Proposed Registration Decision for the New Active Ingredients Double-stranded Ribonucleic Acid Transcript Comprising a *DvSnf7* Inverted Repeat Sequence Derived from Western Corn Rootworm (*Diabrotica virgifera virgifera*) and *Bacillus thuringiensis* Cry3Bb1 Protein and the Genetic Material (vector PV-ZMIR10871) Necessary for Their Production in MON 87411 Corn (OECD Unique Identifier: MON-87411-9)

Approved by:

Jack E. Housenger, Director
Office of Pesticide Programs

Date:

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1. Background

On February 7, 2014, the U.S. Environmental Protection Agency (EPA) received a registration application for MON 87411, a corn plant-incorporated protectant (PIP),[1] from Monsanto Company (Monsanto). Monsanto produced MON 87411 by *Agrobacterium tumefaciens*-mediated transformation of corn tissue using the plant transformation vector PV-ZMIR10871. MON 87411 expresses the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein, which confers tolerance to glyphosate, and the following new pesticidal active ingredients:

(1) Double-stranded ribonucleic acid (dsRNA) transcript comprising a *DvSnf7* inverted repeat sequence from western corn rootworm (*Diabrotica virgifera virgifera*) and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (*DvSnf7* dsRNA).

(2) *Bacillus thuringiensis* (*Bt*) Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (*Bt* Cry3Bb1 protein).[2]

These active ingredients control several coleopteran corn pests (i.e., corn rootworm complex) by two different modes of action, one well known (*Bt* Cry3Bb1 protein) and the other novel amongst pesticides (*DvSnf7* dsRNA).

In general terms, when a susceptible insect larva ingests a *Bt* delta-endotoxin protein like Cry3Bb1, the protein acts on that pest by the Cry toxicity pathway (Knowles and Ellar, 1987; OECD, 2007):

(1) The insect's midgut solubilizes the protein, thereby releasing protoxins.
(2) The insect's proteases cleave these protoxins and release the active toxin.
(3) The active toxin binds to specific receptors on the insect's midgut epithelium.
(4) Toxin subunits form pore structures that inject into the insect's midgut membrane.
(5) Ions and water pass through the pores, resulting in swelling, cell rupture, and eventually the insect's death.

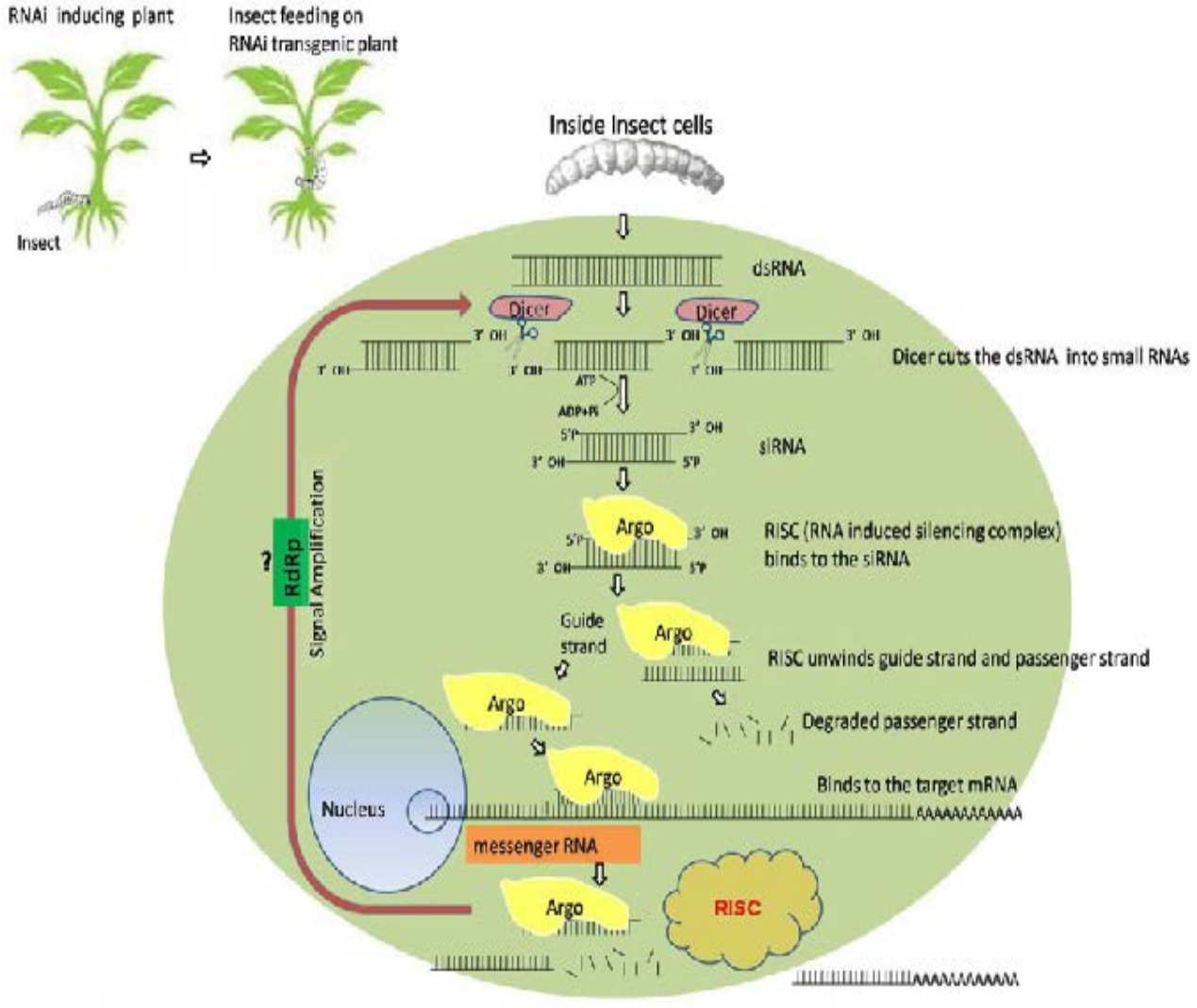
Since 2003, other PIPs that express the *Bt* Cry3Bb1 protein have been used to control insect pests (i.e., MON 863 and MON 88017; see U.S. EPA (2010a)). The EPA has an extensive amount of product characterization, toxicology, and ecological data and information on *Bt* proteins expressed by PIPs and completed a reassessment in 2010 for most of the currently registered corn PIPs (e.g., MON 88017 expressing the *Bt* Cry3Bb1 protein), concluding that these corn PIPs would not cause unreasonable adverse effects on the environment.[3]

[1] In accordance with 40 CFR § 174.3, "plant-incorporated protectant" is defined as follows: "[A] pesticidal substance that is intended to be produced and used in a living plant, or in the produce thereof, and the genetic material necessary for production of such a pesticidal substance. It also includes any inert ingredient contained in the plant, or produce thereof."

[2] Although the EPA registered PIPs expressing the *Bt* Cry3Bb1 protein in 2003 (MON 863 corn) and in 2005 (MON 88017 corn), the EPA considers this *Bt* Cry3Bb1 protein, consistent with past practice, to be a new active ingredient due to its origination from a different genetic event (MON 87411 corn).

[3] Search for "EPA-HQ-OPP-2010-0607" at *http://www.regulations.gov* to access the documents associated with the public process for the 2010 reassessment.

Although ribonucleic acid interference (RNAi) is the mechanism of action in other registered PIPs (i.e., New Leaf® Plus Potatoes and C5 Honeysweet Plum; see U.S. EPA (2000), U.S. EPA (2010b), and U.S. EPA (2013)), the active ingredients in these PIPs involve a targeted dsRNA with specificity for a viral ribonucleic acid (RNA) encoding of either a replicase enzyme or a coat protein and do not control macro-organism pests like *DvSnf7* dsRNA. *DvSnf7* dsRNA is ingested by the insect and recognized by the insect's RNAi machinery, resulting in down-regulation of the targeted *DvSnf7* gene and leading to the insect's death. More specifically, the RNAi pathway is initiated by cleavage of *DvSnf7* dsRNA into short interfering RNAs (siRNA) by the nuclease Dicer (Fire *et al*., 1998). The siRNAs then bind to a complex of proteins known as the RNA-induced silencing complex (RISC), and this leads to specific suppression of the target messenger RNA (mRNA). Since the mRNA encodes a protein with an essential function within the insect, in this case a vacuolar sorting protein belonging to the Endosomal Sorting Complex Required for Transport (ESCRT)-III complex, this suppression causes lethality. A diagram of this process generally, which is from Kola *et al*. (2015), follows immediately below.



Cleaves the target site and no more protein is synthesized. Larval growth and metabolism consequently arrest

(Kola VSR, Renuka P, Madhav MS, and Mangrauthia SK (2015) Front. Physiol. 6:119)

Due to uncertainties of dsRNA pesticide active ingredients identified in the literature and potentially associated with human and nontarget organism risks, the EPA consulted with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) for guidance in understanding and addressing these uncertainties. The meeting of the SAP was held January 28, 2014, with minutes published in May of that year.[4] The SAP consultation was held independently of any registration involving a dsRNA-based pesticide, including MON 87411.

MON 87411 is proposed for seed increase/breeding purposes only (no commercial release at this time) with a time limitation of two years and a per-season acreage cap of 15,000 acres.

2. Evaluation

In evaluating a pesticide registration application, the EPA assesses a wide variety of studies to determine the likelihood of adverse effects (i.e., risk) from exposures associated with the proposed use of the product. Risk assessments are developed to evaluate how the compound might affect a wide range of nontarget organisms, including humans and terrestrial and aquatic wildlife (plants and animals).

Based on these assessments, the EPA evaluates and approves language for each pesticide label to ensure the directions for use and safety measures are appropriate to mitigate any potential risk. In this way, the pesticide's label helps to communicate essential limitations and mitigations that are necessary for public safety. In fact, the pesticide law has a provision that indicates it is a violation to use a pesticide in a way that conflicts with the label.

2.1 Assessment of Risk to Human Health

In order to assess a PIP's risk to human health, the EPA requires allergenicity and toxicity data/information, generally consisting of amino acid sequence homology comparisons to known allergens and toxins, heat stability testing, an acute oral toxicity test at maximum hazard dose, and an *in vitro* digestion assay in a simulated gastric environment. As purified test substance is used in the acute oral toxicity test and the purified test substance often needs to be produced in an alternate production system (e.g., within a yeast or bacterium) to obtain enough for testing, the EPA also requires that the microbially produced and plant-produced substances be shown to have similar biochemical characteristics and bioactivity. On a case-by-case basis, the EPA may require data other than what is described in this document in order to be able to fully evaluate a PIP in accordance with our safety standards. For *DvSnf7* dsRNA and the *Bt* Cry3Bb1 protein expressed in the proposed time- and acreage-limited seed increase registration of MON 87411, the database of studies required to support the assessment of risk to human health is complete.

[4] See *http://www.epa.gov/scipoly/sap/meetings/2014/january/012814minutes.pdf*.

A. Risk to Humans from the *Bt* Cry3Bb1 Protein Expressed in MON 87411

The data submitted and reviewed for the *Bt* Cry3Bb1 protein as expressed in MON 87411 justify bridging the existing findings and conclusions from the human health assessments conducted for MON 88017 (EPA Reg. No. 524-551; see U.S. EPA (2010a)) and support its inclusion in the existing tolerance exemption (40 CFR § 174.518).

The EPA concludes that there are no unreasonable adverse effects and there is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the *Bt* Cry3Bb1 protein and the genetic material necessary for its production in MON 87411. This includes all anticipated dietary exposures as a result of the proposed registration and all other exposures for which there is reliable information. The EPA has arrived at this conclusion because no toxicity to mammals has been observed, and there is no indication of allergenicity potential for the PIP from available information.

B. Risk to Humans from *DvSnf7* dsRNA Expressed in MON 87411

The EPA reviewed a 90-day oral toxicity study with corn grain from MON 87411 at up to 33% in the diet and a 28-day oral toxicity study with purified *DvSnf7* dsRNA at doses up to 100 mg/kg. Based on molecular and functional characteristics (i.e., northern blot analyses and sequence comparison) provided by Monsanto, the EPA considers *DvSnf7* dsRNA produced by *in vitro* transcription and *DvSnf7* dsRNA expressed in MON 87411 to be equivalent; therefore, the use of *DvSnf7* dsRNA produced by *in vitro* transcription as a test substance in the 28-day oral toxicity study is acceptable. Neither study indicated adverse effects in the animals receiving test diet or test substance as there were no treatment-related effects on clinical signs, mortality, body weight parameters, food consumption, functional observation battery, food efficiency, organ weights, gross pathology, histologic pathology, clinical pathology, or microscopic pathology. Collectively, these results show the absence of any dietary hazard associated with *DvSnf7* dsRNA at very high doses.

In addition, bioinformatic searches were conducted to determine *DvSnf7* dsRNA's sequence match to human genes (open reading frames/coding regions). Monsanto conducted a search of all of the possible 21 base pair (bp) small RNAs encoded by the 240 bp *DvSnf7* dsRNA sequence to evaluate whether there were any matches to human transcripts. This search did not produce any matches between *DvSnf7* RNA and human transcripts. This bioinformatics dataset demonstrates that MON 87411 does not contribute any additional small RNAs to the diet that have identity to human transcripts. These data also serve to illustrate the lack of significant sequence homology between human transcripts and *DvSnf7* dsRNA. Empirical toxicology data also reviewed as part of the human health assessment further provides evidence that the ingestion of *DvSnf7* dsRNA does not impact the health of test animals and is safe for consumption.

DvSnf7 dsRNA is an insecticidal nucleic acid component of MON 87411. Nucleic acids (i.e., RNA, dsRNA, and DNA) are present in all living organisms and are routinely consumed as a part of human and animal diets with no apparent adverse effects (Ivashuta *et al*., 2009; Parrott *et al*., 2010). Nucleic acids are considered to be "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (FDA) (U.S. FDA, 1992) and exempt from the requirement of a

tolerance under the Federal Food, Drug, and Cosmetic Act (FFDCA) by the EPA. The EPA also recently affirmed the following: "DNA and RNA are common to all forms of plant and animal life, and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to their consumption as a component of food" (U.S. EPA, 2010a).

The EPA concludes that there are no unreasonable adverse effects and there is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to *DvSnf7* dsRNA and the genetic material necessary for its production in MON 87411. This includes all anticipated dietary exposures as a result of the proposed registration and all other exposures for which there is reliable information. The EPA has arrived at this conclusion because no toxicity to mammals has been observed and because of the lack of significant sequence homology between human transcripts and *DvSnf7* dsRNA.

For more information on the human health risk assessment of *DvSnf7* dsRNA and the *Bt* Cry3Bb1 protein expressed in the proposed time- and acreage-limited seed increase registration of MON 87411, please see the supporting document in the associated regulatory docket (search for "EPA-HQ-OPP-2014-0293" at *http://www.regulations.gov*).

2.2 Assessment of Ecological Risk

To assess risk to the environment for PIPs, the EPA requires nontarget organism toxicity data/information, generally consisting of testing with birds; mammals; freshwater and marine/estuarine fish and invertebrates; nontarget insects, including honey bees; nontarget plants; and soil invertebrates. As purified test substance is used in some of the testing and the purified test substance often needs to be produced in an alternate production system (e.g., within a yeast or bacterium) to obtain enough for testing, the EPA also requires that the microbially produced and plant-produced substances be shown to have similar biochemical characteristics and bioactivity.

In addition to the toxicity data, other data are also considered regarding the environmental persistence of PIPs, as well as the potential for gene flow and development of weediness. The EPA requires laboratory data demonstrating the degradation of the PIP in soils typical of agronomic areas where the PIP crop is grown. To assess gene flow and potential for development of weediness, the EPA considers several lines of evidence related to characteristics of the crop plant, including reproductive capability, presence of wild relatives, and the potential for containment or other mitigating measures to reduce or eliminate establishment in the environment.

On a case-by-case basis, the EPA may require data other than what is described in this document in order to be able to fully evaluate a PIP in accordance with our safety standards. For *DvSnf7* dsRNA and the *Bt* Cry3Bb1 protein expressed in the proposed time- and acreage-limited seed increase registration of MON 87411, the database of studies required to support the assessment of risk to the environment is complete.

A. Risk to Nontarget Organisms from the *Bt* Cry3Bb1 Protein Expressed in MON 87411

Monsanto cited studies previously submitted to support the registration of MON 88017 (EPA Reg. No. 524-551). MON 88017 expresses a *Bt* Cry3Bb1 protein that is equivalent to the *Bt* Cry3Bb1 protein expressed in MON 87411. The EPA previously determined that the *Bt* Cry3Bb1 protein expressed in MON 88017 is equivalent to the *Bt* Cry3Bb1 protein expressed in MON 863 (EPA Reg. No. 524-528, no longer an active registration), and much of the data submitted to support MON 863 was bridged to support the registration of MON 88017. Expression of the *Bt* Cry3Bb1 protein in MON 87411 does not exceed that of either MON 863 or MON 88017.

Acceptable data cited showed that adverse effects are not expected to occur for birds, wild mammals, freshwater fish and invertebrates, honey bees (*Apis mellifera*), nontarget insects (including Monarch butterflies), and soil invertebrates. Additional supplemental studies on the insect activity spectrum, a preliminary Tier IV field evaluation, and summaries of various higher tier laboratory and field studies were also submitted. These data were sufficient to allow the EPA to determine that the *Bt* Cry3Bb1 protein expressed in MON 88017 presents minimal risk to nontarget organisms (see U.S. EPA (2010a) for more details). Therefore, adverse effects are similarly not expected to result from exposure to the *Bt* Cry3Bb1 protein expressed in MON 87411. The EPA also previously concluded that adverse effects to federally listed threatened and endangered ("listed") species were not anticipated for the *Bt* Cry3Bb1 protein, and made a "no effect" conclusion for direct and indirect effects to listed species and their designated critical habitats (U.S. EPA, 2010a).

B. Risk to Nontarget Organisms from *DvSnf7* dsRNA Expressed in MON 87411

i. Birds and Mammals

The EPA typically does not quantify exposure of nontarget vertebrates to PIPs, and instead relies on toxicity testing that is conducted at exposure levels reasonably expected to equal or, preferably, exceed maximum exposure levels in the field based on expression studies. Corn grain is the plant tissue that birds and mammals are most likely to consume, so if exposure assumptions are limited to grain only, then exposure is relatively simple to understand. It is likely, however, that all species of birds and mammals that inhabit corn agroecosystems are exposed via other sources. While incidental dietary exposures to other corn tissues may occur, exposure through consumption of pest insects or other invertebrates insensitive to the toxins produced by MON 87411 is another route by which birds and mammals may be exposed to *DvSnf7* dsRNA. While *DvSnf7* dsRNA is not expected necessarily to accumulate within invertebrates, other corn tissues potentially consumed by these invertebrates show expression of *DvSnf7* dsRNA at levels several orders of magnitude higher than in grain. While the level of exposure may not be significant, the extent of exposure via this source, or any other possible source, has not been determined. A "worst case" scenario that would reduce uncertainty would be based on the highest concentration shown to be expressed in the PIP plant based on expression studies submitted to the EPA. Ideally, exposure in Tier I nontarget organism testing would be derived from these levels.

Current toxicity data available to assess risk for birds consists of a 6-week study of broiler chickens fed 57% MON 87411 grain. If this level of exposure is representative of maximum exposures in the field, then this study is adequate to address potential effects in birds over the period of time observed. As discussed above, actual exposure may be complicated to determine, and consumption of grain does not represent a "worst case" exposure with complete certainty. Nonetheless, even if consumed, physiological barriers exist in birds that significantly limit uptake of *DvSnf7* dsRNA, including salivary and other digestive ribonucleases (Park *et al*., 2006; Stevens and Hume, 1995) and acidic gut environments (Akhtar, 2009; Loretz *et al*., 2006; O'Neill *et al*., 2011). Bioinformatic analyses with *Gallus gallus* and *Columba livia* indicated no exact 21 nucleotide (nt) matches with the *DvSnf7* sequence, providing an additional line of evidence toward expectation of no effects. (The EPA is cautious, however, in interpreting this information as predictive of effects.) Based on these lines of evidence, adverse effects in birds are unlikely.

Data available for mammals include a 28-day study with mice, in which mice were dosed daily at levels up to 105 mg/kg/day, and a 90-day study with rats fed MON 87411 grain at 33% of the diet for the duration of the study. Both studies indicated no adverse effects. The dose of 105 mg/kg/day likely exceeds maximum short term exposure levels to mammals that would occur in the field, and the 90-day exposure to MON 87411 grain provides some insight into longer term exposures at low levels. As with birds, other lines of evidence are available to supplement the data provided, including physiological barriers as discussed above, and bioinformatics analysis with mammalian species. Based on these lines of evidence, the lack of effects observed in the available toxicity studies, and the limits on the proposed seed increase registration, adverse effects to wild mammals are not anticipated to result from the proposed registration of *DvSnf7* dsRNA expressed in MON 87411.

ii. ***Freshwater Fish and Invertebrates***

Exposure in aquatic environments may occur as a result of pollen drift to these areas, and also potentially through leaf or other postharvest crop residue movement off cultivated fields. The EPA previously concluded that exposure to Cry protein PIPs in aquatic systems is limited. In response to the EPA's request for data on *DvSnf7* dsRNA breakdown in plant tissue, Monsanto provided a discussion of the studies supporting this conclusion and the low likelihood of exposure to senescent corn plant tissue in aquatic environments. This information is sufficient to support this assumption for the proposed seed increase registration of MON 87411.

A channel catfish study provided some information on the potential dietary toxicity of *DvSnf7* dsRNA to freshwater fish, but the usefulness of this study is limited by the assumptions of exposure. This study showed that adverse effects as observed are not expected under the exposure conditions tested (diet consisting of 33% grain fed for a period of eight weeks). Since exposure in aquatic environments is assumed to be minimal based on the EPA's current assumptions for Cry protein PIPs, then this study likely is sufficient to capture potential acute or subchronic adverse effects of *DvSnf7* dsRNA to freshwater fish at realistic environmental exposure levels. Exposure is also expected to be reduced by the proposed acreage and time limitations on the registration, and expected rapid breakdown of dsRNA in soils. Additionally, the EPA notes that expression of *DvSnf7* dsRNA generally declines over the growing season and

is reduced by several orders of magnitude in corn stover and senescent root. Therefore, levels of *DvSnf7* dsRNA available in corn plants postharvest are expected to be lower compared to the growing season. Based on all of this information, adverse effects to freshwater fish are not expected to occur as a result of the proposed seed increase registration for *DvSnf7* dsRNA expressed in MON 87411. Similarly, since exposure is not expected to be significant in aquatic environments for this proposed registration, adverse effects on freshwater invertebrates are not anticipated.

iii. ***Marine and Estuarine Fish and Invertebrates***

Significant exposure to *DvSnf7* dsRNA in corn tissue is not expected in marine and estuarine environments because plantings of MON 87411 would likely be limited temporally and spatially. Given that exposure is expected to be extremely limited in these environments, adverse effects are therefore not anticipated for fish or invertebrates.

iv. ***Nontarget Plants***

While it is unclear at this point whether *DvSnf7* dsRNA may be present in root exudates of MON 87411 corn, based on soil degradation data submitted to the EPA, it is likely that any *DvSnf7* dsRNA that enters the soil via this source or by release from plant tissues will rapidly break down. Therefore, adverse effects to plants are not expected, since exposure is unlikely to be significant.

v. ***Nontarget Insects and Other Invertebrates***

Monsanto presented an extensive discussion of the specificity of *DvSnf7* dsRNA for its target pest, western corn rootworm, and its close relative, southern corn rootworm. Primary work to demonstrate specificity for the target pest is described in Bachman *et al*. (2013). In this study, bioassays were conducted with either *DvSnf7* dsRNA or dsRNA developed from an ortholog of the *Snf7* gene in several close relatives. This study showed that *DvSnf7* dsRNA was only active within the Galerucinae Subfamily of the Chrysomelidae Family of coleopterans, which indicates high specificity of *DvSnf7* dsRNA for the insecticidal action on its target pest.

Exposure to nontarget insects for PIPs is typically based on expression levels within plant tissues. Expression levels can differ between plant tissues and also over time, and the plant tissue with the most relevance to the insect diet is typically used to determine dosing levels or diet concentration in nontarget testing. Expression levels in pollen are often used, since many nontarget insects will consume pollen, and expression in leaf may also be considered. As described above for birds and mammals, there is some uncertainty with the actual exposure in the field given the varying diets within arthropod communities. Therefore, a conservative approach to estimating exposure of nontarget organisms to PIPs would be to utilize the highest expression levels measured among all tissues based on dry weight measurements. While this may overestimate exposure for certain organisms, it reduces uncertainty around actual exposure in the field.

In the environmental risk assessment submitted by Monsanto, no observed effect concentration (NOEC) values determined from nontarget testing were compared to maximum expected environmental concentrations (MEEC) to calculate margins of exposure (MOE). If MOEs are >10x the MEEC, then risk is assumed to be negligible. Utilizing the highest mean dry weight expression level measured (84.8×10^{-3} µg/g whole plant tissue from V3–V4 development stage), NOEC values of 1,000 ng/g determined in nontarget insect testing would produce a MOE of 11.8, which, if using this approach, would indicate a low likelihood of adverse effects. The MOE for bees would be >3,000, assuming the maximum recorded expression in pollen represents the MEEC (maximum dw value measured was 0.292×10^{-3} µg/g). This approach has been found to be acceptable by the EPA in previous risk assessments; however, given that the studies are limit dose tests in which only one concentration was tested, there is uncertainty around the biological meaning of the NOEC. Without testing at additional higher concentrations, it is not possible to know how close the NOEC is to levels at which effects that might be observed. Nonetheless, the EPA considers the level at which toxicity was tested to be sufficiently above expected environmental concentration, and the studies submitted are reliable tests of potential effects to nontarget insects at realistic field exposure levels.

Monsanto supplemented the above data with a discussion of barriers to exposure in invertebrate species. It is clear from some studies of dsRNA in invertebrates that some physiological barriers exist that can significantly limit uptake in insects. These barriers include ribonucleases in saliva (Allen and Walker, 2012; Christiaens *et al*., 2014) and DNA/RNA non-specific nucleases in the hemolymph (Garbutt *et al*., 2013). Other evidence of some barriers are demonstrated in studies requiring certain conditions for delivery of dsRNA, such as by microinjection (Whyard *et al*., 2009) or nanoparticle encapsulation (Sarathi *et al*., 2008). While these barriers are not fully understood and information is not yet available to extrapolate between insect taxa, it is apparent that such barriers reduce bioavailability in some insects.

Based on the specificity of the intended effect, invertebrate studies submitted, and other information provided from the literature, the EPA concludes that adverse effects to nontarget insects and other terrestrial invertebrates are not expected to occur as a result of the registration of *DvSnf7* dsRNA expressed in MON 87411 as proposed. Honey bees and other pollinators are included in this conclusion, and the results of testing with both larvae and adult honey bees provide strong evidence in support. Additionally, Monsanto has provided results of bioinformatics analyses with both honey bee and bumble bee, neither of which identified exact 21 nt matches with *DvSnf7*. As noted above, these data provide supplemental information to support this conclusion, but the EPA recognizes that they are not necessarily predictive of effects.

The potential for off-target and other unintended effects, which were discussed as an uncertainty by the FIFRA SAP, appears to be unlikely based on the data and other information provided. Understanding completely all of the potential physiological mechanisms providing barriers to uptake or leading to unintended effects cannot be known without extensive research, which is beyond the scope of the EPA's ecological risk assessments. Off-target and other unintended effects of *DvSnf7* dsRNA, if they occur, are expected to be more evident in insects, since they are more closely related to the target pest; however, despite testing three coleopterans and other insects, no adverse effects have been observed.

vi. *Outcrossing and Weediness*

The EPA previously determined that there is no significant risk of gene capture and expression of any *Bt* endotoxin by wild or weedy relatives of corn in the U.S., its possessions or territories (see extensive discussion in U.S. EPA (2010a)). Since these conclusions are based on the nature of pollination, survival of hybrid offspring, and weediness in corn and its relatives, these conclusions also apply to *DvSnf7* dsRNA.

vii. *Overall Conclusions for DvSnf7 dsRNA*

Based on the data and rationale obtained and reviewed in support of this registration, the EPA does not anticipate adverse effects to nontarget organisms, including endangered and threatened species, as a result of the proposed seed increase registration of *DvSnf7* dsRNA expressed in MON 87411. For PIPs, a seed increase registration like this one is approved to allow an applicant to produce seed in advance of a commercial registration, and it therefore has certain limitations. In this case, MON 87411 will be limited to 15,000 acres per year over 2 years.

In order to evaluate a commercial registration for MON 87411, the EPA would require additional data to confirm its characterization of potential risk to nontarget organisms, as discussed in the supporting risk assessments. As is typical when a seed increase registration is amended for commercial-scale, generally the limitations imposed on this seed increase registration would not remain in effect. For *DvSnf7* dsRNA, should Monsanto submit an application for a commercial registration of MON 87411, the EPA will obtain additional data to confirm its characterization of potential risk to nontarget organisms. The EPA believes that this additional information would likely be sufficient to address any remaining uncertainties and allow the EPA to formalize its effects determination for endangered and threatened species prior to acting on an application that addresses commercial-scale use involving distribution or sale of MON 87411. Because the existing database is already sufficient to support the conclusion that there is likely no risk of effects to listed species at environmentally relevant concentrations, the EPA believes these data will simply serve to support our preliminary conclusion and provide for a robust effects determination.

C. Risk to Nontarget Organisms from the *Bt* Cry3Bb1 Protein and *DvSnf7* dsRNA Expressed in MON 87411

To satisfy data requirements for the *Bt* Cry3Bb1 protein and *DvSnf7* dsRNA expressed in combination in MON 87411, data must either be submitted on the combination, or data must be submitted to show that toxicity is not expected to be higher as a result of the expression of both PIPs. Expression levels of the *Bt* Cry3Bb1 protein in MON 87411 do not exceed those previously considered in ecological risk assessments, which allows for bridging to data supporting previous registrations of the *Bt* Cry3Bb1 protein from other events. A synergism study conducted with a sensitive test species, a synergism study with another coleopteran pest species (Colorado potato beetle), and preliminary data provided from an additional synergism study on growth inhibition in corn rootworm indicate that synergism is unlikely. Therefore, data developed on the individual proteins to support the proposed registration of MON 87411 for a

time- and acreage-limited seed increase registration is appropriate, and the conclusions for the *Bt* Cry3Bb1 protein and *DvSnf7* dsRNA individually also apply to the combination of these active ingredients in MON 87411 for this seed increase registration.

For more information on the environmental risk assessment of *DvSnf7* dsRNA and the *Bt* Cry3Bb1 protein expressed in the proposed time- and acreage-limited seed increase registration of MON 87411, please see the supporting document contained in the associated regulatory docket (search for "EPA-HQ-OPP-2014-0293" at *http://www.regulations.gov*).

3. Alternatives

Given that MON 87411 will be for seed increase/breeding purposes only and will not be for commercial release, the EPA did not conduct an alternatives analysis.

4. Benefits and Public Comments

Biopesticides are pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. PIPs, a class of biopesticides, are pesticidal substances that plants produce from genetic material that has been added to the plant and may have the following benefits:

- Usually are inherently less harmful than conventional pesticides.
- Generally affect only the target pest and closely related organisms, in contrast to broad-spectrum conventional pesticides that may affect many different organisms (e.g., birds, insects, and mammals).
- Often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.
- Can greatly decrease the use of conventional pesticides while crop yields remain high, when used as a component of integrated pest management programs.
- Can offer another tool for pest management in areas where environmental concerns limit the use of conventional pesticides.

The EPA previously assessed the benefits of corn rootworm PIPs like MON 87411 and more thoroughly described many of the benefits summarized in the bullets directly above and others, including durability extension[5] of PIP control measures for corn rootworm (U.S. EPA, 2010a, 2010d, and 2010e). Benefits for PIPs in general are also discussed in the public literature. For example, Klümper and Qaim (2014) carried out a meta-analysis of 147 studies that reported on the impact of genetically modified crops on yield, pesticide use, and/or farmer profits and found that, "[o]n average, [genetically modified] GM technology adoption has reduced chemical pesticide use by 37%, increased crop yields by 22%, and increased farmer profits by 68%." Additionally, Coupe and Capel (2015) describe a substantial reduction in the use of insecticides on corn from the introduction of GM plants in 1996 (8.5 million kilograms of pesticidal active

[5] See *http://www.epa.gov/scipoly/sap/meetings/2013/120413meeting.html* for more details on recent issues related to corn rootworm resistance and *Bt* corn PIPs.

ingredient used) until 2009 (1.8 million kilograms pesticidal active ingredient used), cautioning that some of this decrease could be attributed to other factors like regulatory restrictions on conventional pesticides and adjustments to farming practices. Although the benefits of MON 87411 would not be realized immediately because, at this time, it is not proposed for commercial release, the EPA believes that many of the same benefits already described and discussed for PIPs in its documents and public literature would apply in the future if the technology is commercialized and fully adopted by growers.

Overall, the EPA has provided/will be providing the public two opportunities to comment on the MON 87411 registration action.

In the Federal Register of August 13, 2014 (79 FR 47453), the EPA announced receipt of an application to register a pesticide product containing the new active ingredients *DvSnf7* dsRNA and *Bt* Cry3Bb1 protein and opened a 30-day public comment period. The EPA received approximately 500 comments on this publication. Most of the comments were from private citizens through what appears to be a letter-writing campaign, while one was received from the Pollinator Stewardship Council. A summary of these comments, and the EPA's response to these comments is below.

Human Health-Related Comments

The comments were uniformly negative and against approving the action and ranged from people concerned about children with allergies to GM foods and an increase in children with "special needs" to an autistic child with Crohn's disease to a person with pancreatic cancer. These commenters also objected to the lack of food labeling for GM-containing food. Most of the commenters stated that "the incomplete understanding of this field has hampered their translation into successful therapeutic strategies, with many mammalian studies highlighting the potential lethality and/or toxicity of RNA-based treatments" but did not provide any scientific rationale or citations to justify the statement about adverse mammalian effects. The one article cited by the commenters was published by Monsanto scientists to describe the mode of action of *DvSnf7* dsRNA against the corn rootworm. This article contained no information about effects on mammalian species.

While the EPA is concerned about the safety of any pesticidal product presented for registration and performs a rigorous assessment of the data presented to justify its safety findings, including special consideration for children, it was unable to discern any new substantive information in these comments to add to its safety considerations.

Ecological-Related Comments

The comments were negative, with some comments simply opposed to the registration or genetically modified organisms in general. Most comments generally expressed concern for uncertainties related to this new technology and the need to gather additional information on nontarget effects and environmental fate. Several comments were focused more specifically on concern for honey bees and other pollinators. Most comments did not provide background to support claims of potential effects. One comment was received from the Pollinator Stewardship Council (PSC), expressing concern for bees and other nontarget organisms and citing to both published and unpublished data.

Specifically, the PSC stated the following:

(1) Need more definitive assessment of the spectrum of activity.
(2) Need more comprehensive assessment of environmental fate of small RNAs.
(3) Risk needs to be explored under real scenarios.
(4) Data developed by pesticide companies is conflict of interest.

Regarding the concern for review of data developed by pesticide companies, the EPA directs commenters, including the PSC, to its website where this issue has been raised and addressed previously.[6]

Regarding the other concerns raised by the PSC, the EPA believes that it has evaluated data that addresses these concerns and that no additional substantive information was presented that requires consideration at this time. *DvSnf7* dsRNA's insecticidal effect appears to be highly specific as testing shows effects only to nontarget insects of the Galerucinae Subfamily of Family Chrysomelidae within Order Coleoptera (beetles). Further, multiple lines of evidence suggest a lack of adverse effects from *DvSnf7* dsRNA to pollinators like bees (i.e., larval and adult honey bee testing and bioinformatics with honey and bumble bees). Please see the "Nontarget Insects and Other Invertebrates" section in this document (on pages 9–11) and the environmental risk assessment in the regulatory docket for more details.

Other Comments
Most comments focused on human health- and/or ecological-related issues, but some focused on other topics, such as a dislike for corporations, claims that the EPA's decision-making process for pesticide registrations is unduly influenced by pesticide applicants/registrants, and reference to the regulatory processes in other countries where biotechnology is not accepted. Although the EPA appreciates the input and diverse perspectives of all of the commenters, these other comments did not provide any additional data or information that the EPA could analyze as part of its scientifically based and deliberate risk assessment and decision-making processes under FIFRA and FFDCA.

Because Monsanto's registration application involves two new active ingredients, the EPA is opening a 15-day public comment period in accordance with a policy, first implemented in October 2009, designed to provide a more meaningful opportunity for the public to participate in major registration actions.

5. Regulatory Decision

The database for MON 87411 is comprised of approximately 122 studies (submitted or cited) and, for this particular application with the limitations mentioned in the paragraph immediately below, is considered complete. The proposed use of *DvSnf7* dsRNA and the *Bt* Cry3Bb1 protein expressed by MON 87411 is supported by this database. In considering the assessed risk to

[6] See the following questions on the EPA's website: Why does EPA rely on studies submitted from pesticide companies when the Agency is considering whether or not to register a pesticide? Shouldn't the government be performing independent studies?

human health and the environment, the EPA concludes that *DvSnf7* dsRNA and the *Bt* Cry3Bb1 protein expressed by MON 87411 meet the regulatory standard under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Therefore, the EPA is proposing to grant the unconditional registration of MON 87411 that expresses *DvSnf7* dsRNA and the *Bt* Cry3Bb1 protein under FIFRA section 3(c)(5).

One corn PIP is proposed for registration: MON 87411. Although MON 87411 has been transformed to control several species of corn rootworm, the registration will be for seed increase/breeding purposes only and will not be for commercial release, will be time-limited to two years, and will have a per-season acreage cap of 15,000 acres.

The risk assessments supporting this proposed decision can be found in the associated regulatory docket (search for "EPA-HQ-OPP-2014-0293" at *http://www.regulations.gov*).

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