



DvSnf7 in Event MON 87411 PIP Case Study

Nina Ortiz, Ph.D., Biologist
Emerging Technologies Branch
Biopesticide and Pollution Prevention Division
EPA Office of Pesticide Programs

EPA regulates pesticidal traits in plants

- ▶ Plant-incorporated protectants (PIPs)
 - ▶ PIPs are comprised of the pesticidal substance produced and used in the living plant and the genetic material necessary for its production.
 - ▶ Also included in the regulatory definition are any inert ingredients present in the plant used to confirm the presence of the pesticidal substance, e.g., herbicide resistance trait.
 - ▶ PIPs can be naturally occurring in plants and moved by breeding or engineering
- ▶ Two overarching statutory authorities
 - ▶ Under FIFRA: EPA evaluates pesticides including PIPs for their effects on the environment and human health and regulates their development, sale, distribution, and use.
 - ▶ Under FFDCA: EPA evaluates PIPs that are proposed for use in food or feed. In its assessment the Agency considers all anticipated dietary exposures, residential and other outdoor uses.

MON 87411 as found in SmartStax PRO

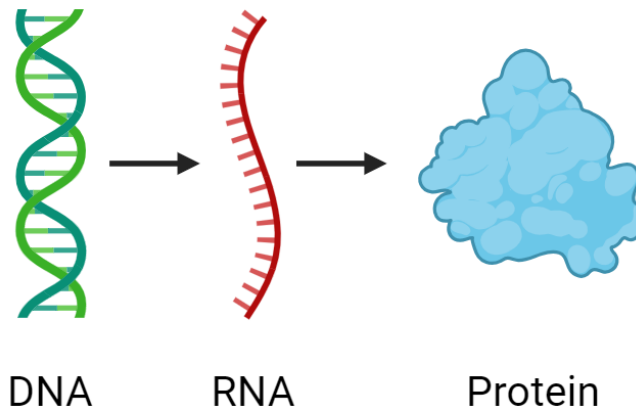
- ▶ Corn plant incorporated protectant (PIP) developed for the control of corn rootworm
- ▶ Event MON 87411 contains two pesticidal active ingredients:
 - ▶ Cry3Bb1
 - ▶ protein derived from the bacterium *Bacillus thuringiensis*
 - ▶ DvSnf7 dsRNA
 - ▶ Double-stranded ribonucleic acid (dsRNA) transcript comprising a DvSnf7 inverted repeat sequence from western corn rootworm (*Diabrotica virgifera virgifera*)
- ▶ This presentation will focus on DvSnf7 dsRNA and EPA's human health and environmental risk assessments

RNAi-based PIPs

- ▶ RNAi (RNA interference) works as a pesticide by silencing the activity of a targeted gene
- ▶ DvSnf7 dsRNA in MON 87411 is the first RNAi-based PIP to target an insect pest.
- ▶ Prior to MON 87411, commercial registrations of RNAi-based PIPs had targeted plant pathogens:
 - ▶ Honeysweet plum (plum pox virus)
 - ▶ X17-2 papaya (papaya ringspot virus)
 - ▶ New Leaf Plus potato (potato leaf roll virus)
- ▶ Understanding how DvSnf7 dsRNA in MON 87411 exerts a pesticidal effect on CRW requires a brief overview of molecular biology

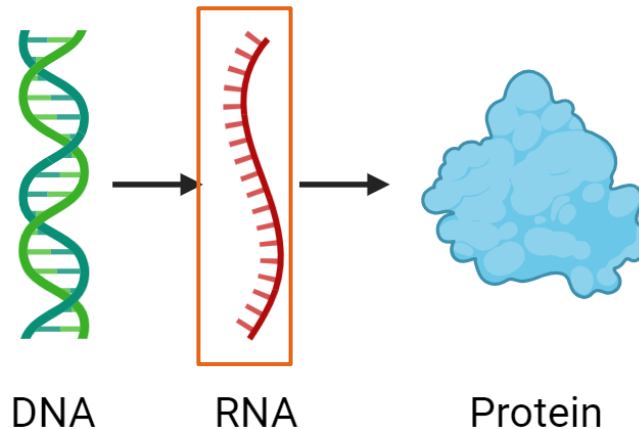
dsRNA Background

- ▶ Generally speaking, living organisms require three different types of molecules to perform key functions in the cells:
 - ▶ DNA - encodes all of the genetic instructions to carry out the functions necessary to life. The means through which the genetic instructions are passed from the parents to the offspring
 - ▶ RNA - performs multiple functions in the cell, the best known is the transfer of the information encoded in the DNA to the cell machinery that makes proteins (e.g., mRNA, dsRNA)
 - ▶ Proteins - carry out catalytic and structural functions



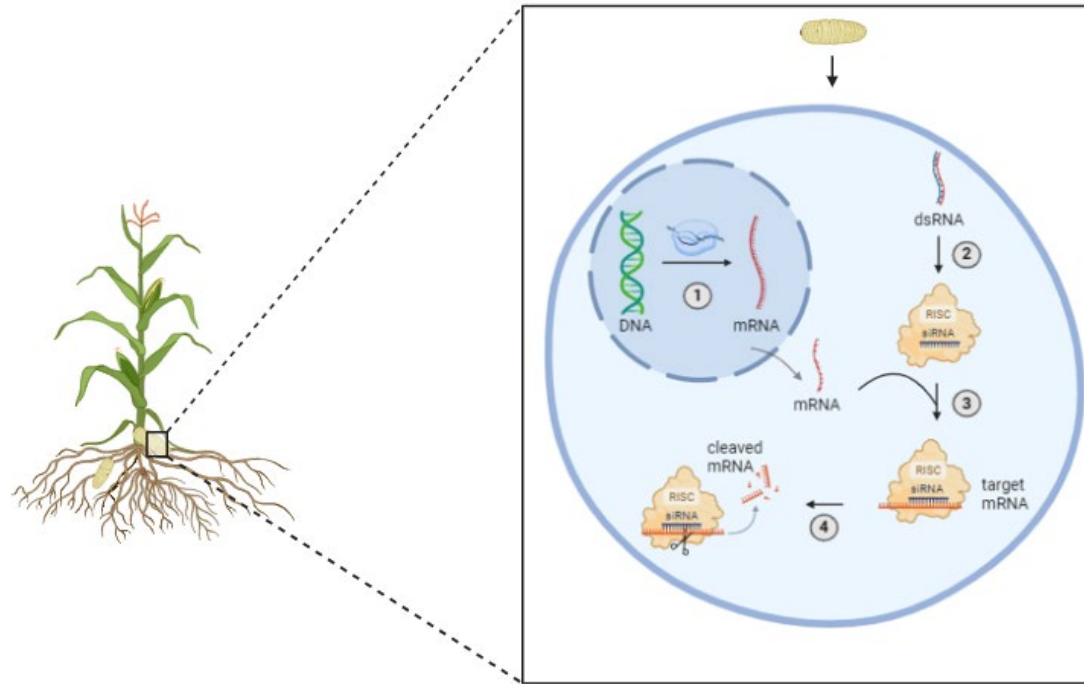
RNAi and dsRNA

- ▶ RNA-interference (RNAi) functions by specifically targeting and degrading mRNA, resulting in reduced protein levels:
 - ▶ Each mRNA is composed of a specific sequence instructing the protein-making machinery to make a specific protein.
 - ▶ Additionally, each dsRNA is composed of a specific sequence indicating which mRNA in a cell it regulates.
 - ▶ RNAi, in turn, is a highly specific mechanism regulating cellular functions



MON 87411: using dsRNA to control CRW

- ▶ MON 87411 corn is engineered to produce DvSnf7 dsRNA
 - ▶ DvSnf7 dsRNA is ingested by the insect and subsequently recognized by the insect's RNAi machinery.
 - ▶ This results in down-regulation of the targeted DvSnf7 gene and ultimately leads to the insect's death.
- ▶ Emphasis on Specificity
 - ▶ DvSnf7 dsRNA is a PIP with a very narrow spectrum of activity, affecting only CRW and a few closely related relatives.

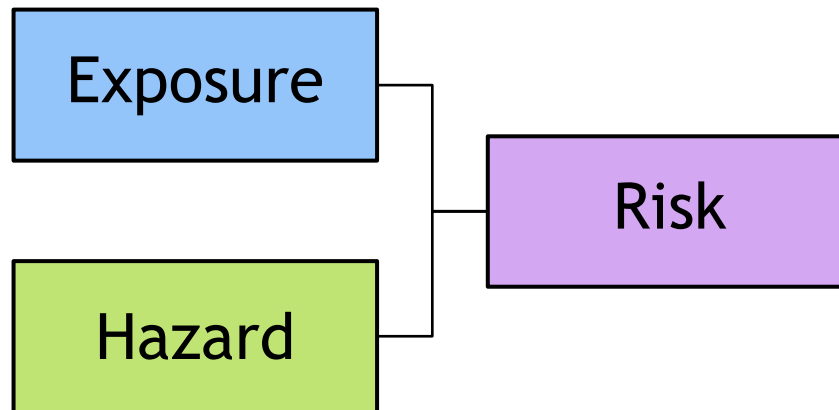


MON 87411: Assessing Risk for RNAi PIPs

- ▶ The help of a FIFRA Scientific Advisory Panel (SAP) was enlisted in the risk assessment process for DvSnf7 as found in MON 87411
 - ▶ Guidance from the SAP meetings was used to help inform data needed for the risk assessment of DvSnf7 dsRNA
 - ▶ Additional data was also submitted by the applicant
- ▶ SAP timeline amid risk assessment process:
 - ▶ 2014: convened to provide general guidance on risk assessments for RNAi-based pesticides
 - ▶ 2016: held to provide a peer review of the Agency's risk assessments for MON 87411's commercial use
- ▶ Assessing risk for dsRNA included using the current framework in place for Bt PIPs, with additional considerations specific to dsRNA in human health and the environment

MON 87411: Assessing Risk for RNAi PIPs

- ▶ EPA assesses risk according to the following equation: Risk = Hazard x Exposure
- ▶ The data reviewed for MON 87411, mentioned in the following slides, informs on each component of the risk equation to determine the risk outcome



Risk Assessment: Overview of Supporting Human Health and Environmental Data

- ▶ Product Characterization
- ▶ Human Health/Toxicology
- ▶ Environmental Effects
 - ▶ Non-target organisms
 - ▶ Environmental fate
- ▶ Insect Resistance Management

MON 87411: Product Characterization

- ▶ Sequence Analysis
 - ▶ confirm the presence and intactness of the inserts and flanking DNAs
- ▶ Southern Blot Analyses
 - ▶ confirm the presence of all DNA inserts in the combined trait maize product
- ▶ Assessment of RNA Levels in Maize Tissues Produced in the United States Field Trials During 2013
 - ▶ determine the DvSnf7 RNA expression levels reported as $\mu\text{g/g}$ dry weight and fresh weight

MON 87411: Human Health/Toxicology

- ▶ Established tolerance exemption for nucleic acids that are part of a PIP
- ▶ Additionally, the following supporting information was reviewed:
 - ▶ 28 Day Oral Toxicity Study in Mice
 - ▶ Subchronic 90 Day Oral Toxicity Study in Rats
 - ▶ History of safe exposure to plant-derived RNA - physiological barriers that exist that minimize exposure such as nucleases in saliva and the digestive tract, acidic gut environments, membrane barriers, rapid elimination from the blood, bioinformatics

MON 87411: Human Health/Toxicology

- ▶ Overall human health risk conclusions:
- ▶ No unreasonable adverse effects and reasonable certainty that no harm will result from aggregate exposure to DvSnf7 dsRNA and the genetic material necessary for its production in MON 87411

MON 87411: Environmental Effects

Terrestrial Environments

- ▶ Expression of DvSnf7 dsRNA was measured in various plant organs and tissues collected from trials within the U.S. and Argentina
 - ▶ Primary source of exposure of terrestrial nontarget organisms to the *DvSnf7* is expected to be the corn tissue, which is ultimately expected to be deposited on or in the soil in terrestrial environments.
 - ▶ Soil degradation study indicated that most of the DvSnf7 dsRNA degraded within approximately 2 days after application to soil, regardless of texture, pH, clay content and other soil differences.
- ▶ Based on these results, DvSnf7 dsRNA is unlikely to persist or accumulate in the environment

MON 87411: Environmental Effects

Aquatic Environments

- ▶ DvSnf7 primarily expected to reach aquatic habitats through deposition of post-harvest plant debris by the action of wind and water.
 - ▶ EPA determined that dsRNA would have fate in aquatic environments similar to that of Cry proteins, i.e., they would leach out of plant debris and break down rapidly in the water; exposure via consumption of plant debris in the water determined to be insignificant
 - ▶ Rapid degradation of DvSnf7 dsRNA in soil is expected to limit its presence in runoff.
 - ▶ Study on persistence of DvSnf7 dsRNA in water and sediment showed rapid degradation
- ▶ Based on available data, EPA determined that, under realistic environmental conditions, DvSnf7 dsRNA would degrade rapidly in aquatic systems

MON 87411: Environmental Effects Non-Target Organisms

- ▶ Birds
 - ▶ Broiler chicken 6 week study
 - ▶ Northern Bobwhite 14-day study
 - ▶ No adverse effects in either study
 - ▶ Bioinformatics analyses

MON 87411: Environmental Effects Non-Target Organisms

- ▶ Freshwater Fish and Invertebrates
 - ▶ study in channel catfish fed a diet of 30% MON 87411 grain indicated no adverse effects at this exposure level.
 - ▶ bioinformatic analysis

MON 87411: Environmental Effects

Non-Target Organisms

- ▶ Bees, Non-target Insects, and Other Invertebrates
 - ▶ DvSnf7 dsRNA is only active within the Chrysomelidae Family of coleopterans and specificity of the intended effect of DvSnf7 dsRNA is expected to be limited to its target species.
 - ▶ EPA reviewed data submitted on the following non-target invertebrates and each study reported no adverse effects on survival, development, growth, or reproduction:
 - ▶ lady beetle (*Coleomegilla maculata*) parasitic wasp (*Pediobus foveolatus*), insidious flower bug (*Orius insidiosus*), carabid beetle (*Poecilus chalcites*), green lacewing (*Chrysoperla carnea*), honey bee (*Apis mellifera*), earthworm (*Eisenia andrei*), and springtail (*Folsomia candida*)
- ▶ Based on specificity of the intended effect and the lack of adverse effects, EPA concluded adverse effects to nontarget insects and other terrestrial invertebrates are not expected to occur as a result of exposure to DvSnf7 dsRNA

MON 87411: Environmental Effects Non-Target Organisms

- ▶ Endangered Species Conclusions:
 - ▶ “No effect” determination for DvSnf7
- ▶ Overall Environmental Conclusions:
 - ▶ Adverse effects to nontarget organisms are not expected due to high specificity
 - ▶ No anticipated gene flow or development of invasiveness

Conclusion

- ▶ Plant-Incorporated Protectants (PIPs) are regulated by EPA according to FIFRA and FFDCa
- ▶ DvSnf7 dsRNA in MON 87411 is the first RNAi-based PIP to target an insect pest and the mode of action is highly specific to CRW
- ▶ EPA assesses risk according to the equation: Risk = Hazard x Exposure
- ▶ Data submitted to support registration of MON 87411 informs EPA on each component of the risk equation
- ▶ Assessing risk for DvSnf7 included using the current framework in place for Bt PIPs, with additional considerations specific to dsRNA in human health and the environment



Thank you for your time!

CONTACT INFO:

**Nina Ortiz
Biologist**

**EPA Office of Pesticide Programs
Biopesticide and Pollution Prevention Division
Emerging Technologies Branch**

Ortiz.Nina@epa.gov