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Ecological risk assessment for DvSnf7 RNA: A plant-incorporated protectant with targeted activity against western corn rootworm

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ABSTRACT

MON 87411 maize, which expresses DvSnf7 RNA, was developed to provide an additional mode of action to confer protection against corn rootworm (*Diabrotica* spp.). A critical step in the registration of a genetically engineered crop with an insecticidal trait is performing an ecological risk assessment to evaluate the potential for adverse ecological effects. For MON 87411, an assessment plan was developed that met specific protection goals by characterizing the routes and levels of exposure, and testing representative functional taxa that would be directly or indirectly exposed in the environment. The potential for toxicity of DvSnf7 RNA was evaluated with a harmonized battery of non-target organisms (NTOs) that included invertebrate predators, parasitoids, pollinators, soil biota as well as aquatic and terrestrial vertebrate species. Laboratory tests evaluated ecologically relevant endpoints such as survival, growth, development, and reproduction and were of sufficient duration to assess the potential for adverse effects. No adverse effects were observed with any species tested at, or above, the maximum expected environmental concentration (MEEC). All margins of exposure for NTOs were >10-fold the MEEC. Therefore, it is reasonable to conclude that exposure to DvSnf7 RNA, both directly and indirectly, is safe for NTOs at the expected field exposure levels.

1. Introduction

Over the past decade, a number of food crops utilizing RNA interference (RNAi), have received regulatory approvals from United States agencies such as the Environmental Protection Agency (U.S. EPA) and Department of Agriculture (USDA), as well as approval in other countries such as Canada, Mexico, Australia, New Zealand, Japan, Korea, and Brazil (CERA, 2012). The RNA-based products approved thus far have conferred resistance to specific viruses (e.g. plum-pox virus), extended produce quality (e.g. Arctic Apple) or nutritional enhancement (e.g. alfalfa, soy) (Auer and Frederick, 2009; CERA, 2012). Recently, genetically engineered (GE) insect-protected plants that confer resistance via RNA-based gene regulation have been developed and reported in the scientific literature (Bachman et al., 2013; Baum et al., 2007; Bolognesi et al., 2012; Mao et al., 2007). These plants express double-stranded RNAs (dsRNAs) targeted to suppress mRNA levels in a specific species or a small group of closely related species by utilizing the RNAi pathway. The sequence specific nature of RNAi allows these products to target pest species with a high level of specificity, while mitigating risk to non-target organisms (NTOs) (Bachman et al., 2013; Burand and Hunter, 2013; Whyard et al., 2009). Monsanto Company has developed a GE maize, MON 87411, that confers protection against corn rootworm (CRW) (*Diabrotica* spp.) utilizing RNAi as the mechanism of insecticidal action (Bolognesi et al., 2012). The DvSnf7 RNA expressed in MON 87411 is composed of a 968 nucleotide sequence containing 240 base pair dsRNA component plus the addition of a poly A tail (Urquhart et al., 2015) designed to target the western corn rootworm (*Diabrotica virgifera virgifera;* WCR) *Snf7* gene (DvSnf7). Upon consumption, the plant-produced RNA in MON 87411 is recognized by the CRW's RNAi machinery, which results in a rapid decrease of DvSnf7 mRNA and protein levels leading to growth inhibition followed by mortality (Bolognesi et al., 2012; Levine et al., 2015). It has been established that after ingestion of DvSnf7 by WCR, suppression of the DvSnf7 mRNA occurs within 24 h, followed by suppression of DvSNF7 protein and onset of mortality by day 5 (Bolognesi et al.,

Abbreviations: dsRNA, double stranded RNA; RNA, ribonucleic acid; RNAi, RNA interference; NTO, non-target organism; ERA, ecological risk assessment.

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2012). MON 87411 also contains a *cry3Bb1* gene that produces a modified *Bacillus thuringiensis* (subsp. *kumamotoensis*) Cry3Bb1 protein to protect against CRW larval feeding. The spectrum of activity of the Cry3Bb1 protein has previously been reviewed by the U.S. EPA and, at the levels expressed in GE maize, activity was only evident in the family Chrysomelidae within the order Coleoptera (U.S. EPA, 2010a). Corn rootworm active *Bt*-technologies, such as the Cry3Bb1 and Cry34Ab1/Cry35Ab1 proteins, have been safely marketed for over a decade, and have provided significant value to farmers (Prasifka et al., 2013). In addition, incorporation of multiple modes of action against CRW by pyramiding *Bt* and RNA-based traits will offer increased efficacy and durability of a product while maintaining a high degree of specificity for the target pest and environmental safety (Baum and Roberts, 2014).

A critical step in the deregulation and/or registration of a GE plant incorporated protectant (PIP) is performing an ecological risk assessment (ERA) to evaluate the potential for adverse ecological effects from cultivation. Assessment of potential ecological impacts, associated with the introduction of a PIP, is based on the characteristics of the crop and the introduced trait. The approach for evaluating ecological risks from pesticides is a multi-step iterative process (Romeis et al., 2013; U.S. EPA, 1998). Key steps include problem formulation, analysis of exposure and potential effects, and risk characterization. During problem formulation, the assessor defines protection goals, prepares a conceptual model to aid in identification of the relevant assessment and measurement endpoints, and then develops an analysis plan that serves as the basis for a risk characterization. Important information that was used to inform the problem formulation step for MON 87411 included the biology and familiarity with the crop and the trait, the mode of action (MOA), the spectrum of activity, the tissue specific expression profile, routes of exposure for ecological receptors and an assessment of potential persistence in the environment. In general, the scope of the ecological safety assessment for a PIP can be reduced when the MOA is well characterized, there is a narrow spectrum of activity, and expression levels of the trait are well characterized (Romeis et al., 2013). The MOA of DvSnf7 RNA has been well characterized (Bolognesi et al., 2012; Ramaseshadri et al., 2013) and has been shown to have a narrow spectrum of activity with activity only evident within a narrow subset of beetles, the Galerucinae subfamily in the order Coleoptera (Bachman et al., 2013). This limited range of activity reduces the potential for non-target effects and can narrow the scope of ecological testing. Additionally, the DvSnf7 RNA and Cry3Bb1 protein have been shown to act independently which allowed for Cry3Bb1 and DvDnf7 RNA to be tested and assessed independently (Levine et al., 2015). Taken together, information on the MOA, spectrum of activity, expression profile, lack of interaction, and routes of potential exposure were used to help inform and define the scope of NTO testing used for this ERA.

For the MON 87411 assessment, the protection goals were identified as the maintenance of ecological functions of NTOs ‘in-field’ and biodiversity of species ‘off-field’ that contribute to the structure and function of the environment. Ecological functions to be protected include pollination, predation and parasitism (i.e., biological pest control, referred to herein as biocontrol), decomposition of soil organic material, and soil nutrient cycling. Additional confirmatory data was collected to address regulatory requirements and to provide empirical data for a broad range of taxa for this first in class insecticidal RNAi product. This included a broader range of avian and other non-target vertebrate populations where a plausible risk hypothesis would typically not require such data given barriers to exposure in these taxa (see section 4.1 in Discussion). An important assessment endpoint for PIPs is the abundance of taxa within functional groups of NTOs. Primary

Table 1
The relationships between protection goals, assessment endpoints, indicators of effect, and measurement endpoints utilized in the DvSnf7 RNA/MON 87411 ecological risk assessment.

Non-target organism		Protection goals	Assessment endpoints	Indicators of effect	Measurement endpoints
Honey bee adult and larvae	*Apis mellifera*	Pollinationservices and pollinator biodiversity	Population size and function, biodiversity	Laboratory larval & adult bee toxicity studies	Adults (worker) survival Larval survival and development to adult
Ladybird beetle	*Coleomegilla maculata*	Biocontrol by non-target arthropods	Population size and function, biodiversity	Laboratory toxicity studies initiated neonates or adults	*C. maculata, P. chalcites, and O. insidiosus* survival, growth and development to adult
Rove Beetle	*Aleochara bilineata*				*A. bilineata and C. carnea* adult survival and reproduction
Ground Beetle	*Poecilus chalcites*				
Green Lacewing	*Chrysoperla carnea*				
Insidious Flower Bug	*Orius insidiosus*				
Parasitic Wasp	*Pediobius foveolatus*				*P. foveolatus* adult survival
Earthworm	*Eisenia andrei*	Nutrient cycling by soil biota	Population size and function, biodiversity of soil macro-organisms	Laboratory toxicity studies initiated with neonates or adults	*E. andrei* survival and body weight
Collembola	*Folsomia candida*				*Collembola* survival and reproduction
Beneficial soil microorganisms			Functionality of microbially-mediated soil processes	Laboratory toxicity studies with soil microorganisms	Carbon and nitrogen transformation
Bobwhite quail	*Colinus virginianus*	Avian and wild mammal populations	Population size, biodiversity	Toxicity studies with bobwhite quail, chickens, and catfish	Survival and growth
Chicken	*Gallus domesticus*				
Catfish	*Ictalurus punctatus*				