National Honey Bee Advisory Board

Promoting Honey Bee Sustainability

To protect $50 billion in pollinated food production through

\- Balanced Pesticide Policy - Evidence Based Decisions - Proactive Education -

All members of NHBAB are professional beekeepers. All have experienced pesticide related mortality in their respective honey bee operations. They were chosen to serve this industry-wide capacity because of their personal and professional qualifications.

Members

David Hackenberg,
Co-chair
Lewisburg, PA 813.713.1239
buffybee@sunlink.net

Bret Adee Co-chair
Bruce SD 661.303.5031
badeehoney@gmail.com

Steve Ellis, Secretary
Barrett, MN 651.357.8280
nhbabsellis@gmail.com

Jim Frazier,
Scientific Advisor
Penn State University PN
814.599.5143
jfrazier@psu.edu

Jeff Anderson
Eagle Bend, MN 209.480.3256
jsa.cmhf@juno.com

Manley Bigalk
Cresco, IA 563.380.4056
grhoney@powerbank.net

Jim Doan
Hamlin NY 585.732.5370
jdoan@rochester.rr.com

David Mendes
N Fort Myers, FL 239.340.0625
davidmendesn@aol.com

Rick Smith
Yuma, AZ 928.210.3726
beezmans@roadrunner.com

Randy Verhoek
Bismarck ND 713.724.5993
r.verhoek@harvesthoneyinc.com

Ex-officio: Gene Brandi, George Hansen, Tim Tucker, Clint Walker

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Animal and Plant Health Inspection Service (APHIS)
Regulatory Analysis and Development
Dr. John Turner, Director
Environmental Risk Analysis Programs
PPD, APHIS Station 3A-03.8
4700 River Rd., Unit 118
Riverdale, MD 20737-1238

Re: Determination of nonregulated status of maize genetically engineered for protection against corn rootworm and resistance to glyphosate

Docket: APHIS-2014-0007

Dear Dr. Turner,

The National Honey Bee Advisory Board (NHBAB) is pleased to offer these comments concerning the availability of petition for determination of nonregulated status of maize genetically engineered for protection against corn rootworm and resistance to glyphosate. (APHIS Petition Number 13-290-01p from the Monsanto Company of St. Loius, MO.)

As agribusiness professionals who support American agriculture through providing pollination services contributing approximately $20 Billion per year to America's food system, we have a fundamental stake in the potential consequences of this technology and its total impact on American agriculture. We have greatly benefited from independent scientific input, upon which these comments are based, and urge you to continue to investigate this technology before allowing expansion of its use.

Science operates in a highly independent and redundant manner to eventually discover the truth about natural phenomena, their underlying mechanisms and how they operate in the real world. No arena is more challenging than the natural ecosystems of our planet and how man's efforts to produce food have impacted these in often less than sustainable manners. US EPA estimates the current *annual* rebuilding cost to the beekeeping industry from un-intended collateral damage from crop protection products at $300 million dollars. Bee industry leaders believe this number to be quite understated.

It has taken more than fifty years of scientific effort to begin to understand the operating mechanisms behind sustainable soils, plant varieties, pest complexes, and their interactions in our food production systems, yet many details remain to be determined. The fledgling arena of GMO plants and the recent developments of RNA interference (RNAi) technology for plant modification are a most cogent example of theoretical promises not yet realized or understood. The promises for GMO plants to reduce pest insect pressure and weed control along with reduced chemical inputs to the environment, and effective cost savings for farmers have not been realized. Recent publications have documented the lack of cost savings for producers, as well as the increasing herbicide resistance among weeds. The promise of using RNAi technology to alter plants likewise is attractive from a theoretical standpoint, but put in the context of failures of plant GMO use to date, brings major reasons for concern. We are decades away from full scientific understanding to allow sustainable and predictable use of this technology under field conditions.

In addition to the lack of knowledge, this particular arena involves the fundamental modes of plant and insect virus resistance and the rapidly evolving counter adaptations by which viruses overwhelm their hosts to become successful invaders. Our understanding of these molecular interactions is woefully limited. To attempt to use this technology at this point is placing the evolution of viral diseases, plants, animals, and human health at risk. This is totally unacceptable when one evaluates the potential risks of plant and animal health, human health, and ecosystem impact alongside the potentially limited gains in pest protection for food production.

APHIS is the lead agency for collaboration with other agencies to protect U.S. agriculture from invasive pests and diseases. We strongly recommend that APHIS collaborate with EPA in reviewing their research on RNAi and deny the nonregulation of MON 87411 maize containing the Snf7 gene and the cry3Bb1 gene until more of this technology is understood. *"It is still unclear how the individual steps in the RNAi mechanism -- from producing dsRNA in the plant cell via their uptake in the insect gut to the silencing of the detoxification genes -- are accomplished to induce a maximum effect."* ("'Yellow biotechnology': Using plants to silence insect genes in a high-throughput manner," Max Planck Institute for Chemical Engineering, Science Daily, 2-2-12)

The National Honey Bee Advisory Board encourages APHIS to work with their collaborative agency, the EPA, and review the recent findings of the EPA Scientific Advisory Panel (SAP) concerning RNAi technology that "not all aspects of the fate of dsRNA in the environment and potential effects on nontarget organisms are necessarily understood."

EPA asked their Scientific Advisory Panel to provide them with their expertise concerning this new pesticide technology. The scientists stated in their White Paper of Sept. 30, 2013, "Better understanding of the mechanisms influencing uptake, particularly if they can be extrapolated to other organisms, would reduce uncertainty in exposure assumptions and help to focus risk assessments on the most appropriate organisms."

The SAP includes scientists working in entomological fields to human studies from acclaimed universities across the United States. The FIFRA SAP concluded in their report, "The new categories of dsRNA products, however, will present additional hazard and risk assessment challenges due to their unique modes of action and other toxicological endpoints that cannot be measured using the traditional testing paradigm."

Honey bees, as the Scientific Advisory Panel stated, could be greatly impacted by the RNAi pesticide technology. They expressed their concerns that "not all aspects of the fate of dsRNA in the environment and potential effects on nontarget organisms are necessarily understood." They advised that it is unclear how RNAi technology can translocate throughout the environment, but possible transmission may include dust from degraded plant material, soil, plant pollen taken to bee hives, and even mammals consuming the plants and depositing the digested food far from the initial treatment area. The nontarget exposure opportunities present many concerns. For honey bees specifically, "The factors influencing the possibility of exposure by this pathway (e.g. longevity of dsRNA once consumed, concentration resulting within the herbivorous insect) are not known."

Huvenne and Smagghe in the Journal of Insect Physiology concluded:

> *"All the presented data suggest that there are at least two pathways for dsRNA uptake in insects. One is based on the transmembrane SID-1 channel protein, as best known from C. elegans. Many sid orthologs have been found in insects, but their precise role in the uptake mechanism of dsRNA often remains to be determined. The second 'alternative' mechanism is possibly based on the endocytosis pathway because it shares several components of its machinery with the dsRNA uptake mechanism. Herein, vacuolar H+ ATPase plays an important role. When studying this endocytosis-mediated uptake mechanism, intriguing parallels with the immune response become visible, thus suggesting the possible origin of the dsRNA uptake mechanism." ("Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: A review," Hanneke Huvenne, Guy Smagghe,* Journal of Insect Physiology *56 (2010) 227–235, 2009*

The researchers clearly state:
the "precise role in the uptake mechanism of dsRNA often remains to be determined;"
"The second 'alternative' mechanism is possibly based on the endocytosis pathway;"
"intriguing parallels with the immune response become visible;"

Most importantly, the researchers exclaim, "To gain further insight on the dsRNA uptake mechanisms in insects, more gene orthologs in different insects should be identified and their roles should be determined . . . in identifying potential genes involved in dsRNA uptake. Continuation of the development of cell lines and even primary cell cultures, especially of the insect midgut microvillar epithelial cells, can help to answer more complex fundamental questions on the uptake mechanism."

Honey bees, integral to the pollination of one third of human food crops, are currently experiencing assaults upon their immune system from pests, pathogens, poor nutrition, and pesticides. Research is also showing effects of pesticides on the mid-gut of honey bees, further weakening their immune system, and increasing their susceptibility to pests and pathogens. To introduce "mechanisms" into agriculture that have unknown pathways, immune response effects, and the developmental delays of insects could be catastrophic to agriculture's beneficial insects, including honey bees.

The National Honey Bee Advisory Board supports the findings of these noted researchers. We agree with EPA's Scientific Advisory Panel, that "the unique nature of dsRNA and RNAi raise several issues of concern with respect to the typical data set submitted for nontarget effects:"

"1) The potential influence of latent effects on results of nontarget testing." "Some studies, such as nontarget insect studies, are carried out for sufficient time to observe effects on reproduction, and latent effects would more likely be observed."

"2) The appropriate life stage for testing." "However, given the range of possible unexpected effects, it is conceivable that an effect could occur in the field that would not be observed in the lab."

"3) The possibility of chronic effects." "Suppression of genes without overt signs of toxicity may be considered insignificant following a single exposure; however, long-term exposure and continuous or repeated knockdown could result in chronic effects."

The SAP's White Paper sums up their concerns succinctly, exclaiming EPA "has not, to date, assessed the hazards or risks of dsRNA applied directly to the environment as components of end-use products intended for pest control under Section 3 of FIFRA." The "screening level assessments currently used for traditional chemical pesticides may not be applicable due to the unique modes of action of dsRNA active ingredients."

APHIS is charged with "protecting and promoting U.S. agricultural health." The SAP White Paper references sixty-four RNA/DNA/RNAi/gene studies which made it clear this new technology "will present additional hazard and risk assessment challenges due to their unique modes of action and other toxicological endpoints that cannot be measured using the traditional testing paradigm." The NHBAB agrees with the EPA's Scientific Advisory Panel and the review of research by Lundgren and Duan (2013) observing "that the current tiered hazard assessment approach used by the Agency, is inappropriate to address the following unique hazards potentially posed by dsRNA products:

Off target gene silencing
Silencing the target gene in unintended organisms
Immune stimulation
Saturation of the RNAi machinery in cells."

The NHBAB agrees with the FIFRA SAP "that accurate, standardized methods for measuring and assessing the aforementioned hazards will be necessary to conduct robust nontarget species risk assessments on dsRNA products."

However, we expressed our concern that EPA granted an experimental use permit in 2013 for a 20,000 acre field study of RNAi corn to study the Snf7 gene directed at the corn root worm before "standardized methods for measuring and assessing the aforementioned hazards" were developed. The FIFRA Scientific Advisory Panel White Paper appears to have been ignored when EPA approved a field test, without applicable testing protocols for this technology. EPA's goal is to "ensure that unreasonable effects do not occur to nontarget populations." This experimental use permit put nontarget organisms at risk. The Scientific Advisory Panel White Paper defined some of those risks:

". . .double stranded RNA (dsRNA) was 10 times or more potent in its effect on gene expression."
"Why some miRNAs trigger transitivity and some do not is not well understood at this time."
". . . the silencing of a gene targeted in one cell can lead to the silencing of a second gene in a distinct cell type."

"Although the details of the RNAi pathways and their outcomes may differ among organisms, what is clear is that the influence of small RNAs on growth, development, defense and even transient heritability of traits is substantial."

"It is unclear at this point whether a dsRNA PIP also would be incidentally present in root exudates, guttation droplets, or nectar, providing additional on-field sources of nontarget exposure."

While RNAi technology may be a useful tool, "uncertainties clearly exist with respect to a complete understanding of all current and future applications of this technology." " . . . The current testing paradigm for nontarget species characterizations, which emphasized limited dose testing and use of mortality as an endpoint, likely will not be adequate to assess adverse effects resulting from off-target gene silencing, silencing of the target gene in unintended organisms, immune stimulation, and saturation of the RNAi machinery in cells."

As the FIFRA SAP committee succinctly stated RNAi "uncertainties clearly exist with respect to a complete understanding of all current and future applications of this technology." RNAi technology must be researched fully to protect bees, to protect human health, and to protect the environment.

The National Honey Bee Advisory Board supports the findings of the EPA Scientific Advisory Panel, and expresses concern APHIS would ignore the recommendations of EPA's Scientific Advisory Panel of national experts.

There are many specific questions that need to be addressed to gain a reasonable understanding of the true risks imposed by the use of this technology in crop plants. Here are but a few of the more obvious ones for which answers do not currently exist:

(1) Since the whole genome sequences of most plants, animals and viruses are currently unknown, how can dsRNA sequences be designed to have minimal off-target impacts?

(2) Since RNAi is a key antiviral pathway in plants, what is the effect of chronic dsRNA exposure on plant immunity?

(3) Since RNAi is a key antiviral pathway in insects, what is the effect of chronic dsRNA exposure on the immunity of the target and non-target species?

(4) What does exposure to dsRNA do to insects, in terms of off-target effects?

(5) Can transgenic plants be designed to reduce exposure? For example, is it possible to produce dsRNA only in specific tissues, at specific times? Is there a way to turn on dsRNA production upon herbivore feeding?

(6) How effective is dsRNA? One concern is that to increase effectiveness, it will be necessary to target multiple genes/transcripts, and/or use very long dsRNA sequences, which will then also increase the likelihood of off-target effects.

The National Honey Bee Advisory Board encourages APHIS to deny the petition by Monsanto for the <u>nonregulation</u> of MON 87411 maize containing the Snf7 gene and the cry3Bb1 gene in order to protect "American animals, plants, and the agricultural industry."

Sincerely,

Dave Hackenberg, Co-Chair
National Honey Bee Advisory Board

Bret Adee, Co-Chair

Randy Verhoek, President
American Honey Producers Association

Tim Tucker, President
American Beekeeping Federation

Bret Adee, President
Pollinator Stewardship Council

Certain pesticides deleteriously impact the health of honey bee colonies, threatening the sustainability of the U.S. beekeeping industry and significantly imperil our national food supply.