ABSTRACTS

OECD Conference on RNAi based pesticides

10-12 April 2019

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Abstracts

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Introductory session

Welcome by the CRP and introduction of the Programme

András Székács – CPR Scientific Advisory Body; National Agricultural Research and Innovation Centre, Hungary

The OECD Co-operative Research Programme: Biological Resource Management for Sustainable Agricultural Systems (CRP) funds cutting-edge research on food, agriculture, fisheries and forestry with a focus on global issues such as sustainability, food security and nutrition, climate change and the inter-connectedness of economies through trade and scientific co-operation. The CRP helps achieve globally agreed policy objectives by facilitating international co-operation among research scientists and institutions. In doing so, it strengthens scientific knowledge and innovation.

With this focus on global issues, CRP-funded research generates benefits for people around the world. To deliver on these overarching challenges, the CRP has three themes into which funding proposals need to fit: Theme 1. Managing natural capital for the future; Theme 2. Managing risks in a connected world; Theme 3. Transformational technologies and innovation.

It achieves its objectives through two distinct activities:

• The CRP funds short-term research projects for individual scientists in other CRP member countries by providing travel bursaries to strengthen the exchange of ideas and increase international co-operation.

• The CRP sponsors international conferences and workshops to keep policy makers, industry and the academic world informed of innovative research, scientific developments and opportunities.

The CRP places a policy emphasis on all the activities it funds, and the findings from the activities provide valuable evidence and information to support policy makers in promoting the sustainable use of natural resources in food, agriculture, fisheries and forestry.

Find out more by visiting the CRP website: www.oecd.org/agriculture/crp.

Session 1: Summary of the State of the Art: dnRNA-based Product Use in Agriculture

An introduction to RNAi technology

Petr Svoboda – Institute of Molecular Genetics of the ASCR, Czech Republic

RNA interference (RNAi) refers to the selective degradation of mRNA induced by double-stranded RNA (dsRNA), first discovered *in Caenorhabditis elegans*. Related silencing pathways are present in almost all eukaryotes. The common feature of all RNA silencing pathways is utilization of small RNA molecules (20-30 nucleotides long), which form ribonucleoprotein complexes with proteins from the Argonaute family. In addition to Argonaute proteins, biogenesis of small RNAs often (but not always) involves activity of RNase III Dicer. Small RNAs in RNA silencing come from many different sources and have diverse biological roles such as regulation of protein-coding genes (genome-encoded microRNAs), innate antiviral immunity (RNAi) or genome protection by repression of mobile elements (RNAi and piRNA pathway in animals). Consequently, the ability of small RNAs to induce a sequence-specific gene silencing has been adapted for specific gene silencing in cultured cells and *in vivo* and became a common experimental strategy, which is being explored also in therapeutic and biotech applications. Importantly, achieving efficient and highly specific silencing requires appropriate experimental design and controls because artificial induction of RNAi may be accompanied with undesirable effects, particularly off-targeting where repression of other genes occurs in addition to the targeted one. In my contribution, I will provide an overview of the molecular mechanism of RNAi and relevant related pathways, will describe basic strategies for silencing using RNAi, and discuss potential non-specific silencing issues and available remedies.

Potential for dsRNA-based management of plant diseases

Karl-Heinz Kogel, Justus Liebig University Giessen, Germany

In plants, small RNA (sRNA)-mediated RNA interference (RNAi) is essential for regulating host immunity against bacteria, fungi, oomycetes, viruses, and pests. Similarly, sRNAs from pathogens and pests also play an important role in modulating their virulence and in developing a disease. Most strikingly, recent evidence supports that some sRNAs can travel between interacting organisms and induce gene silencing in the counter party, a mechanism termed cross-kingdom RNAi.

Our research aims at exploiting the natural role of RNA in plant - microbe /pest interactions as a blue print for novel plant protection strategies based on RNA (for *rev.* Cai et al. 2018, doi 10.1016/j.mib.2018.02.003). In recent years, several reports showed that double-stranded (ds)RNA has the potential to become an important tool for disease-control. In general, RNA can be used in two different technology application strategies. One strategy uses the expression of dsRNA in crop plants, where the dsRNA sequence is partially identical to a devastating target microbial pathogen or pest. This technology is called host-induced gene silencing (HIGS) and has shown unprecedented effectiveness in reducing pest and diseases; traits based on the new technology (such as the dsRNA-producing DvSnf7 transgenic corn) have already been approved by the United States Environmental Protection Agency (EPA) and by the Canadian Food Inspection Agency (CFIA). Alternatively, dsRNAs have been used to control pest and diseases by direct spray applications. Direct application of dsRNAs or sRNAs onto host plants or post-harvest products leads to spray-induced gene silencing (SIGS) of the target microbe/pest by environmental RNAi and may confer efficient disease control. Yet, few examples have been reported in literature. My talk will review the current understanding of cross-kingdom RNAi, its implications for HIGS and SIGS and how these findings can be developed into novel effective strategies to fight diseases caused by microbial pathogens and pests.

RNA interference technologies to control pests and pathogens

Steve Whyard, University of Manitoba, Canada

Chemical pesticides are widely used to protect crops from pests and pathogens and to reduce the spread of insect-borne disease. Increasing incidences of pesticide resistance and growing concerns about the adverse effects of broad-spectrum chemicals on non-target species is driving the need to find safer and more effective alternatives for pest control. RNA interference (RNAi), with its in-built sequence-specific capabilities, is viewed by many research groups as a potentially species-specific method of pest and pathogen control. My research team has been developing a variety of RNAi-based methods to control crop insects, fungal pathogens, and disease-vectoring mosquitoes. Using topically-delivered ingestible dsRNA-based insecticides, we have demonstrated effective control of flea beetles in greenhouse settings, and are now conducting field trials to assess the technology under natural environmental conditions. We are examining the species-specificity of these dsRNAs on non-target species, to ensure that the dsRNAs do not adversely affect beneficial species in the canola farming landscape. We have also been developing topically-applied dsRNA-based fungicides to protect canola against pathogenic fungi. Small-scale field trials will be initiated to assess the dsRNA's effectiveness and specificity to control the pathogens without impacting beneficial fungi within the cropping system.

In addition to developing insecticidal formulations of dsRNA, we are also developing RNAi-based methods of producing sterile male insects for Sterile Insect Technique (SIT) programs. To date, we have focused our efforts on the mosquito *Aedes aegypti*, and on the fruit fly, *Bactrocera tryoni*. Through some of our advances in dsRNA delivery formulations, we have observed improved efficacy of RNAi in these insects, and believe that there is potential for this method of sterilization to complement existing methods to produce sterile males for other SIT programs. The prospect of taking this technology to the field and the various challenges that it will face will be discussed.

RNAi as a novel technology in pest control: current status and challenges

Olivier Christiaens, Ghent University, Belgium

Over the past decade, RNA interference (RNAi), the sequence-specific suppression of gene expression triggered by specific double stranded RNA (dsRNA) molecules, has proven to be a very promising strategy in crop protection. Some of the most alluring aspects of this technology are its species-selectivity and the short persistence in the environment of the dsRNA. Here, we will give an overview of some of the promising results in insect control and the different application strategies, which could be used, in the field, including host-induced gene silencing (HIGS), spray-induced gene silencing (SIGS) and virus-induced gene silencing (VIGS).

Furthermore, we will also address associated sequence-dependent and –independent biosafety aspects and some of the important challenges and barriers that need to be addressed before RNAi could be implemented as a widely used pest control strategy. One of these challenges is a variable efficiency. While some insects show a very robust, efficient and systemic RNAi response, many others show a limited or variable RNAi response. Possible causes for this variability in sensitivity are degradation of the dsRNA in the insect body, insufficient uptake into the cells, an impaired endosomal release, viral interactions or problems with the RNAi machinery in the cells. In recent years, many efforts have been made to increase RNAi-efficiency in some insensitive insects, using different formulations, which could be vital in future sprayable dsRNA applications. An overview of these will be given, with special attention to the use of formulations improving cellular uptake and the dsRNA persistence in the insect body.

Finally, we will also briefly discuss a recent EFSA report which was co-produced by our group, which gave an overview of (cellular) uptake of dsRNA, potential adverse effects, exposure routes, RNAi efficiency in insects and the question whether or not bioinformatics could ever have a role to play in environmental risk assessment of RNAi-based products.

Variation in responsiveness to environmental dsRNA in insects

Ana María Vélez Arango, University of Nebraska, United States

RNA interference (RNAi) is a sequence-specific regulation of gene expression triggered by the presence of double-stranded RNA (dsRNA). RNAi has been explored as a pest management tool for the control of insect pest for the last 12 years. For RNAi to work as an insect management tool, the insect must uptake dsRNA from the environment to regulate endogenous gene expression. The responsiveness to environmental dsRNA in arthropods is highly variable. This variability affects the ability to target different insects orders and the potential effects on non-target species. Coleopterans have shown to be the most susceptible species to environmental dsRNA, while the susceptibility of insects within the orders Hemiptera, Hymenoptera, Diptera, and Lepidoptera is variable. Differences in the response among species have been attributed to the presence of RNases, variations in the processing of dsRNA within the cell, and environmental factors. This presentation will provide an overview of the research to date investigating the factors that affect the responsiveness to environmental dsRNA in different insect orders.

Potential for off-target effects in topically applied dsRNA-based products used for crop protection purposes

Gunter Meister, University of Regensburg, Germany

For efficient gene inactivation, siRNAs are either exogenously applied or expressed as double stranded RNA (dsRNA) precursors from endogenous sources. SiRNAs are 21 nucleotides in length and one strand is incorporated into the RNA-induced silencing complex (RISC) and becomes the guide strand (also referred to as the antisense strand). It directly binds to a member of the Argonaute protein family (Ago proteins) and guides it to fully complementary sequences on target RNAs. A catalytically active Ago protein (Ago2 in mammals, for example) cleaves the complementary target RNA, which is subsequently removed from the cell. This process is generally referred to as RNA interference or short RNAi.

SiRNAs, however, can have off-target effects and target unrelated genes directly or indirectly. Known causes of off-target effects in animals will be summarized and reviewed in the talk. In many animal species, siRNAs mimic endogenous microRNAs (miRNAs) and utilize the miRNA machinery. MiRNAs are incorporated into RISC as well but do not guide it to fully complementary target sequences. Instead, miRNAs bind to only partially complementary sequences and induce mRNA de-adenylation, decapping and decay of the mRNA. The so-called 'seed sequence' spanning nucleotides 2-8 of the miRNA is sufficient to achieve efficient gene knock down. Most siRNAs that are applied possess unwanted seed-matched targets in the cell. In fact, based on our own analysis but also on published results, siRNA screening datasets in human cells are highly dominated by 'miRNA-like' seed matches and thus off-target effects. These data will be presented in the talk. One possibility to overcome such off-target problems, are siRNA-pooling strategies. This allows for a dilution of effects that are specific to individual siRNA sequences. In nematodes and insects, for example, where long double stranded RNA is used as RNAi trigger, such pools naturally occur and seed sequence-based off-target effects are most likely not as problematic as in higher animals such as mammals.

Environmental dissipation of dsRNA in soil, aquatic systems and plants

Pam Bachman, Bayer Crop Science

Determining the rate of biodegradation of double-stranded RNA (dsRNA) in the environment is an essential element of a comprehensive environmental risk assessment (ERA) of an RNA-based agricultural product. This information is used during problem formulation to define relevant routes and durations of environmental exposure for *in planta*–expressed and topically applied dsRNA based on the use pattern of the product. This presentation will examine the technical approaches and results of quantification of dsRNA in terrestrial, aquatic, and plant matrices in context of an ERA. These evaluations include a QuantiGene® assay to quantify the amount of dsRNA, and insect bioassays to measure functional toxicity where appropriate. Although exposure to these dsRNA products in terrestrial and aquatic environment is predicted to be minimal, little is known regarding the fate of dsRNA in these environments. To assess exposure to terrestrial and aquatic environments, a series of studies were conducted to measure the rate of biodegradation of dsRNAs in terrestrial soils and aerobic water–sediment systems. Results support the conclusion that dsRNA-based agricultural products rapidly degrade and consequently are unlikely to persist in terrestrial and aquatic environments. Data are also available that demonstrate barriers to the meaningful uptake of foliarly applied dsRNA, effectively exposing the dsRNA to degradation factors in the environment. Finally, data from *in planta*-expressed dsRNA demonstrates a quantifiable low level of residue can be discerned to inform the regulatory risk assessment.

Validation of RNA interference by RNA-Seq: How to see the big picture

Brenda Oppert, United States Department of Agriculture, United States

Targeting genes via RNA interference (RNAi) has become a successful method to reduce some pest insect populations. Ideally, pest-specific gene expression is targeted with dsRNA to disrupt a gene critical for life function, via spray or oral delivery. Experts have developed working guidelines for the development and regulation of RNAi as a pesticide. In this summary, I argue that an important step in the validation of RNAi is understanding global gene expression in the target pest before and after dsRNA is introduced via RNA-Seq. To support this hypothesis, I provide data from our studies of RNAi in the coleopteran model Tribolium castaneum. These studies have led to new discoveries of insect responses to injected dsRNA, including transcriptome compensation responses that are similar to what has been observed at the protein level. Gene expression compensation can mask RNAi responses and thus can be an obstacle in predicting efficacy of dsRNA treatment. We also have observed differential expression of 52 long noncoding RNAs following knockdown of a cuticle protein gene ($p<0.01$), providing insight into the mechanism of knockdown on overall gene expression. In some cases, we identified new gene interactions that were previously unassociated with the target gene. For example, knockdown of aspartate 1-decarboxylase, a cuticle sclerotization gene, caused increased expression of a dopamine receptor and consequently reduced mobility in RNAi-treated insects. These data emphasize the importance of using RNA-Seq as a tool to understand known and unknown effects of target gene knockdown. This information can be used to predict genetic changes that will impact the efficacy and long-term durability of RNAi products in target pests.

Dietary uptake of environmental dsRNA in humans and other vertebrates

Thais B Rodrigues, Greenlight Biosciences, United States

Products incorporating double-stranded RNA (dsRNA) as the active ingredient have emerged as environmentally friendly biological-based solutions for pest control. These products function by engaging the endogenous RNA interference (RNAi) pathway present in most eukaryotic organisms to silence expression of a specific gene in the target pest. This sequence-dependent mode-of-action contrasts sharply with the broad-spectrum activity of traditional synthetic insecticides and enables development of products designed to kill a specific target pest without impacting non-target organisms. This selective activity, coupled with the rapid degradation of dsRNA in the environment, support a favorable overall safety profile. As a non-transformative spray, dsRNA has the potential to be used in a variety of agricultural applications, ranging from control of specific pests and pathogens on diverse crops, to protection of bees from viral infection. As a new mode of action, dsRNA is also well positioned to add an additional tool to existing pesticide resistance management strategies. However, as with any emerging technology, the potential range of future products, potential future regulatory frameworks, and public acceptance of the technology are all evolving topics. The aim of this presentation is to summarize the published literature related to dietary uptake of external dsRNA in humans and other vertebrates, providing a solid foundation for focused discussion sessions and panel discussions that will help draw recommendations and inform further improvements to regulatory processes. Information gathered from studies examining the effect of dietary uptake of external RNAs by mammals and other vertebrates will be summarized to review our current understanding of the potential impacts and risks on these non-target organisms. Mammalian barriers to uptake and systemic distribution of dietary dsRNA, such as RNA degradation in the digestive system, will be discussed. We will also discuss the potential of ingested RNAs to regulate specific metabolism in mammals, as well as the influence of human health conditions on susceptibility to RNA uptake.

Regulatory experience with antisense oligonucleotides for human use

Frank Holtkamp, Medicine Evaluation Board, The Netherlands

The European Medicines Agency (EMA) is a decentralised agency of the European Union (EU) responsible for the scientific evaluation, supervision and safety monitoring of medicines in the EU. For new medicinal products submitted for marketing authorisation, the EMA reviews whether a positive benefit risk balance can be identified to recommend for possible approval within the EU. EMA has reviewed several antisense oligonucleotides (AONs) submitted for marketing authorisation. These AONs are currently primarily focused on treating rare occurring diseases caused by a genetic problem, therefore the available clinical safety data is relatively limited. Current knowledge on the safety aspects of these AONs will be presented and discussed.

Panel Discussion Session 1

Moderator: Achim Gathmann, Federal Office of Consumer Protection and Food Safety (BVL), Germany

DAY 2

Session 2: Summary of Regulatory & Risk Assessment Experience with dsRNA-based Products

Problem formulation considerations for externally applied dsRNA-based products

Alan Raybould, Syngenta

Risk comprises the probability and severity of harm that may result from undertaking an activity, such as applying a pesticide. Risk is high when severe effects are likely and low when harmful effects are predicted to be rare or trivial, or both. Estimates of risk contribute to decisions about whether to take certain actions; other relevant factors may be estimates of the likely benefits of those actions and the risks from not taking action. Efficient and effective risk assessment relies on problem formulation, which includes several vital steps: 1. agreement on what effects should be regarded as harmful; 2. formulation of hypotheses about how the proposed activity may lead to such harmful effects; 3. tests of those hypotheses with existing data; and 4. a plan to acquire new data for hypothesis testing should tests with existing data be insufficient for decision-making. By beginning with defining harm and how it may be caused by the proposed activity, problem formulation encourages the development of decision-making criteria early in the risk assessment; in effect, risk assessment becomes a test of a hypothesis that the proposed activity does not pose unacceptable risk. This approach may be particularly valuable when assessing uses of innovative products, such as those with new modes of action, where distinguishing between basic research hypotheses ("nice to know") and hypotheses about decision-making criteria ("need to know") may be more difficult than for more familiar products. We illustrate how problem formulation can guide risk assessments for spray applications of insecticides containing dsRNA active ingredients that induce RNAi in target insects.

Ecological assessment of topically applied dsRNA-based products

Jörg Romeis[1], Franco Widmer[2], [1] Research Division Agroecology and Environment, [2]Competence Division Method Development and Analytics, Agroscope, Zurich, Switzerland

RNA interference (RNAi) is an emerging and powerful technology that offers new opportunities for pest control through the silencing of target genes in arthropod pests. Because the RNAi effect is sequence-specific, dsRNA can be designed to have a very narrow spectrum of both activity and target organisms, thus allowing very targeted pest control. While successful RNAi has been reported from a number of arthropod species belonging to various orders, the impact of environmental RNAi (i.e., an RNAi response after dsRNA uptake) is more limited. However, RNAi in pest control may be achieved by applying dsRNA in foliar sprays.

One of the main concerns related to the use of dsRNA in pest control is that it could cause adverse effects on the environment and in particular on valued non-target species. Arthropods form a major part of the biodiversity in agricultural landscapes and contribute to important ecosystem services. This includes, in particular, regulating services such as pollination and biological control of herbivores, supporting services such as nutrient cycling, and cultural services in the case of species of conservation concern. Consequently, environmental risk assessment (ERA) to assess the potential impacts that plant protection products may have on valued non-target arthropods is legally required prior to their placement on the market.

Early in the ERA, problem formulation is used to set the problem context and to develop plausible pathways on how the application of plant protection products containing dsRNA could harm valued non-target arthropod species. In this presentation, potential pathways will be presented and testable risk hypotheses will be identified.

Furthermore, suggestions will be presented on how these hypotheses can be tested in a proportionate and tiered manner.

Review of EFSA's activities on the risk assessment of RNAi-based GM crops

Nikoletta Papadopoulou, Fernando Álvarez-Alfageme, Yann Devos, Anna Lanzoni, Claudia Paoletti, Elisabeth Waigmann, European Food Safety Authority, EU

Genetically modified (GM) plants intended for market release can be designed to induce silencing of specific genes *in planta* or in target pests through RNA interference (RNAi). As part of the pre-market risk assessment (RA), the European Food Safety Authority (EFSA) evaluates any risks that GM plants may pose to the animal and human health and the environment. Potential risks associated with the use of RNAi in GM plants were considered at an international scientific workshop organised by EFSA. Experts from academia, RA bodies and the private sector discussed the biology underlying the RNAi mechanisms, current and future applications of RNAi-based GM plants, and RA approaches. The outcome of the workshop helped determine in which areas the existing approaches for RA are appropriate, and whether complementary or alternative RA strategies need to be developed for RA of RNAi-based GM plants (EFSA, 2014; Ramon et al., 2014, Casacuberta et al., 2015). In addition, limitations in methods to unequivocally identify potential off-target effects, and remaining scientific uncertainties on the likelihood of exposure of humans, animals and the environment to dsRNA and deriving small RNAs (miRNA, siRNA) were addressed at the workshop. To further address these issues, EFSA commissioned separate external scientific reports, in which relevant scientific literature has been reviewed to inform food and feed and environmental risk assessment of RNAi-based GM plants (Paces et al., 2017; Christiaens et al., 2018). Furthermore, the EFSA GMO Panel, considering the available scientific information and the technical limitations for small RNA off-target bioinformatics studies, developed a strategy on how to identify and assess plant off-target effects in RNAi-based GM plants[1], which was recently implemented on the safety assessment of DvSnf7 dsRNA-expressing GM maize (EFSA GMO Panel, 2018).

1) European Food Safety Authority, 2014. International scientific workshop 'Risk assessment considerations for RNAi-based GM plants' (4–5 June 2014, Brussels, Belgium). EFSA supporting publication 2014:EN-705, 38 pp.

2) Ramon M., Devos Y., Lanzoni A., Liu Y., Gomes A., Gennaro A. and Waigmann E., 2014. RNAi-based GM plants: food for thought for risk assessors. Plant Biotechnology Journal, 12 (9): 1271-1273.

3) Casacuberta J.M., Devos Y., du Jardin P., Ramon M., Vaucheret H., Nogué F. 2015. Biotechnological uses of RNAi in plants: risk assessment considerations. Trends Biotechnology, 33(3):145-147.

4) Paces J, Nic M, Novotny T and Svoboda P, 2017. Literature review of baseline information to support the risk assessment of RNAi-based GM plants. EFSA supporting publication 2017:EN-1246, 314 pp. https://doi.org/10.2903/sp.efsa.2017.en-1246.

5) Christiaens O, Dzhambazova T, Kostov K, Arpaia S, Joga MR, Urru I, Sweet J, Smagghe G, 2018. Literature review of baseline information on RNAi to support the environmental risk assessment of RNAi-based GM plants. EFSA supporting publication 2018:EN-1424. 173 pp. doi:10.2903/sp.efsa.2018.EN-1424

6) EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H,Birch AN, Casacuberta J, De Schrijver A, Gralak AM, Guerche P, Jones H, Manachini B, Messean A,Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Ardizzone M,De Sanctis G, Fernandez Dumont A, Gennaro A, Gomez Ruiz JA, Lanzoni A, Neri FM, Papadopoulou N, Paraskevopoulos K and Ramon M, 2018. Scientific Opinion on the assessment of genetically modified maize MON 87411 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-124). EFSA Journal 2018;16(6):5310, 29 pp.

[1] Annex II of the minutes of the 118th GMO plenary meeting https://www.efsa.europa.eu/sites/default/files/event/171025-m.pdf

The European perspective on regulatory aspects and experiences with dsRNA-based products

Achim Gathmann, Federal Office of Consumer Protection and Food Safety (BVL), Germany

RNA interference (RNAi) is a means of reducing or switching-off the expression of individual genes, often described as 'gene silencing'. RNAi is a natural process with important defence and regulatory functions in animals, plants and fungi. RNAi technology is widely used in GM plants. Sprayable dsRNA-based plant protection products are in the pipeline aiming at different targets such as flea beetles in oil seed rape, fusarium diseases in barley, or weed control to overcome resistant weeds.

RNAi constitutes a new mode of action in "conventional plant protection products". In the past, the authorisation process of plant protection products focused on chemical substances. This new type of products places new demands on the authorisation process. While the characteristics of dsRNA as active ingredient might ease the assessment of some risk areas, for other risk areas there is a need for adaptions of existing or the development of new risk assessment tools. Additionally, new formulations to resist rapid degradation of dsRNA after product application such as liquid encapsulation, conjunction with polymers or nanoparticles might challenge the risk assessment.

The presentation will give a short introduction into the authorisation process in the EU. Some aspects regarding specific challenges for risk assessment and risk management of this new type of plant protection product will be highlighted. A short view on activities and experiences gained in the assessment of GM plants using RNAi mechanisms in Europe will complement the presentation considering similarities and differences in risk assessment of biotechnical and classical plant protection products

Ecological Risk Assessment Considerations for in planta Expressed and Exogenously Applied dsRNA at the U.S. EPA

Shannon Borges, US Environmental Protection Agency, United States

Double-stranded RNA (dsRNA)-based pesticides require special considerations for ecological risk assessment (ERA) because of their unique nature and mode of action. The U.S. EPA has developed problem formulations for dsRNAs expressed in planta and applied exogenously. These problem formulations were presented to the FIFRA Science Advisory Panel (SAP) in 2014, and relevant recommendations by this SAP were applied to the ERA for the DvSnf7 dsRNA expressed in planta in corn (a plant incorporated protectant [PIP]). This ERA relied primarily on the results of bioassays, but also considered other lines of evidence, including environmental fate in soil and water, barriers to dsRNA uptake in vertebrates, bioinformatics analyses, and bioassays to determine specificity to the target pest. Additional bioassays beyond the standard data set for PIPs were required to assess the potential for unintended effects, such as off-target gene silencing. The EPA determined that this DvSnf7 dsRNA corn PIP did not present risks to non-target organisms or the environment. While the EPA has not yet conducted an ERA specifically for an exogenously applied dsRNA-based pesticide, many of the lessons learned from this experience are applicable to exogenously applied dsRNA-based pesticides.

A perspective on risks associated with dsRNA-based products

Neena Mitter, Centre for Horticultural Science, Australia

By 2050, it is estimated that up to 10 billion people will populate the planet and in order to meet their daily need of two trillion calories the global agriculture industry will have to sustainably increase production by 70%. The emerging concept of pre- and post-harvest topical application of dsRNA to trigger gene silencing in pest and pathogens offers a paradigm shift in agriculture that promises to contribute to this need. As with any new technology, there are potential risks, including to human health, the environment, and trade. The regulation of dsRNA-based topical products needs to be viewed differently from GM crops. Importantly, the Office of the Gene Technology Regulator (OGTR) in Australia has proposed that topically applied dsRNA be exempt from GMO regulations. The development of dsRNA-based crop protection products leveraging the naturally occurring RNAi pathway share the history of exposure to, and consumption of, small RNAs. The registration of dsRNA-expressing crops such as western corn rootworm-resistant maize provides some regulatory synergy in this area. The presence and persistence of dsRNA in environmental matrices as well as the exposure and uptake by non-target organisms

(NTOs) need consideration. Some of these risks are addressed by available information on barriers to exposure and cellular uptake, potential degradation of dsRNA, instability of dsRNA in soil/environment and within the recipient organism, and the inherent sensitivity of the organism to ingested dsRNA. Bioinformatics-based design to avoid off-target impacts in NTOs mitigates risks further. Potential risks associated with formulation ingredients including carriers for delivering external dsRNA should be examined on a case-by-case basis. Finally, early engagement with all stakeholders will be paramount to acquire consumer acceptance and public support given the fundamental advantages of the new technology.

Session 3: Discussion themes

Background to the draft OECD Working Paper on 'Environmental Risks from the Application of dsRNA-Based Pesticides'

Les Davies,OECD Consultant, Canberra, Australia

The purpose of the working paper is to (1) provide an overview of available scientific information on RNAi relevant to externally-applied dsRNA-based pesticides (including basic biological mechanisms of RNAi, the environmental fate and behaviour of dsRNA molecules, and the possible impacts of small RNAs on non-target organisms); and 2) highlight issues for consideration by regulatory authorities in conducting environmental risk assessments of dsRNA-based pesticide products. The document is organised into sections, which provide an overview of available scientific information relating to RNAi, regulatory experience to date with pesticide products involving an RNAi mechanism, and considerations regarding environmental risk, including environmental fate and non-target effects. While not directly focussing on the risk assessment of plants which have been genetically modified to incorporate the machinery to synthesise dsRNA directed against pest species, regulatory history and risk considerations for in-planta RNAi are discussed in the context of externally-applied dsRNA-based pesticides.

The initial draft was prepared by Les Davies, OECD consultant, and was based on publicly-available scientific and regulatory information.

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This event is supported by the OECD Co-operative Research Programme.