13 January 2017
EMA/CVMP/245941/2015-Corr.[1]
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts) (Bees)

On 1 February 2016 the European Commission adopted a Regulation[2] establishing maximum residue limits ("No MRL required" classification) for purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts) in honey, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts) is intended for use in strips to be placed in bee hive brood frames.

VITA (EUROPE) LIMITED submitted to the European Medicines Agency an application for the establishment of maximum residue limits, on 6 January 2014.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 7 May 2015 the establishment of maximum residue limits for purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts) in honey.

Subsequently the Commission recommended on 4 December 2015 that maximum residue limits in honey are established ("No MRL required" classification). This recommendation was confirmed on 25 December 2015 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 1 February 2016.

[1] A typographical error in the title of the document concerning "lupulus" has been corrected
[2] Commission Implementing Regulation (EU) No 2016/129, O.J. L 25, of 02 February 2016

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts)
Therapeutic class:	Antiparasitic agents / Agents (acting) against ectoparasites
Procedure number:	EMEA/V/MRL/003923/FULL/0001
Applicant:	VITA (EUROPE) Limited
Target species:	Bees
Intended therapeutic indication:	Treatment of varroosis due to *Varroa destructor*
Route(s) of administration:	Topical

1. Introduction

Purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts), also referred to as potassium beta resin, is an active substance intended for use in bees for treatment of varroosis due to *Varroa destructor*. The material, which is prepared using extraction of hop strobiles with supercritical CO_2 followed by purification, includes the beta acids lupulone, colupulone and adlupulone. The final concentration of hop beta acids in potassium beta resin is approximately 48% (these acids make up between 1 and 10% of dried hop strobiles).

Potassium beta resin is intended for use in strips to be placed in bee hive brood frames. Two different strengths of strips are envisaged with the higher strength strip containing 8.25 g potassium beta resin, equivalent to 3.96 g beta acids. Two strips will be placed in the brood chamber and will be replaced after two weeks. A total of 2 applications (4 strips) is envisaged, corresponding to a total of 33 g potassium beta resin, equivalent to approximately 16 g beta acids. The lower strength strip contains 3.96 g potassium beta resin, equivalent to 1.9 g beta acids. Two strips will be placed in the brood chamber and will be replaced after one week and again after a second week, resulting in a total of 3 applications of 2 strips, corresponding to a total of approximately 24 g potassium beta resin, equivalent to approximately 11.4 g beta acids.

Potassium beta resin is not used in human medicine although various hop-based herbal medicines are available for human use.

The CVMP provided scientific advice to the applicant in 2011. The advice was only partially followed.

2. Scientific risk assessment

2.1. Safety assessment

The extraction and purification steps performed to produce potassium beta resin from hop strobiles results in the concentration of beta acids (with a final concentration of approximately 48%) and the removal of alpha acids (with a final concentration of less than 1%). Chemical analyses of batches of potassium beta acids reveal that the remaining (50%) portion of the material is made up of water (10 to 15% of potassium beta resin), ash (11-13%) of which the largest component was potassium, low levels of waxes and fixed oils (2 to 5%) consisting primarily of glycolipids, and a larger fraction referred

to as ‘other resins’ (10 to 30%). The ‘other resins’ make up the second largest component (after beta acids) of the resinous fraction of potassium beta resin.

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties

The active component of potassium beta resin consists of hop beta acids. These are weak organic acids, which repel sucking plant pests including two-spotted spider mites *(Tetranychus urticae* Koch) and hop aphids (*Phorodon humuli* Schrank).

The pharmacodynamic targets of hop beta acids are not known, but it is assumed that they affect feeding and ovipositional behaviour of mites. There are also some indications that hop beta acids could affect other reproductive processes of mites.

Hops are reported to have a number of pharmacological effects, many of which have been associated with particular hop components. Antibacterial effects have been associated with the alpha acids, beta acids and essential oils, sedative effects have been associated with alpha acids, beta acids, essential oils and flavonoids, anti-inflammatory activity has been associated with alpha acids, anti-proliferative activity has been associated with alpha acids, anti-diabetic activity has been associated with alpha acids, anticancer effects have been associated with flavonoids, and ectoparasitic activity has been associated with the beta acids. However, as the extraction and purification steps result in an extract that is almost free from alpha acids, essential oils and flavonoids, of the effects mentioned above it is only the antibacterial, sedative and ectoparasitic properties that are relevant for potassium beta resin.

Data are not available to allow the establishment of no effect levels for pharmacological effects associated with hop beta acids. In addition, it is noted that potassium beta resin includes a significant amount of ‘other resins’ that have not been identified. No information is available on potential pharmacological effects associated with this fraction.

Pharmacokinetic properties

No pharmacokinetic data were provided for potassium beta resin or for beta hop acids.

Calculation of a pharmacological ADI, if relevant

Data that would allow derivation of a pharmacological ADI are not available.

2.1.2. Overview of toxicology

No data are available on the toxicology of the final extract, potassium beta resin. However, some limited data are available on the toxicology of the active component of the resin, namely the hop beta acids. The beta acid component of potassium beta resin consists of lupulone (35 to 70%), colupulone (20 to 55%) and adlupulone (10 to 15%). In light of the structural similarity of these acids it is considered that toxicology data generated with lupulone can be considered to also be relevant for the evaluation of colupulone and adlupulone.

In relation to acute toxicity, reported oral LD_{50} values for lupulone vary from 130 mg/kg bw in guinea pigs to 1500 mg/kg bw in mice and 1800 mg/kg in rats. After intramuscular administration, the LD_{50} was 330 mg/kg bw in rats and 800 mg/kg bw in mice, indicating low acute toxicity.

Limited data on repeated dose toxicity are available from a published literature report, although these data are old (dating back to 1950) and so were not generated in line with current standards. In a short term (13 day) study in monkeys with daily oral doses of approximately 200 mg/kg bw lupulone, no effects were seen on body weight, blood parameters, electrocardiogram or liver function.

No data were provided on reproductive toxicity, including developmental toxicity, or on genotoxicity or carcinogenicity of hop beta acids. In addition, it is noted that potassium beta resin includes a significant amount of other resins that have not been identified. No information is available on potential toxicological effects associated with this fraction.

2.1.3. Calculation of the toxicological ADI or alternative limit

Data that would allow derivation of a toxicological ADI are not available.

2.1.4. Overview of microbiological properties of residues

No data are available on the microbiological properties of the final extract, potassium beta resin. However, various components of hops have been associated with antimicrobial actions, in particular alpha acids, beta acids and essential oils. Lupulone is reported to be active against gram-positive bacteria by interfering with the building and functioning of the cell wall and is inactivated by serum phospholipids.

2.1.5. Calculation of microbiological ADI

Data that would allow derivation of a microbiological ADI are not available.

2.1.6. Observations in humans

As mentioned in section 2.1.1 of this report, a number of pharmacological effects have been associated with hops and particular hop components. Effects associated with hop beta acids include antibacterial, sedative and ectoparasitic properties. Available data suggests that a person routinely taking the maximum dose of some hop-based herbal products might ingest up to 20 mg beta acids per day.

2.1.7. Findings of EU or international scientific bodies

The European Medicines Agency's Committee on Herbal Medicinal Products (HMPC) reviewed hop strobiles in 2008. The report indicates that hops have been used in traditional medicine for over a hundred years and that a variety of preparations are available, including preparations produced using supercritical carbon dioxide extraction. In relation to safety, the HMPC report that the "experimental toxicological data on hop preparations are rather limited and incomplete but as a whole show low toxicity" and states that "In view of its long term use and present use in humans hops is considered to be non-toxic and safe with no significant adverse effects."

In the USA hop beta acids have GRAS (generally regarded as safe) status.

2.1.8. Overall conclusions on the ADI

The available data do not allow establishment of a pharmacological, toxicological or microbiological ADI for potassium beta resin and consequently no overall ADI can be established. It is argued that an ADI is not required for potassium beta resin as the safety of the material can be established by demonstrating that consumers are already routinely exposed to hops and hop derivatives and that any exposure resulting from the use of potassium beta resin for the treatment of honey bees will be relatively unimportant. The validity of this argument is considered in section 3.3 of this report.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

No studies investigating the pharmacokinetics of potassium beta resin in honey bees were provided.

2.2.2. Residue depletion studies

Three residue depletion studies were provided in which strips of the proposed product were applied to brood chambers of hives and the resulting residues of beta acids in honey were monitored. For all three studies the limits of detection and quantification of the analytical method were 0.41 and 2.76 mg/kg, respectively.

The first residue study investigated the levels of beta hop acids in honey collected on five occasions up to 35 days following a single application of two strips of the formulated product per hive (further strips were not applied) to six colonies of bees. A further two colonies served as untreated controls. The strips were placed side by side in the bottom brood frames. No strips were placed in the supers. Four of the six treated colonies had only one box with brood while the remaining colonies had one honey box on top of the brood box. Honey samples were collected from each of the six brood boxes and from the two top boxes where no treatments had been applied. Each sample comprised of the honey from ten cells. On day 3 of the study the strips were observed to be still moist but the bees had chewed the strips and removed about 5% of the strip, which was piled up at the bottom of the hive. All bees were observed to be in good health and hive activity was normal. On day 23 the strips were dry and a significant portion had been chewed with the debris collected at the bottom of the hive. The treated colonies showed signs of good health, solid brood pattern and normal bee behaviour. On day 35 of the study the strips were similar to day 23 but more of the strips had been removed to the bottom of the hive. The colonies showed signs of good health, solid brood pattern and normal hive activity. Hop beta acids were not detected in the two untreated top boxes on any occasion. In five of the six treated boxes hop beta acids were either not detected at all or were detected on one or two occasions but at levels below the limit of quantification. In the remaining treated box hop beta acids were detected on all occasions with maximum levels (9.52 mg/kg) seen on day 14. By day 15 levels had decreased to below the limit of quantification. It is noted that this study is not reflective of the expected conditions of use as only a single application of the product was made (up to three consecutive applications are recommended), and as honey from brood boxes was collected (in practice this honey would not be used for human consumption). The study does demonstrate that hop beta acids did not occur above the limit of detection in honey from the two top boxes (sitting on top of the treated brood box) and only rarely were hop beta acids detected in the honey from treated boxes.

The second study investigated the hop beta acid residues in honey from ten colonies each consisting of two brood boxes and one honey shallow box (super) from which honey was collected. Two strips of the product were placed in each brood chamber and honey samples taken from the shallow box after one week. Ten further colonies served as controls. Extracted honey from each treated and control colony was filtered, thoroughly mixed and retained in a closed container until assayed for the presence of hop beta acids. At 24 hours post-treatment the strips were moist, bees were walking on the strips and had started to chew the strip edges. Some strip debris was present in the bottom of the hive. At 48 hours the strips were still moist. At 72 hours the strips were drier but still moist and there was further evidence of the bees having chewed the edges of the strips. At the end of seven days the strips were removed. Analysis of the honey samples from both treated and untreated hives showed no measurable levels of hop beta acids.

The third study investigated the hop beta acid residues in honey from a shallow box (super) following three applications of two strips per brood box to ten colonies. Ten further colonies were used as controls. On day 1 of the study product strips (two per deep box in brood chamber) were inserted into each of the 10 colonies. After one week honey samples were collected and the product strips replaced with new strips. This was repeated at the end of the second week. Further honey samples were collected at the end of weeks 3 and 4 but new strips were not added. The honey samples were stored at –20°C until analyse for hop beta acid residues. Analysis of the honey samples indicated that in all but one sample no hop acids were detected. In the remaining sample, collected 21 days after the start of treatment, the level of hop beta acids was 0.44 mg/kg, i.e. slightly above the limit of detection but below the limit of quantification. At 28 days after the start of treatment levels of hop beta acids were below the limit of detection.

In all three studies only very small quantities of hop beta acids were detected and all of these were in honey sampled close to the position of the strips in the hive. In none of the studies were hop beta acids found in honey sampled from the supers. However, the HPLC method used to determine beta acid levels in honey in the three residues studies had a limit of quantification of 2.76 mg/kg. By today's standards this HPLC-UV method is not very sensitive and the limit of quantification is too high to satisfactorily demonstrate the absence of hop beta acids. In addition, only hop beta acids were measured and not any of the other components of potassium beta resin. These issues limit the accuracy of any assessment of the intake of residues that can be performed (see section 3.3).

Selection of marker residue and ratio of marker to total residues

A "No MRL required" classification is intended and therefore identification of a marker residue or the ratio of marker to total residues is not relevant.

2.2.3. Monitoring or exposure data

No monitoring or exposure data other than that described elsewhere in this report are available.

2.2.4. Analytical method for monitoring of residues

A "No MRL required" classification is intended and, in line with this, no analytical method for the monitoring of residues has been presented.

2.2.5. Findings of EU or international scientific bodies

In the USA hop beta acids have GRAS (generally regarded as safe) status. In the documentation relating to the allocation of GRAS status it was calculated that average daily intake of beta acids from beer and processed foods is 0.34 mg/person/day.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

Potassium beta resin is not proposed for use in dairy animals and consequently relevant effects are not expected.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

Honey bees are considered a minor species and therefore the guideline on safety and residue data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/SWP/66781/2005) and the note for guidance on the risk analysis approach for residues of veterinary medicinal products in food of animal origin (EMEA/CVMP/187/00-FINAL) were taken into account for the evaluation of the reduced data package submitted. In addition, the availability of veterinary medicines for honey bees is very limited and this was also taken into account for the evaluation of potassium beta resin.

Hops have been cultivated and used by humans for hundreds of years. Hops are used as flavouring agents, most notably in the brewing process, where they contribute a bitter taste to beer. Traditionally hops are added to the wort but these days hop extracts, including supercritical carbon dioxide extracts, may be used in place of whole hops. It is reported that only a small portion (less than 4%) of the beta acids from hops are transferred into beer, with most remaining in the spent hops. These may be included in animal feed. The hop beta acid content of beers is variable but, based on the available data, does not generally exceed 1.0 mg/l.

The European Medicines Agency's Committee on Herbal Medicinal Products (HMPC) reports that "In view of its long term use and present use in humans hops is considered to be non-toxic and safe with no significant adverse effects."

Hop shoots are eaten and are often compared to asparagus.

No additional relevant factors were identified for consideration of the risk management recommendations.

3.3. Elaboration of MRLs

The available residue data demonstrate that, following application of potassium beta resin, hop beta acid levels present in honey will be below 2.76 mg/kg (the limit of quantification of the analytical method used in the residues studies). In addition to demonstrating that consumers will not be exposed to significant levels of hop beta acids it is necessary to ensure that they will not be exposed to significant levels of 'other

resins', which have not been specifically identified or toxicologically characterised. These resins make up 10 to 30% of potassium beta resin.

In relation to the beta acids:

The standard food basket includes a portion of 20 g for the daily intake of honey. Assuming a concentration of 2.76 mg/kg beta acids in honey (the limit of quantification of the analytical method), the worst case intake of beta acids would be 55.2 µg/person.

As data to allow determination of an ADI are lacking there is no standard toxicological reference value against which to compare this worst case intake. However, in view of the long-term and well established use of hop derived products, it is considered that an appropriate approach to use, in this case, is to verify that the intake of residues resulting from the use of potassium beta resin in honey production will not greatly increase the overall consumer intake of hop beta acids.

As indicated earlier in this report, the beta acid content of beers is variable but does not generally exceed 1.0 mg/l. A single small (250 ml) beer may therefore lead to a consumer ingesting 250 µg of beta acids. Also as indicated earlier in this report, hop-based herbal medicinal products may include significant levels of beta acids, and regular users may be exposed to levels in the mg per day range. Finally, in the USA, where hop beta acids have GRAS status, it has been estimated that average daily intake of beta acids from beer and processed foods is 0.34 mg/person. Based on these figures it can be concluded that consumer intake of hop beta acids from honey produced in hives treated with potassium beta resins will represent only a relatively small addition to the overall consumer intake of beta acids and does not raise consumer safety concerns.

In relation to other hop resins:

Potassium beta resin contains 10 to 30% of 'other hop resins'. No residue data are available demonstrating the concentration of these in honey following treatment of hives with potassium beta resin. However, information relating to the manufacture of potassium beta resin demonstrates that the 'other resins' portion of hop resins is consistently present in hop strobiles, in the crude supercritical carbon dioxide extract and in the final purified potassium beta resin at approximately half the level of the beta acids. It is therefore considered reasonable to conclude that the physicochemical properties of the 'other resins' are similar to those of the beta acids, and that consequently, transfer of these substances into honey collected for human consumption will occur at a similar rate to transfer of hop beta acids. Based on this assumption it is estimated that the worst case intake of these 'other resins' will be in the region of 28 µg/person/day. It is also considered reasonable to assume that other hop-based products to which consumers are regularly exposed will also contain these 'other resins' at concentrations of approximately half the hop beta acid concentrations. It is therefore considered that consumer intake of 'other hop resins' from honey produced in hives treated with potassium beta resins will represent only a relatively small addition to the overall consumer intake of these other hop resins and does not raise consumer safety concerns.

The above calculations represent a worst case scenario, which is demonstrated by the fact that, in the residues studies, hop beta acids were present at levels below the limit of detection of 0.41 mg/kg in all but one sample collected from honey supers, and in the remaining sample they were present at a level very close to the limit of detection. It would therefore be reasonable to estimate residue intake using the limit of detection instead of the limit of quantification, which would result in exposure estimates approximately 7-fold lower than those provided above.

Based on the above it is considered that the intake of potassium beta resin residues resulting from the use of potassium beta resin in the treatment of bee hives does not represent a consumer safety concern and that the establishment of an MRL in honey is not necessary for the protection of consumer health.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EC) No 470/2009 the CVMP considered the possibility of extrapolating its recommendation on maximum residue limits for purified semi-liquid extract from *Humulus lupulus L*. containing approximately 48% of beta acids (as potassium salts) in honey to other food producing species and commodities. However, residues in honey are not subject to the metabolic processes to which they may be subjected in other food commodities of animal origin. Consequently, extrapolation of MRLs in honey to other food commodities is not appropriate.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- no ADI can be established for purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts) as appropriate data are not available,
- hops have been cultivated and used by humans for hundreds of years and are currently used for flavouring purposes as well as in herbal medicinal products, where they are considered to be non-toxic and safe with no significant adverse effects,
- limited residue data indicate that intake of residues of purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts) resulting from its use in honey production will result in only a relatively small addition to the overall consumer intake of hop-derived residues,
- the establishment of a maximum residue limit in honey for purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts) is not necessary for the protection of human health;

the CVMP recommends the inclusion of purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts) in table 1 of the Annex to Regulation (EU) No. 37/2010 as follows:

Pharmaco-logically active substance	**Marker residue**	**Animal species**	**MRLs**	**Target tissues**	**Other provisions**	**Therapeutic classification**
Purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts)	NOT APPLICABLE	Bees	No MRL required	Honey	NO ENTRY	Antiparasitic agents / Agents acting against ectoparasites

Background information on the procedure

Submission of the dossier	6 January 2014
Steps taken for assessment of the substance	
Application validated:	5 January 2014
Clock started:	6 February 2014
List of questions adopted:	5 June 2014
Consolidated response to list of questions submitted	4 December 2014
Clock restarted:	15 December 2014
List of outstanding issues adopted:	12 February 2015
Oral explanation	8 April 2015
CVMP opinion adopted:	7 May 2015