis. These gaps can lead to off-target effects being misidentified, because uncharacterized genes or regulatory elements are not incorporated into the bioinformatic models. Furthermore, our understanding of the biological mechanisms influenced by RNAi remains incomplete. Many of the underlying processes and interactions are complex and have not yet been fully elucidated. This limited mechanistic understanding constrains predictive accuracy and may generate false-positive or false-negative results. False-positive predictions may indicate non-existent risks, whereas false-negative results may overlook off-target effects.

Another limitation is the reliance of bioinformatic models on generalized assumptions and data that may not reflect species-specific biological mechanisms, potentially leading to inaccurate predictions. The absence of biological tests to validate bioinformatic predictions poses a further challenge. Targeted experimental studies, such as those conducted for Ledprona, are necessary to reliably verify the actual off-target effects. Such validation is vital for confirming predictions and ensuring product safety.

The integration of bioinformatic methods into the risk assessment of RNAi-based plant protection products provides valuable insights and helps to identify potential risks at an early stage. However, the predictive capabilities are limited by incomplete genome data, insufficient mechanistic knowledge, and the need for biological validation. A comprehensive assessment, therefore, requires the combined use of precise bioinformatic analyses and robust biological tests to ensure the safety and efficacy of RNAi-based plant protection products.

11 OPEN QUESTIONS FOR RISK ASSESSMENT IN THE EU

In the USA, the registration of plant protection products such as Calantha™/Ledprona by the EPA involves the consideration of numerous aspects, many of which may likewise be relevant to the risk assessment procedures for active substances and plant protection products in the EU. The question arises whether the development of RNAi-specific guidance documents within the EU framework would facilitate consistent and transparent assessments. Such guidance could clarify expectations regarding bioinformatic analyses, empirical testing strategies, exposure assessment, and the interpretation of sequence-specific effects. Below we list selected questions that remain open or have not yet been conclusively addressed within the EU Regulatory Framework, and that may warrant further clarification in the context of regulatory assessment and harmonization.

11.1 Data requirements and quality

What additional or specific data are required in the EU that were not generated from US studies? Are there differences in the requirements for toxicity and exposure studies?

11.2 Exposure assessment

How are exposure assessments for Calantha™/Ledprona conducted in the EU, and how do they differ from US assessments?

Are all relevant exposure pathways, including environmental pollution and residues in food, addressed under EU guidance?

11.3 Safety factors for sensitive groups

What additional safety factors are applied in the EU for particularly sensitive population groups (e.g., infants and children)? Do these factors differ from those used in the USA? How are pre- and post-natal effects incorporated in the assessment?

11.4 Cumulative and combined exposure

How is cumulative exposure to Calantha™/Ledprona and other substances that share a common toxicity mechanism assessed in the EU? Do the USA and the EU differ in their methodological approaches to the assessment of cumulative toxicity?

11.5 Long-term risks to the environment and health

How are long-term risks to human health and the environment assessed in the EU, especially regarding possible cumulative effects over extended periods of time? Are there differences in the criteria used to assess the environmental compatibility and long-term effects of Calantha™/Ledprona?

11.6 Regulatory differences

What regulatory differences exist between the EPA and the authorities in other EU Member States about the assessment and approval of active substances and the authorization of plant protection products? How do these differences influence decision-making processes and safety assessment?

11.7 Approval procedures and testing requirements

Under current German and EU regulatory procedures, which testing requirements would RNA-based plant protection products such as Calantha™/Ledprona be required to meet? Are there indications that authorities might envisage adjusting or simplifying the data requirements or testing programs due to the low-risk potential of these active substances?

11.8 Acceptance of studies and data

Are the studies and data originally generated in the USA accepted in the EU, or are there additional requirements for data acceptance? How is the quality and relevance of the submitted studies assessed?

11.9 Risk communication

How are risks to consumers and users communicated in the EU? Are there differences in transparency and access to information compared to communication practices in the USA?

These questions are important to ensure that the approval of active substances such as Ledprona in the EU and the authorization of plant protection products such as Calantha™ in the EU take full account of the health and safety of the population and the environment.

12 CONCLUSION

The approval of Ledprona represents a regulatory milestone for externally applied RNAi-based plant protection products. It demonstrates that dsRNA-based active substances can be evaluated within existing regulatory systems when supported by an appropriate data package. At the same time, the case highlights methodological and regulatory questions that are specific to sequence-based modes of action. These include the role and standardization of bioinformatic screening, the interpretation of

sequence specificity, and the integration of molecular data into ecological risk assessment. Such aspects may warrant further structured discussion within the EU regulatory context. However, the experience gained during the approval process of Ledprona may inform future regulatory approaches and methodological refinement, both within the USA and in other jurisdictions, including the EU.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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