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MEMORANDUM

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SUBJECT: REVIEW OF THE PROPOSED FIELD TRIAL FOR THE BIOPESTICIDE dsRNA OLIGONUCLEOTIDES FOR CONTROLLING HONEY BEE MITE *VARROA DESTRUCTOR*

The Human Health Assessment Branch (HHAB) reviewed the proposed field trials of the biopesticide dsRNA oligonucleotides proposed by Dr. Ruohan Liu (Monsanto Company) on April 6, 2016. This study proposes to do a field trial involving 480 honey bee hives (240 per site x 2 sites at Placer County and Imperial County, California) to evaluate the effectiveness of four different formulations (MON104549, MON104551, MON104550, MON104553) of dsRNA oligonucleotides, a RNA interference technique, on controlling honey bee ectoparasitic *Varroa* mite (*Varroa destructor*). The dsRNA oligonucleotides in different formulations are suspended in a sucrose solution and will be applied to honey bee hives prior to bees being released at a dose of 3.2 g/hive per season starting from May 1, 2016 to October 31, 2016 (every 3-4 weeks per application). Honey bee hives will remain stationary during the trial and treated hives will not be used for pollination and/or harvest of honey or other hive products.

Pesticide RNA refers to the use of ribonucleic acid interference (RNAi), a gene silencing technique, for pest control. The mode of action of RNAi involves introduction of a synthetic double-stranded RNA (dsRNA), designed to targeting specific gene of interest, into cells of pests, which is subsequently cleaved by an RNase III enzyme called Dicer into small interfering RNA molecules (siRNAs). These siRNAs are then incorporated into an active multi-protein RNA-induced silencing complex (RISC) that separate them into single stands, which consequently base-pair to their target messenger RNA (mRNA) and leads to mRNA destruction. Therefore, the mRNA cannot be translated into a functional protein product, which is important for the survival of pests.

Currently, there is no human health risk assessment available for any pesticide RNAs. In January 2014, the U.S. EPA held a scientific advisory panel (SAP) on the issues associated with the use of “RNAi technology as a pesticide: problem formulation for human health and ecological risk

assessment". The panel concluded that available evidence supports the conclusion of no significant absorption of dsRNA in mammals through oral ingestion and minimal likelihood of adverse effects. This conclusion was derived based on the facts that RNA is an intrinsically unstable molecule that can be readily hydrolyzed under both acidic and basic conditions, and human digestive system, extreme acidic pH and abundant RNA nucleases, is likely to ensure all foreign RNA degradation. However, the panel also raised the concerns of uncertainties associated with other routes of exposure (dermal and inhalation), dsRNA stability in individuals that manifest diseases, immune compromised, elderly or children, and potential effects of dsRNA on gut microbiome. In June 2014, the European Food Safety Authority (EFSA) held a two-day workshop on the topic "Risk assessment consideration for RNAi-based GM plants" in Brussels, Belgium. EFSA organized this event as a proactive step to assess whether the RNAi technique could be evaluated with its present approach for risk assessment of Genetic Modified Organisms (GMO). No newer information was presented in this workshop to add to the conclusions reached by the U.S. EPA SAP panel. In addition, the EFSA workshop does not consider the adverse effects such as the saturation of the RNAi machinery and immune stimulatory effects relevant in humans under realistic exposure conditions.

In this proposed study, the applicant indicated that dsRNA pesticide will be directly placed into managed honey bee hives, and precautions (not specified) will be taken to limit exposure to applicators and keep treated commodities from entering the food supply. Based on these assumptions, human exposures through dietary injection (honey or hive products) and dermal (applicators) routes are minimal. There is a potential risk of inhalation exposure to pollens if RNA pesticide was dispersed to flowers by treated bees. However, pollen is generally larger than inhalable particles (< 10 µm), and as such inhaled pollen particles are cleared from the upper airways and result in secondary oral exposure, which poses minimal likelihood of adverse effect based on the U.S. EPA's conclusion. An additional a potential risk of exposure to RNA pesticide and/or its RNAi effector molecules is through bee stingers. It is unknown whether the RNA pesticide uptake by bees is distributed in all tissues including the venom sac or it is only concentrated within the hemolymph system. Although the applicant indicated that the four different formulations did not significantly alter the dissipation profile of dsRNA, it is unknown how these formulations will affect the stability of dsRNA within the bee tissues and whether dsRNA will be used by the ribosome machinery of bee cells to produce various RNAi species (the true effector molecules for gene suppression or silencing). Finally, the target gene of this RNA pesticide is not specified, thus it is unknown whether this pesticide will also silence target gene or off-target genes with sufficient sequence homology in humans.

In summary, HHAB concludes that absorption of RNA pesticide proposed in this field trial in humans through oral ingestion, dermal or inhalation is minimal, however, there is a concern of direct injection of this RNA pesticide and/or its RNAi effector molecules via bee stingers. Additional information on the distribution of dsRNA oligonucleotides and/or their RNAi effector molecules in different tissues of the bee, especially in the venom sac, sequence homology of

target gene to human genes, and whether specific formulations will affect dsRNA stability internally within the bee body are necessary to evaluate potential adverse effect of this specific route of exposure.

References:

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