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Committee for Veterinary Medicinal Products

# *Varroa destructor* calmodulin gene-specific double-stranded interfering RNA EP15 - summary of assessment undertaken to determine whether the substance may be entered into the list of chemical-unlike biological substances considered as not requiring an MRL evaluation

In accordance with Commission Regulation (EU) 2018/782, biological substances other than those identified in Article 1(2)(a) of Regulation (EC) No 470/2009 shall be evaluated on a case-by-case basis where the biological substance is chemical-unlike, and a report describing the scientific basis for the request on whether a full MRL evaluation is required or not shall contain information as set forth in the following sections A to E.

## A. Nature of the biological substance (e.g. cell, tissue, live or killed organism) and a comparison with similar biological substances to which consumers are known to be routinely exposed

The substance is a double-stranded ribonucleic acid (dsRNA) that contains a sequence homologous to a sequence region within the Varroa mite calmodulin gene. The substance belongs to the group of biologic macromolecules (consisting of carbohydrates, amino acids and nucleic acids). It is not produced by chemical synthesis. Based on this information, it can be concluded that the substance is a biological substance to be used in biological veterinary medicinal products.

Exposure to this specific dsRNA and/or siRNA sequences via food commodities is unlikely. In general, RNA is ubiquitous in eukaryotic cells and commonly consumed via plant and animal-derived foods.

## B. Description of the mechanism of action underlying the substance's therapeutic effect and, if available, information on its potency

The dsRNA is planned to be incorporated in a ready-to-use formulation (2 and 4 g/L) containing sucrose to be placed inside a hive. Transfer of dsRNA to mites can occur via bee haemolymph, via feed jelly or by direct contact with the sucrose solution. After uptake in the cells of the mites, the exogenous dsRNA is cleaved in the cytoplasm into several double stranded short sequences of 21 bp (21-mer siRNAs) by so called Dicer proteins. These siRNAs represent the entire length of the exogenous dsRNA. One strand of each siRNA incorporates into a multiprotein RNA-induced silencing complex (RISC), and subsequently it binds to endogenous RNAs carrying homologous sequences (in

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this case the calmodulin of the Varroa mites) resulting in gene silencing. Calmodulin is an essential calcium-binding protein that regulates multiple protein targets. Due to the gene silencing the mortality of the Varroa mites increases and therefore decreases the mite population.

## *C. Fate of the substance in the treated animal (i.e. is it bioavailable, are residues expected in food commodities)*

Residues of dsRNA in honey have not been studied so far. Therefore, it is not known whether residues can be expected / are likely to occur in the food commodity honey.

## *D. Any activity that the substance may have in the human gut (are the residues inactive or do they produce local effects)*

No studies have been submitted to assess the activity in the human gut.

SiRNA and dsRNA are required to enter human cells to have a pharmacological activity, as the function is gene silencing by binding to intracellular RNA. It is expected that in mammals orally ingested dsRNA will not be absorbed and will be rapidly degraded to its constituent nucleotides. In addition, the probability of naked RNA (without a carrier system) overcoming the cell barriers is low due to relatively large molecular weight and its negative charge. Therefore, local effects in the gut affecting the human system are unlikely.

Bacteria have RNA-based regulatory systems, however, the system is not homologous to eukaryotic cells and it is therefore unlikely that the orally ingested dsRNA can interact with this system.

## *E. Systemic availability of residues following ingestion of residues by consumers, along with a worst-case consumer exposure estimate*

It is not known whether residues of dsRNA or siRNA can be found in honey from treated hives. Furthermore, the applied dose per hive and the duration of treatment is unknown yet. Therefore, a theoretic calculation of residues ingested by consumers is not possible.

However, information regarding bioavailability and pharmacological activity in consumers are available:

The dsRNA/siRNAs of concern are non-modified/non-stabilised RNA and are therefore prone to degradation under physiological conditions. Furthermore, they are not attached to a delivery system and the proposed formulation does not contain a specific delivery system that would improve the uptake into the cells. Therefore, they can be seen as similar/comparable to common dietary (ds)RNA. Orally ingested dsRNA/siRNA most likely encounter several physical and biochemical barriers, preventing them from reaching systemic availability in the cytoplasm of dsRNA/siRNA in the human cells in relevant quantities to mediate adverse biological function. Furthermore, it may be assumed that uptake of dsRNA in mammalian cell will be very limited due to the relatively large molecular weight and the negative charge. Therefore, bioavailability is most likely very low.

A bioinformatics analysis (BLAST, MegaBlast, discontinuous Megablast and Burrows wheeler aligner) that allows for comparison of the dsRNA/siRNAs with the human transcriptome to assess the potential for sequence matches with human transcriptome and thus potential off-target matches was performed. No complete alignment with the components of the dsRNA were identified. Based on weight-of-evidence approach, it was concluded that for all but one identified partial alignments with two or less mismatches the likelihood of off-target binding is low. However, considering the low bioavailability, the likelihood that this partial alignment mediates adverse effects is very low.

# Conclusions

Having considered that:

- dsRNA is not modified to change the pharmacokinetic properties (i.e. to increase its stability under physiological conditions),
- consumers orally ingest nucleic acids on a daily basis and there is no known risk associated with this exposure,
- due to the expected rapid degradation of RNA in human stomach and gut, due to size and polar nature of RNA molecules, cell penetration is likely to be very limited and it is overall unlikely that sufficient quantities of dsRNA/siRNA would enter human cells to mediate off-target effects,
- no complete alignment to human transcriptome and only three potential partial alignment off-target sequence have been identified in bioinformatics analysis,
- the siRNA potentially targeting the one identified potential off-target would likely not reach cells in sufficient quantities to mediate a biological effect,

the Committee concludes that no further MRL assessment is necessary for *Varroa destructor* calmodulin gene-specific double-stranded interfering RNA EP15 and that the substance can be included in the list of chemical-unlike biological substances considered as not requiring an MRL evaluation as per Commission Regulation (EU) 2018/782 with the following entry:

*Varroa destructor* calmodulin gene-specific double-stranded interfering RNA EP15 (naked unmodified dsRNA)