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How to regulate new microbial solutions for plant protection in Europe

Biopesticides are at the forefront of innovation in plant protection. Microbial pesticides are the most advanced group of biopesticides, both in terms of discovery and risk assessment. Still there are steps that should be made in risk assessment to unravel the full potential of this new era of plant protection products (PPPs). Microbials are currently based on bacterial, fungal and viral components, but new microbial solutions based on bacteriophages, microbial consortia and protists are at the doorstep of the EU. Despite advances in this area, the risk assessment of new microbial solutions is still lagging behind. We describe the current scene of risk assessment for microbial pesticides and highlight the unique features of new microbial solutions that should be considered for the urgently needed reform of risk assessment of these innovative products.

INTRODUCTION

Synthetic pesticides are still a cornerstone of modern agriculture. However, their extensive use has raised concerns about their impact on the environment and human health, despite the stringent regulatory framework that is in place to control their placement in the EU market. Industrial innovators and the European Commission are investing in the discovery and development of innovative biopesticides such as microbials, semiochemicals /pheromones, botanicals (plant extracts and pure plant derived compounds) and other substances of biological origin (e.g. natural peptides, ds-RNA). Still, the placement in the market of this new era of products has been restrained by Europe's delayed development of relevant regulatory instruments, as the present EU pesticide regulatory framework has been tailored to the needs of synthetic pesticides and does not consider the unique features of biopesticides.In 2022 the European Commission made available for the first time the data requirements for the approval of microbial pesticides, and in October 2023 it provided, through Explanatory notes (in the

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framework of Reg. (EC)No 1107/2009), technical information on how these data requirements can be addressed, or which guidance document or guidelines may apply. In the same context, the European Commission has established the term low-risk substances to put a “safety label” on such products, which is given only after risk assessment is concluded. The criteria for the approval of microbial and non-microbial pesticides as low risk are included in the point 5 of annex II of Regulation EC no 1107/2009. Complementing this, the European Food Safety Authority (EFSA) has proposed the term “low-concern substances”, which includes all substances of biological origin that are potentially of low risk . Microbial pesticides are the most populated and advanced category of biopesticides with currently 71 of them approved at EU level and 26 applications pending**[1]**. Amongst the approved microbials, fungal-based products dominate (40), followed by bacteria (23) and viruses (8). The list of pending microbial PPPs includes the first application of a bacteriophage product, while several other bacteriophage-based plant protection products (PPPs) are in the production pipeline. In addition to bacteriophages, other novel microbial PPPs based on protists and synthetic microbial consortia of variable complexity are under development and expected to be submitted for approval. A major challenge we are currently facing is to provide a novel regulatory framework for a fast track, efficient and scientifically sound risk assessment of existing, and most importantly, of future microbial solutions.

HOW PREPARED ARE WE TO REGULATE THE CURRENTLY AVAILABLE MICROBIAL PESTICIDES?

Microbial pesticides are most probably the best regulated group of biopesticides. Regulation 283/2013 Part B**[2]** describes the requirements that should be met in order for a microbial product to be placed on the market. Recent guidance documents have addressed the main safety concerns for microbial pesticides like:

- The carriage and transmissibility of antibiotic resistance genes (ARGs) (European Commission 2020, SANTE/2020/12260)**[3]**
- The biosynthesis of secondary metabolites of concern (European Commission 2020, SANTE 2020/12258) **[4]**
- The pathogenicity and infectivity potential

Whole Genome Sequencing (WGS) analysis of microbial strains used in PPPs is now recognized as an invaluable tool for early screening and detection of genetic elements of concern and dictates the generation of follow-up data or the rejection of products. Furthermore, advents in sequencing technologies enable the rapid and cost-effective WGS of bacterial and fungal strains, facilitating accurate phylogenetic

identification even at the strain level. This can avoid unnecessary testing based on established knowledge about the safety of microorganisms belonging to certain species or genera. In this frame EFSA assesses the safety of microorganisms used in different applications (food and feed additives, PPPs) based on the Qualified Presumption of Safety (QPS) approach that covers safety concerns for humans, animals and the environment based on the taxonomic identity of the microorganism, related body of knowledge and potential safety**[5]**. However, the main question remains "Do regulators have the necessary knowledge and access to tools that will facilitate risk assessment decisions?" The answer to this question is not trivial. Only few member-state regulatory bodies (less than half) have staff with the expertise to delve into WGS data**[6]**. EFSA made available a guidance document for the assessment of the data obtained from WGS analysis.In addition, EFSA has developed and provided to regulatory bodies the Microorganisms Pipeline (MoPs) tool, a non-open access pipeline that identifies potential functional traits of concern in microbial genomes like virulence factors, resistance to antimicrobials of clinical relevance for humans and animals and biosynthesis of known toxic secondary metabolites. WGS analysis through MoPs is based on state-of-the-art bioinformatic tools, but relies on a limited number of microbial genomes that are present in the MoPs database, while there is limited information on the potential transferability of ARGs, a key component of the risk assessment of microbial pesticides.

Where do we need to focus on in order to improve our risk assessment of the currently available microbial pesticides (bacterial, fungal and viral PPPs)?

1. Mobilization of more regulatory experts with specialization in microbial ecology, environmental microbiology and bioinformatics
2. Improvement of currently available tools or development of novel open-access pipelines that will use the full breadth of curated sequencing data available worldwide.

HOW READY ARE WE TO REGULATE THE EMERGING MICROBIAL SOLUTIONS?

Before we answer this question we need to define which are those emerging microbial solutions that we will be asked to regulate:

- Bacteriophages (or phages)
- Protists
- Microbial consortia or Synthetic microbial communities

No regulatory documents are available for any of these novel microbial solutions except for bacteriophages

for which an Organization of Economic Co-operation and Development (OECD) guidance document has recently been published**[7]**. Risk assessment for these new microbial solutions should be always case by case and in most cases will be qualitative, often using a weight-of-evidence approach. Expert judgment will be needed to determine what should be and what should not be considered a foreseeable risk. For this, good knowledge of the ecology and biology of the particular microbial solution is highly relevant.

Bacteriophages are viruses that specifically infect bacteria. Their main characteristics that will be relevant for risk assessment innovation are summarized below:

- Many phages are highly specific to their bacterial host (sometime even at the strain level), making them a potentially ideal and highly selective tool in crop protection**[8]**.
- The use of mixtures of phages (so-called phage cocktails) with different infection profiles is a scientifically and commercially promising strategy to combat multiple disease-causing bacterial strains and to limit the emergence of resistance.
- Phages that reproduce using only the lytic life cycle, infect and kill the bacterial cell directly. This life cycle is considered preferable and more relevant for crop protection applications than the lysogenic life cycle, in which the phage integrates into the bacterial genome with the risk of transferring virulence genes to the host. Environmental signals can trigger a switch from lysogeny to lysis.
- There are established methods for quantification including classic microbiological and molecular methods.
- WGS approaches can be used for identification, detection of antibiotic resistance genes and prediction of life cycles and functions.
- Phage survival and infectivity are highly sensitive to environmental parameters and this should be considered in the mode of application.
- Phages are not infectious to eukaryotic cells and are not known to produce secondary metabolites.
- Production of phages is straightforward but requires a bacterial host. The presence of the host should be checked and considered in the risk assessment.
- Phage-dedicated collections are available, allowing deposition, which is a prerequisite for product registration.
- Non-target effects on the microbiome should be tested under different environmental scenarios (e.g. high or realistic pressure on bacterial populations).

Phage PPPs are currently available in the USA market for the control of (a) Xanthomonas campestris pv. vesicatoria and Pseudomonas syringae pv. tomato (b) Xyllela fastidiosa (c) Pectobacterium carotovorum (Erwinia carotovora) and (d) Xanthomonas citri pv. citri while several others are either pending authorization or are in the pipeline of production for the market**[9][10]**.

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Protists are the most morphologically and phylogenetically diverse group of microorganisms**[11]**. They encompass a range of lifestyles spanning from saprotrophy (i.e. feeding on dead organic matter) and phototrophy (i.e. using light as primary energy source) to predation, the latter being the most common lifestyle and the one most relevant for crop protection. Most predatory protists feed on bacteria but feeding on fungi is also ubiquitous**[12]**. Certain characteristics of protists that should be considered relevant for risk assessment innovation are summarized below:

- WGS analysis as a tool in risk assessment is becoming possible due to recent sequencing of 10000 protist genomes**[13]**.
- The mode of action of protists could be direct (via predation) or indirect (via stimulation of microbes with enhanced phytopathogen inhibition capacities, plant growth promoting or plant protecting traits) and should be treated differently in the risk assessment.
- There is little knowledge about the ability of protists to produce toxins, secondary metabolites and to carry ARGs. WGS analysis could facilitate early detection and clarify data requirements.
- Protists also include human and animal pathogens and parasites, while predatory protists could be pathogenic under certain conditions.
- Compared to phages, protist predation on bacteria is rather non-specific, although cases of strain-specific predation have been postulated and feeding preferences and selective grazing have been shown.
- The mass production of protists might require feed with prey (single or multiple), which may raise concerns about the presence of prey cells as contaminants in the product, while also axenic growth (i.e. without bacterial prey) is possible for certain protists.
- Large-scale isolation and the cultivation from soil remain difficult and another major barrier is the lack of dedicated culture collections that are necessary for product registration.

Currently, there are no protist-based crop protection products on the market in the EU and the USA. However, several recent reports of their activity as controllers of soil-borne bacterial pathogens (e.g. Ralstonia solanacearum)**[14]** are likely to stimulate interest in the development of protist-based PPPs.

Microbial consortia of different complexity could also be a viable and novel microbial solution in the new era of crop protection. They can be characterized by low or high complexity. In the former category we include consortia constructed by combining different well-characterized strains of bacteria or fungi (intra-kingdom SynComs), or bacteria and fungi (trans-kingdom SynComs) that have complementary modes of action or different optima of activity expected to maximize efficiency compared to individual strains.

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In the latter category we could consider naturally isolated enrichment cultures of bacteria (mostly) and/or fungi (rarely) that are stable in composition with often more than 10 distinct members. Regarding microbial consortia an amendment to the Regulation 283/2013 with Regulation 1439/2022**[15]** considered for the first time in a regulatory context the application of products that are composed of "a qualitatively defined combination of strains as they occur naturally or by manufacture". In addition, the first considerations of risk assessment for microbial consortia were set out. The risk assessment of synthetic microbial consortia of low complexity seems to be rather straightforward with key notes:

- Data requirements will be defined according to the characteristics of the consortium and the intended use.
- A qualitative definition of the consortium and the range of content (minimum and maximum) of each member should be requested.
- All components should be deposited in culture collections.
- WGS analysis per consortium member is required to define risks (pathogenicity, infectivity, antibiotic resistance, toxin and secondary metabolite biosynthesis).

Less consideration has been given to natural microbial consortia derived from enrichment cultures, that may be characterized by higher complexity, compared to synthetically produced consortia, and may involve other unique features that should be taken into consideration in risk assessment innovation. Compositionally stable enrichment cultures could be fully defined qualitatively and certain features relevant for risk assessment (virulence factors, ARGs, gene clusters coding for the biosynthesis of secondary metabolites) could be clarified by whole (meta)genome sequencing and bioinformatics.

However, it is often the case that enrichment cultures could not be disentangled fully to their members due to the limited cultivability of individual members or nutritional and metabolic interdependencies (e.g. provision of vitamins and amino acids) **[16]** which limit axenic cultivation. This is a limitation that contrasts with the requirements for deposition of individual consortium members to culture collections. Specific adjustments to this requirement should be considered to allow deposition and also testing for the whole consortium rather than for individual members. Another important aspect for naturally derived consortia is the mode of action and the definition of the functional role of its individual member. These could be determined by more advanced omic tools (metatranscriptomic, metaproteomic, meta-metabolomic) followed by demanding bioinformatic analysis. It is often the case that only one (or a few) of the members of a microbial consortium have a plant protection relevant mode of action while the rest confer supportive services to the coherence of the consortium adhering to the widespread auxotrophy amongst prokaryotes**[17]**.

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INNOVATION IN THE RISK ASSESSMENT OF NEW MICROBIAL SOLUTIONS: WHAT ELSE IS NEEDED?

Risk assessment of novel microbial solutions requires fast and efficient steps to match the pace of research and industrial innovation. Amongst the upcoming microbial solutions, phages primarily and microbial consortia secondarily are on the doorstep of the EU market. Despite that, we do not have regulatory procedures in place to address their characteristics and meet the requirements for a proper risk assessment scheme. On the other hand, PPPs based on alive protists are not yet, as far as we know, in the production pipeline, but scientific evidence suggests that they might be possible candidates for developmentif certain safety limitations at technological or biological level are addressed. Therefore, we urgently need to develop guidelines and recommendations for the risk assessment of those novel microbial solutions that will consider all their specific features and cases deployed above. This will encourage and benchmark innovation in the discovery and development of novel biobased solutions for crop protection.

This project has received funding from the European Union's Horizon Europe Research and Innovation programme under Grant Agreement No. 101084163. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

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TERMINOLOGY

Auxotrophy: the inability of an organism to synthesize de novo a particular organic biomolecule required for growth.

Botanicals: are active substances which carry pesticide activity and they are obtained by processing material of botanical origin. Botanical pesticides could be a mixture of several different plant-derived compounds or purified substances.

Semiochemicals: are substances emitted by plants, animals, and other organisms that evoke a behavioural or physiological response in individuals of the same or other species. Amongst them pheromones are produced by individuals of a species and modify the behaviour of other individuals of the same species.

Metagenome: the sum of the genomes of all microorganisms of a microbial consortium.

Metatranscriptome: the sum of all transcripts of all microorganisms of a microbial consortium produced under a specific growth condition.

Metaproteome: the sum of all proteins of all microorganism of a microbial consortium produced under a specific growth condition.

Metametabolome: the sum of all metabolites of all microorganisms of a microbial consortium produced under a specific growth condition.

Whole genome sequencing: sequencing of the entire genome of an organism including chromosome, plasmids, mitochondria and other organelles.

Synthetic microbial communities (SynComs): carefully chosen microbial strains that are grown together in a single community to produce the desired microbiome function (e.g. plant protection, pollutants degradation, human and animal protection)

RATION
Funded by the European Union

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RISK ASSESSMENT FOR DSRNA-BASED PESTICIDES

The agricultural sector is currently facing challenges such as decreasing availability of conventional pesticides, rapid pesticide resistance evolution, and climate change impacts, leading to a growing demand for sustainable pest management solutions.

INTRODUCTION

Double-stranded RNA (dsRNA)-based pesticides have emerged as a promising alternative, leveraging RNA interference (RNAi) to precisely target and silence specific genes in pest organisms. dsRNA acts more selectively than conventional pesticides, which often harm non-target species. When dsRNA is taken up by eukaryotic cells, it is processed by Dicer endonucleases into 20-25 nt short interfering RNAs (siRNAs) (Figure 1).These siRNAs guide Argonaute (AGO) proteins in the RNA-Induced Silencing Complex (RISC) to identify and degrade complementary mRNAs, leading to post-transcriptional gene silencing (PTGS).

If the targeted gene is essential for organismal development, this can result in lethality. This specificity reduces the possibility of undesirable effects on non-target organisms, positioning dsRNA-based pesticides as a significant advancement in sustainable agriculture (1-3). However, RNAi-based pest control has limitations in efficacy against certain pest orders, such as lepidopterans (4), and rapid resistance development has been observed in coleopteran pests (5-8), indicating a need for resistance management strategies to protect the technology.

Given the promising potential of dsRNA-based pesticides, there is a pressing need for regulatory frameworks to adapt to this new technology.

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Current regulations may not adequately address the unique properties and mechanisms of dsRNA products, while it is essential to ensure their safety, efficacy, and responsible use in agriculture. Establishing clear guidelines can help facilitate the integration of dsRNA solutions into modern pest management strategies, contributing to sustainable agricultural practices.

The regulatory landscape for dsRNA-based pesticides is complex, particularly in the European Union, where they are classified as chemical pesticides under Regulation (EC) No. 1107/2009.

Consequently, dsRNA products undergo the same stringent safety and efficacy assessments as conventional pesticides, delaying their approval and commercial availability (2, 3). This pesticide-based regulatory approach does not consider the biological specificity of dsRNA and its rapid degradation in the environment, which may make it safer than traditional chemicals.

In contrast, the U.S. Environmental Protection Agency (EPA) has begun approving dsRNA products like Calantha/Ledprona (CAS Number: 2433753-68-3, Docket ID: EPA-HQ-OPP-2021-0271), setting a precedent for more flexible regulatory frameworks that recognize the biological nature of dsRNA (9).

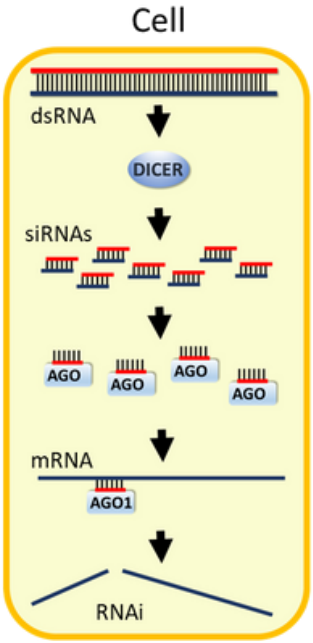

Figure 1. Schematic representation of the RNAi mechanism induced by dsRNAs

REGULATORY AND SCIENTIFIC CHALLENGES

The deployment of dsRNA-based pesticides faces a range of regulatory and scientific challenges that must be addressed to ensure their safe and effective use. The Organisation for Economic Co-operation and Development (OECD) has made significant contributions by publishing comprehensive documents that tackle both environmental and human health considerations related to dsRNA products (1,10). These reports underscore the critical importance of conducting thorough evaluations of potential toxicological hazards associated with dsRNA application. Specifically, assessments must focus on various exposure routes, the

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potential risks presented to applicators, bystanders, and consumers who may come into contact with or consume treated product. Potential risk assessment challenges have to do with knowledge gaps related mainly to hazard identification and characterization. Pro-actively identifying off-targets and non-targets is of paramount importance and for this sake bioinformatics may prove extremely helpful, especially when considering the ever-growing availability of genome and transcriptome data. The golden standard for assessing the likelihood of off-target hits is the presence of a 20-nt sequence identity between a given dsRNA pesticide and the target's genome and/or transcriptome. Yet, more nuanced criteria need to be developed, because RNAi efficiency in various organisms is dependent more factors, including e.g. the overall size of sequence identity, the number of allowed mismatches between a small RNA and its mRNA target, the number of small RNA hits on the genome/transcriptome, the topology of hitting site. Clearly, the bioinformatics criteria need to be nuanced and are currently far from being established. A surprisingly unexplored facet of RNAi is a possible induction of unintended epigenetic events, especially at the crops of application. DsRNA may trigger DNA methylation at genome loci sharing an overall 70-80% sequence identity with the dsRNA (11). Then, de novo DNA methylation may occur at CG, CHG and CHH context; of these, methylation at CG context may be trans-generationally maintained (even at the absence of homologous dsRNA) and may affect endogenous transcription (12), This is not of minor importance and highlights that bioinformatics tools need to be optimized also for these worst-case scenarios. In that case, of course, these tools would need to employ not only on transcriptome but also genome data. Finally, besides RNAi-specific and sequence-dependent events, dsRNA may also trigger RNAi-unspecific and sequence-independent events, such as immune system stimulation and even saturation of the endogenous RNAi machinery, both of which may negatively affect the organism (2,13). Importantly, the scenario where a dsRNA affects non-target species in the environment needs to be addressed.

Besides hazard identification and characterization, risk assessment relies upon exposure assessment. DsRNA environmental persistence is influenced by various ecological factors, including microbial activity, levels of ultraviolet (UV) exposure, and the composition of the soil in which it is applied (2,3). While dsRNA typically undergoes rapid degradation in agricultural soils (e.g. DT90<35 h) (14) and aquatic microcosms (e.g. DT90 <96h) (15) its stability may be greatly increased upon the use of formulations such as LDH nanoclays, carbon dots, chitosan etc. (16). Such formulations are meant to increase dsRNA's bioactivity (stability and uptake) but may also increase

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bioavailability to non-target organism or exhibit toxicity themselves. Evidently, the impact of formulation substances needs to be addressed in future risk assessment schemes, where (eco)toxicological tests and environmental persistence assays should (also) be performed with a given dsRNA in its formulated form.

Indeed, the OECD document highlights the necessity of understanding the stability and bioavailability of dsRNA in environmental contexts. These factors play a pivotal role in determining non-target organisms exposure levels and overall risk profiles associated with the use of dsRNA products (1,10). To this end, novel methods for accurate and specific dsRNA quantification need to be developed. So far, detection and quantification of dsRNA pesticides is achieved by QuantiGene assay, a hybridization probe-based method that allows the direct measurement of dsRNA molecules, using signal amplification rather than target amplification. Yet, although specific and sensitive, this method exhibits low versatility, since, for each new sequence/dsRNA, newly customized probes need to be designed, synthesized and provided to the user; moreover, the availability of expensive equipment such as Luminex instruments for the detection of the signal is required. But analytical methods, intended to support regulatory decision making, require a high level of standardization and versatility. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is a widely used method to detect and quantify different types of transcripts, such as messenger RNA, ribosomal RNA, non-coding RNA etc. As a method, RT-qPCR is characterized by established standardization protocols, reproducibility, versatility of detecting a wide range of dsRNA concentrations in divergent environmental matrices and low reaction costs. Development of personalized PCR-based protocols adjusted to the technical challenges of isolation and quantification of dsRNA from environmental samples will most likely prove extremely helpful. Coupling RT-qPCR assays with small RNA sequencing where detection of dsRNA degradation products such as small RNAs is achieved would provide the most stringent data on dsRNA (and its active degradation intermediates) persistence.

The potential effects of dsRNA targeting essential and highly conserved genes on non-target organisms, particularly beneficial species such as pollinators and essential soil organisms, remain a significant area for ongoing research. These considerations highlight the pressing need for thorough and comprehensive risk assessments that consult a wide range of parameters, including environmental persistence, degradation rates, uptake by non-target species, their susceptibility to RNAi, and the likelihood of off-target effects. This multifaceted approach is essential to ensure that dsRNA-based pesticides can be used safely and sustainably in modern agricultural practices (2,3).

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ADVANCES IN RESEARCH AND APPLICATIONS

Recent developments in dsRNA-based pesticides are showing remarkable potential, but also its limitations in the pest management landscape. Field trials have provided compelling evidence of the effectiveness of dsRNA against notorious pests such as the Colorado potato beetle, whereas other pest groups such as lepidopterans and hemipterans are largely not amenable to RNAi under applied conditions (yet). Notably, these innovations cause minimal collateral damage to non-target species, emphasizing their ecological safety. Furthermore, dsRNA has shown the potential for not only controlling pest populations but also for tackling the pathogens they carry, which may significantly broaden its role in integrated pest management strategies.

A key driver of this progress is the innovation in formulation technologies, particularly the development of nanoparticle-based delivery systems and bioclay formulations. These novel approaches have dramatically improved the stability and bioavailability of dsRNA in agricultural settings (17).

One of the primary challenges faced by dsRNA-based pesticides is ensuring their effectiveness across varying environmental conditions, including fluctuations in UV exposure and microbial activity. Research has indicated that nanoparticles can shield dsRNA from accelerated degradation while simultaneously enhancing its uptake by insect pests, thus boosting its overall efficacy. Additionally, Bioclay formulations have been successfully utilized to stabilize dsRNA, further improving its performance in outdoor environments (17).

Despite the encouraging advancements in dsRNA formulation and field applications, the pathway to regulatory approval remains a vital hurdle. The approval of Calantha/Ledprona by the EPA marks a significant milestone, underscoring the ability of dsRNA-based products to conform to stringent safety and efficacy standards. This case serves as a crucial reference point for other regulatory bodies, illustrating the necessity for robust yet adaptable regulatory frameworks capable of accommodating these innovative pesticide solutions.

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RECOMMENDATIONS FOR COMPREHENSIVE RISK ASSESSMENT

In light of the growing interest in deploying dsRNA as a pesticide, it is crucial to establish a robust framework for risk assessment that ensures both safety and efficacy in the long term. Several key recommendations must be meticulously considered:

Hazard identification and characterization:
Develop appropriate bioinformatic tools that will provide detailed and in-depth analysis of the potential for unintended effects by comparing dsRNA sequences with genomic and transcriptomic databases of non-target organisms. This analysis could be considered a basis for planning testing strategies of given dsRNA molecules destined for use in plant protection.

Determination of Envrionmental fate:
Assess the environmental persistence and degradation of dsRNA under various conditions (e.g., UV exposure, microbial activity, soil type) to understand its stability, degradation kinetics, and potential for accumulation. Additionally, study its environmental fate, including mobility through runoff, leaching, or atmospheric deposition, and identify any degradation products that may pose ecological or human health risks.

The potential of mathematical models, currently available for conventional chemical pesticides, to determine environmental exposure levels for dsRNA molecules should be evaluated and new tools might be required in case low prediction accuracy is shown.

Non-target and Ecological Impact Assessment:
To address the unique characteristics inherent to dsRNA molecules, the adaptation of standardized testing protocols may be necessary. While the existing OECD test guidelines offer a variety of standard test species, it is important to verify whether potentially susceptible species identified through bioinformatics analysis are included, especially those related to ecologically significant organism groups and phylogenetically similar organisms. Investigate potential trophic transfer and potential long-term effects due to epigenetic effects.

Resistance Management and Human Health:
Monitor target pest populations for resistance development and assess gene silencing efficacy to ensure continued effectiveness. Evaluate potential human exposure routes (inhalation, dermal contact, ingestion)

and conduct toxicological studies to assess immunogenicity, allergenicity, and other adverse effects, particularly for agricultural workers and consumers.

Investment in Training and Capacity Building:
Regulatory agencies must prioritize the enhancement of their scientific and technical understanding of RNA interference mechanisms and the potential ecological ramifications of dsRNA applications. This investment entails providing targeted training programs and resources for regulatory personnel and stakeholders in the agricultural sector. An emphasis on interdisciplinary collaboration is vital, fostering partnerships between regulatory bodies, scientific researchers, and industry representatives. Such cooperation will ensure comprehensive evaluations of dsRNA products that consider both the direct impacts on target pests and the possible indirect effects on ecosystem dynamics, including predator-prey interactions and shifts in community structure.

Promoting Public Engagement and Transparency in dsRNA technology development and application:
For dsRNA-based pesticides to gain widespread acceptance, it is essential to foster public engagement and transparency in their development, assessment and use. Openly communicating the benefits, potential risks, and underlying science of dsRNA products is fundamental to building public trust. Engagement strategies should include informative outreach efforts directed at various stakeholders, including farmers, environmental groups, and the general public. Additionally, the establishment of open-access databases that share data on dsRNA sequences, risk assessments, and research findings will facilitate informed dialogue and collaborative efforts. By ensuring that the development of dsRNA products is transparent and participatory, the agricultural community can promote responsible usage and mitigate concerns regarding safety and environmental impact.

By implementing these detailed recommendations, the journey toward safe and effective utilization of dsRNA-based pesticides can be navigated with greater confidence and responsibility.

This project has received funding from the European Union's Horizon Europe Research and Innovation programme under Grant Agreement No. 101084163. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

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LITERATURE

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