Pollinator Stewardship Council
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Sept. 11, 2014

Biopesticides and Pollution Division (BPPD)
Robert McNally
Office of Pesticide Programs, EPA
28221T
1200 Pennsylvania Ave, NW
Washington, D.C. 20460-0001

Re: *Bacillus thuringiensis* Cry1A.105 and Cry2Ab2 proteins and the genetic material (Vector PV-GMIR13196) necessary for their production in MON 87751 soybean.
Docket: EPA-HQ-OPP-2014-0456

Re: Double-stranded ribonucleic acid (dsRNA) transcript comprising a DvSnf7 inverted repeat sequence derived from western corn rootworm (*Diabrotica virgifera virgifera*) and *Bacillus thuringiensis* Cry3Bb1 protein and the genetic materials (vector PV-ZMIR10871) necessary for their production in MON 87411 corn
Docket: EPA-HQ-OPP-2014-0293

Dear Mr. McNally,

While it may appear that RNAi is a promising technology, we have limited experience with the widespread deployment of genetically engineered crops that express pesticidal RNAs. This limited experience, and the potential hazards and exposures, lead us to object to the approval of dsRNAs as a pesticide or in genetically modified crop plants, until a consensus can be reached by researchers in the field which should include academia.

Humans and animals consume small, interfering RNAs with every meal. To our knowledge these ingested small molecules apparently do not affect us, but this is based on very little data. This argument is central to the presumption that pesticidal RNAi will be safe to non-target organisms. The fact that ingested, pesticidal small RNAs kills or harms animals (the target insect) negates this presumption. If ingested small RNAs never had effects on higher organisms, then pesticidal small RNAs would not be a viable means for controlling pest insects. Therefore, it is necessary to assume that small RNAs have effects on higher organisms.

As pointed out in the recent EPA Scientific Advisory Panel report (January 28, 2014), it is crucial that a risk assessment paradigm that can proactively anticipate potential non-target effects be developed for pesticidal

RNAs *prior to the lifting of deregulation of this technology*. Some key points of this risk assessment would include:

A more definitive assessment of the spectrum of activity for RNAi. Most eukaryotic species (including humans, livestock, crops, and honey bees) possess the cellular machinery to use dsRNA in their maintenance and protection. As such, any organisms that ingest a pesticidal dsRNA are potentially affected. These non-target effects have been identified in several insects for other corn rootworm-specific dsRNAs; namely the Colorado potato beetle (Baum et al. 2007), other species of *Diabrotica* (Baum et al. 2007; Bachman et al. 2013), and lady beetles (at least *Hippodamia convergens*, unpublished data from Blair Siegfreid's group in Nebraska). Not all species are affected, yet we have limited experience with the widespread deployment of genetically engineered crops that express pesticidal RNAs. The data presented is by the registrant, and peer-reviewed research is extremely limited.

Experts in the field indicate a likely hazard posed by dsRNAs and siRNAs will be unintended gene suppression. *(Lundgren, JG and Duan, JJ., 2013, RNAi-Based Insecticidal Crops: Potential Effects on Nontarget Species, BioScience 63:657–665; doi:10.1525/bio.2013.63.8.8)* It is statistically likely that non-target organisms will share a critical level of gene homology with the target gene in the target pests, especially when one considers that the functional unit of RNAi (the siRNA) is as little as 21 nucleotides long and full sequence homology does not appear to be necessary for gene suppression. How much sequence homology to the dsRNA is required to enact unintended gene suppression in non-target species is a critical question that needs to be answered. Also, there needs to be a more robust way to proactively assess unintended gene silencing of an siRNA in non-target organisms, as well as predict the phenotypic outcome of this unintended gene suppression. Hazards may also come from saturation of cellular machinery involved in RNAi and potential immune effects of RNA on cells. The doses of dsRNA and siRNA required for instigating these hazards on non-target organisms, especially honey bees and flowers, need to be determined prior to commercialization of this technology.

We fear that without a better prediction of the spectrum of activity of pesticidal dsRNAs, and proactive approaches to mitigate these effects, there is a potential for non-target effects in the use of this technology.

A more comprehensive assessment of the environmental fate of small RNAs. Our knowledge of the environmental fate of dsRNAs and siRNAs in various soil types and other matrices is necessary to understand how these chemicals will affect ecosystems. It seems sensible that single stranded RNA, and even dsRNA, would degrade rapidly in the environment. However, there are scenarios where these nucleic acids might persist. A first important consideration is the molecular structure of the nucleic acid in question- hairpins seem to help persistence of the dsRNA in soil, but this remains to be better understood. Whether these RNAs expressed in GM plant tissues persist for longer periods in the crop residue than as naked molecules also needs to be explored in research. Finally, new research out of Florida suggests that these naked pesticidal dsRNA molecules can be fed into trees through irrigation lines, and the resulting bioactivity of these pesticides persists within citrus trees for months. This gives question over whether plants adjacent to cropland will pick up these molecules as an additional source of exposure to organisms like pollinators and honey bees.

Risk needs to be explored in realistic scenarios. Non-target organisms like honey bees are currently challenged by multiple stressors including pesticide exposures, diseases, pests, and a lack of bee forage. As proposed, dsRNA-expressing corn will not be present in the environment as a single toxin. Rather, it will be coupled with Cry3Bb1, and likely expressed in corn treated with neonicotinoid and fungal seed treatments and exposed to glyphosate. **Laboratory toxicity assays that focus solely on dsRNA are not sufficient to predict**

the effects of this toxin against honey bees. First, many of the effects of RNAi resulting from unintended gene suppression would manifest themselves as sublethal effects. Second, non-target insects that are stressed from other environmental contaminants will have a different susceptibility to RNAi than healthy individuals. Realistic hazard tests that examine multiple fitness endpoints are important for accurately predicting the effects of this new technology. The complete Risk Cup must be evaluated before deciding to add additional stressors to an ecological system that is already breaking down. Remember, EPA's charge is to "prevent unreasonable risk to man and the environment." Making our environment a testing ground for potentially unsafe technologies does not fall under that charge.

Finally, it is noteworthy that much of the safety data used to support the deregulation of dsRNA was supplied by the registrant themselves. We understand that these types of experiments are technical and costly, and that the industry is well-positioned to conduct these types of experiments. But there is an important conflict of interest underlying these data. It would be beneficial if these experimental results could be validated by publicly funded scientists at public institutions.

Sincerely,

Dave Hackenberg, Co-Chair Bret Adee, Co-Chair
National Honey Bee Advisory Board

Randy Verhoek, President
National Honey Producers Association

Tim Tucker, President
American Beekeeping Federation

Bret Adee, President
Pollinator Stewardship Council