January 13, 2024

Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Ave. NW
Washington, DC 20460-0001.
RE: EPA-HQ-OPP-2023-0561-0001 Pesticide Tolerance Petition, Application from GreenLight Biosciences for Vadescana product

To Whom It May Concern:

We are writing to express profound concerns regarding the potential risks associated with the use of RNA interference (RNai) products on pollinator populations and their ecosystems. There is insufficient evidence that RNAi-based treatments do not yield unwanted effects, and the honeybee, already burdened with various environmental challenges, may face additional risks from the introduction of gene-silencing viruses. In order to ensure an approach that combines technological advancement with ecological health, we strongly urge undertaking further research so that we may avoid catastrophic harm and unintended consequences associated with RNAi-based technology. As we move forward in our understanding of RNAi-based technology, it is of utmost importance that we do not neglect our environmental responsibilities. We must continue to uphold the harmony of nature and work towards creating a sustainable future for our environment.

We do not believe the Vadescana product [Vadescana (CAS No. 2643947-26-4)] meets the criteria for tolerance exemption and we request that the application for exemption be denied.

One concern is that the entire premise of the exemption request is not supported by a selection of scientific research as is required by the EPA but instead, is based on the concept: “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.” [Vadescana (CAS No. 2643947-26-4)] In reviewing the application for exemption, there appears to be little to no data as outlined by the EPA requirements including “test data developed by the petitioner, publically available literature and data, including peer-reviewed assessments and journals (e.g., WHO, OECD SIDS, IUCLID, EPA HPV, etc.), modeled data, analog/surrogate data.” (https://www.epa.gov/minimum-risk-pesticides/need-tolerances-and-tolerance-exemptions-minimum-risk-pesticides)

Additionally, we are highly concerned by the possibility of recombination/genetic sharing.   Even in a system where recombination rarely occurs it only has to happen enough times for the Vadescana product to become virulent to the very creature that it is trying to protect as it is an insecticide. For example, despite the expectation that recombination should be rare in rotavirus A, there are nevertheless numerous reports of recombination among rotaviruses in the literature (Suzuki et al. 1998; Parra et al. 2004; Phan et al. 2007a,b; Cao et al. 2008; Martinez-Laso et al. 2009; Donker, Boniface, and Kirkwood 2011; Jere et al. 2011; Esona et al. 2017; Jing et al. 2018).

RNA interference (RNAi) stands as a powerful method for effectively suppressing target genes. Nevertheless, its extensive application in reverse genetic analysis and genetic manipulation is curtailed by the potential off-target effects. While rules governing the specificity of siRNA-based silencing have been elucidated, particularly beneficial for diverse applications in humans, the understanding of the specificity of dsRNAs—more suitable for deployment as insecticides—remains insufficient. One recent study states …”our experiments in several species of insects showed that the dsRNA specificity rules we established in T. castaneum apply to non-target species, and the conserved RNAi mechanism among eukaryotes might fund the base for this situation. Thus, dsRNA used in the field needs to take cross-species off-target effects into account. **At present, there is no perfect evaluation system for insecticide dsRNAs. Therefore, evaluation systems for understanding of the non-target effects and potential ecotoxicology of dsRNA treatments are urgently needed.”** (Chen, 2021)

Further, Vadescana works by inhibiting the calmodulin protein from properly being transcribed which is essential for the mites to locate their mates and thus this interrupts their breeding cycle. Bees also use calmodulin which is critical for foraging and there is insufficient research to ensure that there is no risk of interference as the similarities in DNA coding present a potential for off-target effects. Calmodulin is extremely important in binding odor receptor proteins in honeybees as bees rely on their sensitive olfactory system to perform foraging activities in the surrounding environment. The off-target effects could prevent proper pollination or foraging behavior, causing a colony to lose the ability to create food. This ability is associated with the existence of olfactory receptors (ORs). One study identified the AcerOr2 (ortholog to the Orco) protein in Apis cerana which contains a conserved, putative calmodulin (CaM)-binding site (CBS) indicating that CaM is involved in its function. (Guo, 2022)

This product is particularly concerning as the varroa dsRNA has a 99% nucleotide match to the Varroa mite calmodulin mRNA (S2 Fig) and a 74% nucleotide match, which includes a contiguous sequence of 14 nucleotides, to the honey bee calmodulin mRNA. In one study intended as a positive control on the product Vadecana in which insects sharing 100% of targeted genes would experience dramatic mortality or other off target effects, the opposite occurred which shows that Vadescana does not perform as predicted or indicated and substantial further research is required. The study found that "...The lack of mortality and sublethal effects following dietary exposure to dsRNA with 21-base pair and 100% base pair match to mRNAs that correspond to regulatory genes suggest monarch mRNA may be refractory to silencing by dsRNA or monarch dsRNase may degrade dsRNA to a concentration that is insufficient to silence mRNA signaling." (Krishnan, 2021) This research proves that Vadescana is unpredictable even to those who have designed it. RNA is the most mutagenic gene sequence that exists, with existing mutation rates of 1 for every gene replaced which allows for a wide range of variation to the original strands, exacerbated by the unique proteins that honeybees produce for RNA integration and usage. One of the biggest factors for RNA gene shift is the environment: as the virus increases frequency with the host, the more likely for mutation to occur and infect the host. Honeybees have a unique ability to transfer RNA, and it would be a matter of time before this RNA would be expressed in the honeybee with catastrophic results.

(Krishnan, 2021) Additionally, research found unspecific gene downregulation depending on both the dsRNA used and the different tissues; RNAi experiments in the honeybee require rigid controls and carefully selected dsRNA sequences to avoid misinterpretation of RNAi-derived phenotypes. (Jarosch, 2021).

Another major concern is that honeybees have a unique system of horizontal gene transfer between RNA molecules in which they secrete RNA binding proteins to aid in the gene transmission and uptake of RNA molecules. One protein, Major Royal Jelly Protein 3 or MRJP-3, is particularly effective at this. Research has shown that MRJP-3, an abundant jelly ingredient, is a secreted non-sequence-specific RNA-binding protein and that multivalent RNA binding mediates the transition of MRJP-3 into extracellular RNP (eRNP) granules that concentrate, stabilize, and enhance environmental RNA uptake. (Mayori, 2019) The research informs us that honeybees create a very RNA specific protein that binds to any RNA and makes it more bioavailable for transcription of gene transference which ultimately could lead to the improper silencing of crucial genes in the honeybee that would have devastating effects to the colony. In a conversation with one of the authors of this research, Dr. Eyal Maori from University of Cambridge stated "I suspect you will have cross RNAi reactivity. You can align the varroa transcript against the bee transcript and, hopefully, identify sequence stretch that is not conserved between the two. Do you have to target this varroa gene?" As an expert in the field of RNAi therapy and application, it is highly concerning that he feels there will be unintended consequences to using this specific dsRNA (Vadescana).

Given the immense importance of pollinators for agriculture and biodiversity, prioritizing their well-being and health is absolutely crucial before implementing RNA-based technology. The precautionary principle, espoused by the International Union for Conservation of Nature (IUCN) and other reputable organizations, underscores the need to anticipate and prevent potential harm to the environment and living organisms in the face of scientific and technological advancements, as outlined by the IUCN in 2014. In the case of RNA-based technology, it is imperative not to overlook or underestimate the possible detrimental effects it may have on pollinators.

> "Because pesticidal RNAi poses risks to non-target organisms that are different from other pesticides, a risk assessment framework has been proposed to proactively assess these risks using a series of steps (FIFRA-SAP, 2014; Roberts et al., 2015). Indeed, the United Nations employs the precautionary principle when conducting risk assessment of genetically modified organisms to ensure that these products do not adversely affect the environment…" (Mogren, 2017).

It is critically important to understand that Greenlight Biosciences has stated repeatedly that they are aware that their Vadescana product is unsafe for honeybees and other invertebrates. A selection of statements from forms and letters published and available on the Securities and Exchange Commission website includes the following statements:

"A potential challenge with the EPA approval is that **EPA typically seeks a 10x dose safety factor. At these doses, however, we have observed significant bee mortality that we do not yet understand.** Ensuring that we can meet applicable EPA safety factor requirements while protecting bee populations is a significant challenge to commercializing our product...If we cannot reduce bee mortality, we may not be able to obtain EPA approval to market our product."

“Unless we are able to develop clear correlations between Vadescana use and specific successful outcomes in beehives, Vadescana (assuming it obtains regularly approval) may not have strong or any commercial prospects.”

“Our Vadescana product is intended to be used in commercial beehives and used in a fashion which will expose the product only to bees and the Varroa destructor mite. **If Vadescana is used inappropriately and is consumed by invertebrates other than the Varroa destructor mite, it could be harmful to those invertebrates.”**

“**There is a dose-response in bees and mortality increases.** While our product is targeted to impact mites and not bees, **there may be unanticipated impacts on bee health that we do not yet understand** which could be related to product viscosity or the product replacing other nutrients. We **have observed some evidence of a relationship between the dose of our product and bee mortality at rates that are significantly higher than the necessary therapeutic dose**. A significant relationship between our product and bee mortality may undercut our product’s intended function of protecting bees.”

“There is an established history of safe consumption of both exogenous and endogenous RNA molecules in human and animal food that suggests that there is no negative biological effect of ingested RNAs and supports human and animal safety of these molecules for use as active ingredients for biopesticides. Notwithstanding this history of safe consumption in vertebrates like humans, **our Vadescana product negatively impacts ladybugs and could also negatively impact other invertebrates** if our use instructions are ignored, and the invertebrates gain access to and consume the Vadescana product. Moreover, the **honey from hives using Vadescana will have trace elements of Vadescana which could be harmful to invertebrates** consuming that honey.”

Beekeepers are keenly aware of the damage caused by varroa mites and would welcome a scientifically proven safe treatment method to mitigate our losses. However, it is paramount that we undertake further research and implement precautionary to ensure the sustainability and well-being of pollinators. Introducing a new gene-silencing virus without comprehensive studies on its impact could be devastating to an already overloaded creature. To address this concern, studies should be conducted in isolated locations and across multiple seasons to assess the long-term effects of these mite deterrents on honeybee foraging and pollination activities. Moreover, considering the multitude of challenges honeybees currently face, it is essential to prioritize their well-being over the expedited adoption of mite deterrents. There are many natural and synthetic methods currently available that kill 80-99% of the mites at a low cost with little to no damage to honeybees. Bees have demonstrated resilience over the years, and withholding the use of Vadescana for further study seems a prudent approach. The potential harm caused by unforeseen effects from inadequate testing should not be underestimated.

We strongly urge the scientific community and regulatory bodies, including the Environmental Protection Agency (EPA), to collaborate on extensive and transparent research into the safety of RNai products, particularly those designed as mite deterrents for honeybees. This includes evaluating the unintended consequences on honeybee behavior, foraging patterns, and overall colony health. We request the tolerance exemption for Vadescana be denied and further research will be taken to ensure the thorough evaluation and safety of mite deterrents before their widespread use.

Sincerely,

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www.pollinatorstewardship.org

American Beekeeping Federation
480 Town Center St. N, PMB #253, Mooresville, IN 46158
https://abfnet.org/

American Honey Producers Association
PO Box 435 Mendon, UT 84325
https://ahpanet.com/

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