CHARGE TO THE SEPTEMBER 27-28, 2016 FIFRA SCIENTIFIC ADVISORY PANEL

Human Health and Ecological Risk Assessments for dsRNA DvSnf7 in the Combination PIP MON 89034 x TC1507 x MON 87411 x DAS-59122-7 Corn

DATE: AUGUST 29, 2016

Purpose

This meeting of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) is to consider the information provided to the U.S. Environmental Protection Agency (EPA) for determining the safety in corn of a Plant-Incorporated Protectant (PIP) expressing a double stranded RNA molecule (dsRNA), DvSnf7, to control the corn root worm (CRW) complex, also known as MON 87411 corn.

This SAP is also requested to comment on the information provided for DvSnf7 in response to the recommendations of the SAP at the January 28, 2014, meeting, which assessed the data needed to inform outstanding issues for pesticidal products using RNA interference (RNAi).

A number of documents, related to a number of regulatory events, are relevant to the EPA charge to the September 27-28, 2016 SAP. EPA indicates after each charge questions the most relevant pages in the most relevant documents. EPA provides a list of the documents and describes their relevance to the September 27-28, 2016, SAP in the tables immediately following the charge questions.

Product Characterization and Human Health Risk Assessment

(Attachments #3, #5, #7 and #8)

Question 1. The 2014 SAP in general agreed that there were few issues with dietary exposure to dsRNA molecules in mammalian species but recommended several issues be examined to confirm these assumptions. These recommendations include: (1) confirm that special dsRNA forms (e.g., hairpins, super coils) may not degrade as quickly as simple dsRNA; (2) the blood of animals consuming food containing an RNAi-PIP should be examined for the presence of the dsRNA or pieces of dsRNA: (3) although bioinformatics would not give definitive answers, it can be predictive, and as such a useful tool, depending on the search methods and completeness of the database; and (4) consider the possibility that special subpopulations such as the elderly, children or people with gastrointestinal tract illnesses (e.g., Crohn's disease, colitis, or irritable bowel syndrome) may present a different pattern of uptake of dsRNA from the diet.

a) Please comment on the feasibility of creating and successfully deploying in plants, dsRNA structures likely to present greater stability (e.g., supercoil, viroid-like structures). Please comment on EPA's conclusion that the single hairpin structure of the dsRNA for DvSnf7 is one of the simpler structures expected for RNAi inducing molecules, and that this structure is unlikely to present any unique stability to RNA degrading enzymatic attack. (See, in particular, pages 7-8 of Attachment 7)

b) Recent evidence suggests that RNAs, including miRNAs, are normally present in blood and are transported to various target tissues and organs as part of normal homeostasis (Freedman et al., 2016). Please comment on this evidence. How might this evidence relate to the subchronic and 28-day studies data supplied in support of this registration that show no effects on whole animals of the DvSnf7 dsRNA. What additional support might confirmatory testing in mammalian blood provide to a risk assessment? (See, in particular, pages 8-9 of Attachment 7)

c) Assuming that the SAP continues to believe that bioinformatics can be a useful tool, can the Panel offer advice on what level of similarity might trigger a biologically significant effect? Please comment on what RNA properties, in addition to sequence identity match, (for example, sequence length, context, or biophysical properties) are relevant in assessing the potential for a dsRNA molecule to mediate gene suppression. (See, in particular, pages 9-12 of Attachment 7)

d) Please comment on EPA's analysis regarding stability and potential for uptake of DvSnf7 dsRNA by special subpopulations with altered absorption or digestion (e.g., Crohn's disease, colitis, IBS). (Pages 13-14 of Attachment 7)

Question 2. Please comment on EPA's risk assessment of the combination PIP product including the evaluation of the DvSnf7 gene with regard to product characterization and human health. Does the SAP have any suggestions to improve it? (Attachments 7, and 3 and 5)

Ecological Risk Assessment **(Attachments #4, #6 and #8)**

Question 3. Environmental Fate and Exposure. The 2014 SAP recommended an exposure-based model for testing related to dsRNA based pesticides, which places emphasis on the environmental fate and exposure data and analyses. To inform the environmental fate and exposure analysis for the DvSnf7 dsRNA, EPA has reviewed data submitted by Monsanto Company that describes degradation in soil, water, sediment, and DvSnf7 expression levels, and has also examined information from public literature. Based on these data, EPA has concluded that exposure in the terrestrial environment primarily is limited to organisms that consume plant tissue directly and to those that may be exposed secondarily through consumption of herbivorous arthropods. In the aquatic environment, EPA has determined that exposure to DvSnf7 in corn detritus is minimal, and while some exposure may occur in the water column, the exposure will be short lived and not significant.

a) Please comment on the completeness of the data set considered for determining exposure and environmental fate of DvSnf7 in both terrestrial and aquatic environments, taking into consideration the scope of EPA's needs for environmental risk assessment and the recommendations of the 2014 SAP. (Pages 4-12 of Attachment 6)
b) EPA has based its determination of the aquatic environmental fate of DvSnf7 on assumptions developed for *Bacillus thuringiensis* derived Cry proteins, which are largely based on information from the literature. Please comment on the applicability of these assumptions to DvSnf7 and describe any additional or alternative information and/or analyses that EPA should consider. (Pages 10-12 of Attachment 6)
c) Due to the nature of the data provided, EPA has based estimates of nontarget organism exposure on environmental concentrations. However, as indicated in several publications, a certain threshold of molecules is required to induce RNAi and subsequent gene silencing. An analysis considering these thresholds was included in Monsanto Company's white paper on human safety of DvSnf7 (MRID 49724001, see pages 20-21 of Attachment 5). EPA recognizes that a similar analysis for nontarget organisms might provide more refined exposure estimates for DvSnf7 and for future risk assessments of dsRNA pesticides; however, it is unclear whether sufficient data are available to develop a similar analysis for nontarget organisms. Please comment on the risk assessment for DvSnf7 by such a threshold of exposure based approach, considering the scope of EPA's ecological risk assessments, and any additional information needed to implement such a threshold based analysis. (Attachment 6)

Question 4. Nontarget Organism Hazard. EPA has reviewed nontarget hazard data developed for DvSnf7 on two species of birds, two mammal species (from human health testing), a

freshwater fish, seven species of nontarget arthropods, an earthworm, and honey bees, and has included these studies in its consideration of nontarget organism risk. This approach for dsRNA is consistent with EPA's testing framework for *Bt* derived PIPs, with additional data required on nontarget insect reproduction. EPA has also included assumptions about common barriers to dsRNA uptake in vertebrates and bioinformatics analysis as additional lines of evidence in this consideration. Based on the whole of these data, EPA has concluded that adverse effects to nontarget organisms are not anticipated to result from cultivation of MON 89034 x TC1507 x MON 87411 x DAS-59122-7 corn.

a) Please comment on the completeness of the nontarget organism hazard data reviewed for DvSnf7 as it pertains to the needs of the environmental risk assessment and the recommendations for testing made by the 2014 SAP. (Pages 12-27 of Attachment 6)
b) EPA has concluded that DvSnf7 is unlikely to cause adverse effects to vertebrate nontarget organisms. This conclusion relies in part on an assumption that biological barriers that limit uptake in mammals (see the human health risk assessment for DvSnf7) would also apply to other vertebrates. Please comment on the biological barrier assumption as a line of evidence supporting EPA's conclusion of minimal risk to vertebrate nontarget organisms. (Pages 28-31 of Attachment 6)
c) EPA concluded that off-target and other unintended effects related to dsRNA exposure are unlikely in nontarget organisms, based on the lack of effects observed in nontarget testing. Please comment on EPA's conclusions regarding these effects. (Pages 34-35 of Attachment 6)

Question 5. Synergism. EPA requires data to demonstrate that PIPs expressed in combination within the same plant are not synergistic. The purpose of these studies is to allow bridging of data developed on individual PIPs to the combined trait product; otherwise, new data generation would be required on the combination to determine nontarget risk. EPA reviewed five studies examining synergism among DvSnf7 and the Cry proteins expressed in MON 89034 x TC1507 x MON 87411 x DAS-59122-7 and determined that no synergism is expected. Please comment on EPA's analyses of these data and the scientific value of these data in the risk assessment. (Pages 45-50 of Attachment 6)

Insect Resistance

Although the EPA has not requested comment from previous Scientific Advisory Panels on the potential for resistance in corn rootworm to RNAi-based pesticides, the Agency believes this to be an important consideration in its regulation of such pesticides, and uses this opportunity to collect advice and information.

Question 6. Corn rootworm have demonstrated an ability to adapt to a wide range of chemical and cultural controls, including Bt Plant-Incorporated Protectants. Please discuss the likelihood

and potential mechanisms by which corn rootworm could develop resistance to an RNAi-based pesticide such as DvSnf7 dsRNA.

Reference

Freedman, J.E., Gerstein, M., Mick, E., Rozowsky, J., Levy, D., Kitchen, R., Saumya, D., Shah, R., Danielson, K., Beaulieu, L., Navarro, F.C.P., Wang, Y., Galeev, T.R., Holman, A., Kwong, R.Y., Murthy, V., Tanriverdi, S.E., Koupenova, M., Mikhalev, E., and Tanriverdi, K. 2016. Diverse human extracellular RNAs are widely detected in human plasma. Nature Communications/7:11106/DOI:10.1038/ncomms11106/www.nature.com/naturecommunications

Document	Relevance	
Attachment #1 – RNA-Interference FIFRA Scientific Advisory Panel White Paper	EPA White Paper written to provide context for its charge questions to the SAP January 28, 2014 meeting. When the Environmental Protection Agency (EPA) became aware that an RNAi based plant-incorporated protectant (PIP) was close to being ready for submission for regulatory approval for control of insect pests, the Agency developed a proposed approach for assessing the risk of this type of pesticide. EPA's proposed approach was based on 25 years of experience with the regulation of PIPs, most of which was based on insecticidal proteins from the microorganism *Bacillus thuringiensis*, and microbial and biochemical pesticides. The information forming the nucleus of EPA's proposed approach to assessing RNAi-based PIP products for risk revolved around potential effects on humans from consumption of dsRNA, interaction of dsRNA with non-target organisms, and the environmental fate of dsRNA when part of a PIP. To ensure that EPA's proposed assessment program met the primary objective of ensuring that this type of pesticide is safe for human health and the environment, EPA requested the SAP to evaluate whether the Agency's proposed risk assessment approach was adequate for this type of PIP[1], and offer advice on prospective issues that might arise as the technology develops and finds wider application in agriculture. The SAP response to the EPA request can be found in Attachment #2.	
Attachment #2 – RNAi Technology: Program Formulation for Human Health and Ecological Risk Assessment	**SAP recommendations to EPA charge questions** The January 28, 2014 SAP considered the Agency's proposed approach and found that for the most part, the proposed approach formed a solid basis for assessing risk. The SAP did, however, offer additional comments on prospective issues in product characterization, human and mammalian health and ecological considerations. Specific recommendations are summarized below:	**EPA response to SAP recommendations** EPA's specific point-by-point response to each of the SAP's recommendations has been addressed in the risk assessment documents for DvSnf7: • Environmental Risk Assessment for a FIFRA Section 3 Registration of MON 89034 x TC1507 x MON 87411 x DAS-59122-7 Combined Trait Maize Expressing Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34/35Ab1 *Bacillus thuringiensis* Derived Insecticidal Protein, and DvSnf7 Double Stranded RNA (dsRNA) **(Attachment #6)**

[1] EPA posed the same question for the biochemical type of dsRNA (e.g., sprayable) to the SAP at the January 28, 2014 meeting, however, there are currently no products of this type before EPA, and the SAP is not asked to consider this type of pesticide in its deliberations.

Attachment #2 – RNAi Technology: Program Formulation for Human Health and Ecological Risk Assessment (continued)		• Transmittal of Compilation of Key Product Characterization and Mammalian Safety Information to Support Registration of DvSnf7 for the FIFRA SAP Meeting to be held on September 27-28, 2016 **(Attachment #7)** See below for the references to EPA responses to specific SAP recommendations.
	SAP recommendations to EPA charge questions Briefly, for human health considerations and product characterization, while the majority of the findings of the Panel agreed with the Agency that there were few issues with dietary exposure for PIP dsRNA products, in the disciplines of product characterization and mammalian safety for dsRNA in PIPs and other pesticidal products, the SAP recommended several areas where additional information for a PIP dsRNA product might be appropriate:	**EPA response to SAP recommendations**
	• Bioinformatics can be predictive, but would not give absolute answers. It can however be a useful tool, depending on search methods and completeness of database.	• EPA Response: Pages 9-12 of Attachment 7
	• Available evidence supports a conclusion of no significant absorption of dsRNA in mammals. But the SAP recommended that EPA:	
	o Obtain additional information on dsRNA abundance and tissue distribution in plants.	o EPA Response: Pages 4-5 of Attachment 7
	o Require testing of animal blood and exposed tissue to ensure more resistant forms of dsRNA do not enter body.	o EPA Response: Pages 8-9 of Attachment 7

Attachment #2 – RNAi Technology: Program Formulation for Human Health and Ecological Risk Assessment (continued)	• dsRNA likely to be degraded no matter its structure in digestive process. But the SAP suggested EPA confirm: o Stability of special dsRNA forms (e.g., hairpins, super coils) – verify same degradation occurs as with simple dsRNA o The ability of different structural forms to survive degradation in dermal and inhalation routes of exposure o Stability and possible uptake by special subpopulations, e.g., elderly, children, people with GI tract illnesses (e.g., Crohn's disease, colitis, IBS) o Whether dsRNA, or pieces of dsRNA, can be detected in the blood of animals consuming food containing an RNAi-PIP. Briefly, for ecological fate and effects, the SAP advised: • Effects on Nontarget organisms of varying degrees of relatedness to the target organism should be assayed • Assess the potential for and severity of unintended effects on nontargets, particularly those most likely to be exposed, including for sublethal, latent, chronic and off-target effects • Develop information on environmental fate, persistence and bioavailability of dsRNA.	o EPA Response: Pages 7-8 of Attachment 7 o EPA Response: Pages 12-13 of Attachment 7 o EPA Response: Pages 13-14 of Attachment 7 o EPA Response: Pages 8-9 of Attachment 7 • EPA Response: Pages 39-43 of Attachment 6 • EPA Response: Pages 37-43 of Attachment 6 • EPA Response: Pages 36-37 of Attachment 6

Attachment #3 - Review of Product Characterization and Toxicity Data in Support for a Seed Increase Section 3 Registration *Attachment #4 - Environmental Risk Assessment for a FIFRA Section 3 Limited Seed Increase Registration*	The risk assessments for a seed increase registration. Product expresses DvSnf7 dsRNA and Bt Cry3Bb1 insecticidal protein. Subsequent to the January 28, 2014 SAP meeting, Monsanto submitted a request for a registration of their RNAi-based product, MON 87411 in maize (OECD unique identifier MON 87411-9). MON 87411 maize protects against corn rootworm (CRW; *Diabrotica* spp.) and tolerates the herbicide glyphosate. MON 87411 contains a cassette that expresses an inverted repeat sequence designed to match the *Snf7* sequence of western corn rootworm (WCR; *Diabrotica virgifera virgifera*). MON 87411 also contains a *cry3Bb1* coding sequence that produces a modified *Bacillus thuringiensis* (subsp. *kumamotoensis*) Cry3Bb1 protein to protect against CRW larval feeding. EPA issued to Monsanto on October 29, 2015, a seed increase registration, which allows for an increase in the amount of MON 87411 seed, not for production of grain intended for human or animal consumption. Under the registration the company could grow seed on 15,000 acres (0.02% of corn acreage in the U.S.) per year for two (2) years. EPA relied on the risk assessment process it had developed for RNAi-based PIPs, taking into account certain of the recommendations offered by the January 28, 2014 SAP. Because a seed increase registration is time and acreage limited, EPA only needed information to satisfy a subset of the SAP recommendations to support the seed increase registration. EPA indicated that it would need additional information, as recommended by the SAP, to support the full commercial registration. Briefly, for human health considerations and product characterization risk assessment, the company should supply for a full commercial registration: • Confirmatory data tracking DvSnf7 dsRNA decay in whole blood. Briefly, in the 2015 ecological risk assessment, EPA indicated the company should supply the following information to support the full commercial registration: • Additional data/information to confirm the results of already submitted dietary or contact toxicity studies done with Nontarget insects, honey bees, and soil invertebrates • Additional study to confirm no synergism between Bt Cry3Bb1 and DvSnf7 • Confirmatory feeding with birds conducted at a maximum hazard concentration • Studies to address potential effects on reproduction in two additional insect species, preferably species that are closely related to the target pest.

Attachment #5 - Human Health Risk Assessment for a FIFRA Section 3 Full Commercial Registration (Monsanto) *Attachment #6 – Environmental Risk Assessment" for a FIFRA Section 3 Full Commercial Registration (Monsanto)* *Attachment #7 – Product Characterization and Mammalian Safety Assessment to Support Registration of DvSnf7 PIP (Monsanto)* *Attachment #8 – Review of Product Characterization, Human Health, Environmental, and Insect Resistance Management Data Cited for a FIFRA Section 3 Full Commercial Registration (Dow)*	The risk assessments for the full commercial registration. These documents were produced specifically for this meeting, and the DvSnf7 analyses in them are the primary focus of the charge questions. Monsanto Company and Dow AgroSciences LLC subsequently applied for full commercial registrations for their stacked PIP products containing MON 87411. The stacked product contains MON 89034 x TC1507 x MON 87411 x DAS-59122-7, expressing Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1 Cry34/35Ab1 *Bacillus thuringiensis* derived insecticidal protein and DvSnf7 double stranded RNA. In order to focus specifically on the human health implications of DvSnf7, EPA extracted from Attachments #3 and #6 data and information key to the assessment of DvSnf7 to create Attachment #7. Attachment #7 also includes EPA's response to the SAP recommendation related to human health considerations.