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Committee for Medicinal Products for Veterinary Use

Committee for Medicinal Products for Veterinary Use (CVMP)

Withdrawal assessment report for HopGuard Gold

EMEA/V/C/002836/0000

Common name: purified semi-solid extract from *Humulus lupulus L.* containing approximately 48% of beta acids as potassium salts

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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Introduction

The applicant Vita (Europe) Limited submitted on 22 July 2016 an application for a marketing authorisation to the European Medicines Agency (The Agency) for HopGuard Gold, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (new active substance).

The eligibility to the centralised procedure was agreed upon by the CVMP on 8 November 2012 as HopGuard Gold contains a purified semi-solid extract from *Humulus lupulus L*. (also referred to in this report as potassium beta resin or KBR) containing approximately 48% of beta acids as potassium salts, a new active substance which was not authorised as a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004. An MRL for the active substance is included in Commission Regulation (EU) No 37/2010 under the name of "Purified semi-solid extract from *Humulus lupulus L*. containing approximately 48% of beta acids (as potassium salts)".

The applicant applied for the following indication: Treatment of varroosis due to *Varroa destructor*.

The active substance of HopGuard Gold, the potassium beta resin containing hop beta acids (as potassium salts) as the constituents with known therapeutic activity, is an antiparasitic product to be used in honey bees for treatment of varroosis. HopGuard Gold is presented as bee-hive strips for in-hive use in packs containing 12 strips or 24 strips.

The applicant (Vita (Europe) Ltd) is registered as an SME pursuant to the definition set out in Commission Recommendation 2003/361/EC.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

On 12 April 2018, Vita (Europe) Ltd withdrew the application at day 203 of the procedure. In its letter notifying the Agency of the withdrawal of application, the applicant stated the reason for the withdrawal: CVMP considered that the data provided do not allow the Committee to conclude on a positive benefit-risk balance.

Scientific advice

The applicant received scientific advice from the CVMP in May 2013 (EMA/CVMP/SAWP/52845/2013) and further clarification in July 2013 (EMA/CVMP/SAWP/356637/2013). The scientific advice pertained to the establishment of the MRL, the quality and the safety (general, user, environmental, consumer and target animal) of the dossier.

The CVMP considered that the applicant, in general, followed the scientific advice; any deviations from the advice given were adequately justified.

MUMS/limited market status

The applicant requested classification of this application as MUMS/limited market by the CVMP, and the Committee confirmed that, where appropriate, the data requirements in the relevant CVMP guideline(s) on minor use minor species (MUMS) data requirements would be applied when assessing the application. MUMS/limited market status was granted as honey bees are considered a minor species.

Part 1 - Administrative particulars

Prescription status

The applicant applied for exemption from veterinary prescription under Article 2 of EU Directive 2006/130/EC. The CVMP considered that the assessment of the application would need to confirm that the product complies with all the criteria prescribed in Article 2 of Commission Directive 2006/130/EC. However, in view of remaining unresolved issues, no final decision on this request was taken (see Part 5 – *Conditions or restrictions regarding supply and use* as part of the benefit-risk assessment for HopGuard Gold).

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (dated February 2018), which fulfils the requirements of Directive 2001/82/EC. Based on the information provided, the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse event occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the active substance and testing before importation to Europe takes place outside the EU. Some further testing of the active substance, the manufacture of the finished product and the control of the dosage form takes place within the EU.

Batch release was within the EU at a site which holds a manufacturing authorisation issued by the relevant competent authority.

A declaration of compliance of the active substance manufacturing site with the standards of the Guideline on *Good Agricultural and Collection Practice (GACP) for starting materials of herbal origin (EMEA/HMPC/246816/2005)* is provided. However, a declaration signed by the QP at the EU batch release site would still need to have been provided.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

The GMP status of the finished product manufacturing sites has been satisfactorily established and is in line with legal requirements. For the active substance, a declaration signed by the Qualified Person (QP) at the EU batch release site would however still need to have been provided.

Part 2 - Quality

Composition

The finished product, HopGuard Gold, is a veterinary medicinal product of herbal origin which is presented as bee-hive strips comprised of multi-layered cardboard strips impregnated with a liquid phase.

The liquid phase contains a potassium beta resin (abbreviated in this report as 'KBR') as the active substance together with several excipients. KBR is a purified semi-solid extract from *Humulus lupulus L*. produced by supercritical carbon dioxide extraction. KBR contains approximately 48% (range 45%–65%) of hop beta acids (as the potassium salts). Hop beta acids (as the potassium salts) are the only constituents of KBR with known therapeutic activity. The KBR content in the finished product is standardized to ensure 3.36 g of hop beta acids per bee-hive strip (equivalent to 16%).

The support for the liquid phase is a strip made of a multi-layered cardboard (which does not contain any colouring agents).

The product is packaged in bags containing either 12 or 24 multi-layered cardboard strips and 300 g or 600 g of liquid phase respectively (the unit dose is one impregnated strip.)

Containers

The primary packaging is a heat-sealed multi-layered bag consisting of 3 layers: (outer layer) polyethylene terephthalate (PET)/(middle layer) aluminium-PET/(inner layer) linear low density polyethylene (LLDPE). A question regarding the quality of the proposed bag's material was still pending.

Each pack contains 12 or 24 bee-hive strips and as not all the strips may be used at one time it would have been necessary before authorisation to modify the bag to allow the beekeeper to open and close the bag on several occasions. This issue remained outstanding at time of withdrawal of the application.

The component materials of the bag comply with the relevant European Pharmacopoeia (Ph. Eur.) and EU requirements.

There is no outer packaging.

Development pharmaceutics

The formulation for HopGuard Gold was based on an earlier formulation for a bee-hive strip which had been authorised outside the EU. This earlier formulation had the same active ingredient and excipients but a different cardboard strip material, and it was used in some of the laboratory and field studies. However, it was found that its absorptive properties were not appropriate to support the proposed treatment period, so that to solve this problem, a new multi-layered cardboard strip was developed.

The inherent antimicrobial nature of the formulation was confirmed.

All the excipients are well known pharmaceutical ingredients and their quality is compliant with the respective Ph. Eur. standards. Regarding the multi-layered cardboard strips, information has been

provided to justify the selection of this material and to further explain the behaviour of the strip in the hive.

At the time of withdrawal of the application some information/data still remained to be provided concerning pharmaceutical development and including the volume of liquid in the bags.

Method of manufacture

The manufacturing process consists of only two main steps and is considered to be a standard process. Firstly, the bulk liquid phase is prepared by heating the KBR and then mixing it with the excipients to obtain the HopGuard Gold liquid phase. The second step is filling of the bags with the cardboard strips and the HopGuard Gold liquid phase, and then closing the bags by heat-sealing.

The concentration of hop beta acids in the active substance (KBR) can vary. Therefore, in order to obtain the target amount of 3.36 g of hop beta acids per strip (equivalent to 16%), the KBR content of the finished product is standardized in the manufacturing process.

The manufacturing steps have been clearly described and appropriate in-process controls have been established at each step.

The main steps in the manufacturing process have been validated by a number of process validation studies, although some deficiencies were observed and some further data would have been required.

No stability data was provided to support any holding time for the bulk liquid phase.

Control of starting materials

Active substance

The active substance, which is not described in the EU pharmacopoeia, is a herbal preparation, a potassium beta resin (KBR), which is defined as a “Purified semi-solid extract from *Humulus lupulus L.*, containing 45%–65% of hop beta acids (as potassium salts). The extraction solvent was in accordance with the CVMP Guideline *on the declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products (EMA/HMPC/CHMP/CVMP/287539/2005 Rev. 1)*. Due to the long length of the formal name, the abbreviation ‘KBR’ has been proposed for use in this application and is used in this report accordingly.

KBR is an amber to brown coloured highly viscous oleoresin. The general properties of KBR have been described, including the pH of a 1% solution, its UV visible absorption spectrum, solubility in water and organic solvents, and density.

KBR contains hop beta acids, as the potassium salts (45–65%), together with other resins, waxes and fixed oils, inorganic compounds and water. The hop beta acids are however the only constituents in the KBR with any therapeutic activity. The other constituents are inert and are not considered as impurities. The hop beta acids are chemically defined active resins (lupulone, colupulone and adlupulone) with similar chemical structures. Lupulone is the main beta acid in the hop beta acids mixture.

KBR exhibits neither stereoisomerism nor polymorphism as it is a mixture of components.

In-house specifications have been proposed for the KBR and these were generally satisfactory. The specified impurities, which are present at higher levels than the qualification threshold established in VICH guideline GL39, were justified by their presence in human foodstuffs (beer). Some questions were still pending at time of withdrawal regarding the addition of some controls and the final KBR specifications remained to be provided.

Characterisation of the active substance and its impurities is in accordance with the EU guidelines on herbal preparations (*Guideline on Quality of herbal medicinal products/traditional herbal medicinal products, CPMP/QWP/2819/00 Rev. 2* and the HMPC *guideline on Specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products, CPMP/QWP/2820/00 Rev. 2*). Potential and actual impurities were well discussed with regards to their origin and were sufficiently characterised.

KBR is obtained by extraction from hop strobiles (the starting material). The manufacturing process steps were all defined and standardized. The process starts with production of the hop vines followed by harvesting of the hop strobiles. Details of the rest of the manufacture and processing steps are given. No organic solvents are used in the manufacture of the active substance.

The description of the preparation of the starting material, hop strobiles, includes information regarding their production under GACP, and the production of the hop vines is duly regulated by the appropriate department. Adequate descriptions of both the preparation processes used for the starting material and the in-process controls are provided.

Specifications are provided for the starting material, although some questions remain to be satisfactorily resolved.

Batch analyses data from three production batches of the starting material (hop strobiles), have been provided. Adequate in-process controls are applied during the extraction of the active substance from the hop strobiles. The specifications and control methods for the raw materials, intermediate products and reagents have been presented. Many analytical methods used for the starting material, hop strobiles, are those of the relevant Ph. Eur. monographs. All other methods have been sufficiently validated in accordance with VICH guideline GL2.

The analytical methods used for the KBR starting material are the general methods of the Ph. Eur. whenever applicable; however, at the time of withdrawal of the application some issues still remained to be resolved.

Information on the reference standards used for the assay and impurities testing has been presented, although some additional information on one the reference standards used is still pending.

Information on the container used for storing the KBR has been presented.

Batch analyses data from 3 batches of KBR have been provided. Stability data on five production scale batches of KBR were also provided but the study was not conducted in accordance with VICH guideline GL3 conditions. However, in accordance with the CVMP Guideline on *quality data requirements for veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMEA/CVMP/QWP/128710/2004)*, testing of the KBR to its full specification is proposed immediately before use in the manufacture of the final medicinal product. This is satisfactory.

Excipients

The excipients in the liquid phase of HopGuard Gold are well known pharmaceutical ingredients and their quality is compliant with current Ph. Eur. monographs.

The multi-layered cardboard strips which are used as a support for the liquid phase each consist of six layers. Each strip is folded and the inner side of the strip is corrugated. The material of their construction is not described in any pharmacopoeia and in-house specifications have been proposed. Due to the importance of the nature of the strip in the behaviour of the medicinal product, their manufacturer and the materials used for their construction have been declared, and confirmation provided that only those exact strips would be used. However, the dossier had not been fully amended to include this information at time of withdrawal of the application.

The nitrogen used in the manufacture of the finished product complies with the Ph. Eur.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

None of the starting materials used for the active substance or the finished product are risk materials as defined in the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products. A valid TSE declaration from the applicant has been provided.

Control tests on the finished product

The specifications proposed at release and at the end of shelf life were provided and were mostly appropriate to control the quality of the finished product although some parameters and limits still required amendment (or further justification) at time of withdrawal of the application, including two major concerns.

In general, the analytical methods used have been adequately described and appropriately validated in accordance with the relevant VICH guidelines.

One outstanding issue concerns the absence of the control of degradation products in the finished product specifications at release since it has not yet been demonstrated that degradation does not occur during the manufacturing process.

Batch analyses results are provided for three batches of each pack size. Although the batches are slightly smaller than pilot scale, no significant differences are expected on scale-up due to the simplicity of the manufacturing process. However, before the data could be accepted some questions remained regarding the specifications and one of the analytical methods.

Stability

Stability data are provided for the three batches of each pack size of the finished product mentioned above. The batches were stored under long term conditions for 36 months at 25 ºC/60% RH and at 30 ºC/65% RH, and for up to 6 months under accelerated conditions at 40 ºC/75% RH, in accordance with VICH guideline GL3. The batches of the finished product were packaged in the primary packaging proposed for marketing.

Based on the available stability data, the proposed shelf life period of 3 years when stored below 25 ºC might have been acceptable when, furthermore, assurance was provided that the first three

production batches would have been placed on stability to confirm the stability results obtained (which is in agreement with the CVMP Guideline on *quality data requirements for veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMEA/CVMP/QWP/128710/2004))*. Compliance with the *CVMP Guideline on start of shelf life of the finished dosage form (EMEA/CVMP/453/01)* was confirmed.

No photostability testing has been performed with the liquid impregnated cardboard strips, but based on the CVMP Guideline on *quality data requirements for veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMEA/CVMP/QWP/128710/2004)* the storage condition 'Store in the original container' was proposed and considered acceptable as the primary packaging is opaque and offers adequate protection from light.

At the time of withdrawal of the application no in-use shelf life period had been satisfactorily justified.

Overall conclusions on quality

Information on the development, manufacture and control of the active substance and the finished product has been presented. The results of tests carried out could have indicated consistency and uniformity of important product quality characteristics, which could have led to the conclusion that the product should have a satisfactory and uniform performance in clinical use, provided that remaining outstanding issues had been satisfactorily resolved.

The manufacturing process is considered a standard manufacturing process due to its simplicity. The main steps in the manufacturing process and in-process controls have been described and validated by a number of process validation studies, although some deficiencies were observed and some further data would have been required to justify the proposed batch size and studies would have been required to establish a holding time for the bulk liquid. The active substance is an herbal preparation, a potassium beta resin (abbreviated as KBR). The information provided on the KBR is comprehensive regarding the general information and the description of the extraction process, confirming that the proposed route of extraction is able to produce the desired extract with a suitable quality of the hop beta acids, which are the constituents with the therapeutic activities. The remaining constituents of the KBR are inert. Potential and actual impurities were well discussed with regards to their origin and they were sufficiently characterised. The description of preparation of the starting material (hop strobiles) includes information regarding their production, harvesting, etc, including the facilities, processes of preparation of the starting material, raw materials and in-process controls. Some questions had been raised regarding the control of the hop strobiles, and also the KBR, which would have needed to be resolved prior to a marketing authorisation being granted. Batch analyses data from three production batches of the starting material (hop strobiles) have been provided but their validity is compromised whilst there are unresolved questions regarding the specifications of the starting material. Batch analyses data from 3 batches of KBR have been provided, however, their validity is compromised as questions remain regarding the KBR specifications. Regarding the stability data for the active substance, although some data have been provided, there was no appropriate stability study performed in accordance with VICH guidelines. Nevertheless, based on the CVMP Guideline on *quality data requirements for veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMEA/CVMP/QWP/128710/2004)*, full testing of the active substance to its full specification immediately before its use in the manufacture of the final medicinal product was confirmed.

The excipients used in the liquid phase are well-known pharmaceutical ingredients and their quality is compliant with the relevant Ph. Eur. standards.

Information has been provided on the multi-layered cardboard strips used as supports for the liquid phase. Due to the importance of the nature of the strip in the behaviour of the medicinal product, their manufacturer and the materials used for their construction have been declared, and confirmation provided that only those exact strips would be used. However, the dossier had not been fully amended to include this information at time of withdrawal of the application.

The product does not contain any materials derived from human or animal origin.

Specifications proposed to control the finished product at release and during shelf life were generally acceptable but still some outstanding concerns remained to be settled. Most of the control methods are described and generally appropriately validated, although all outstanding issues have not yet been fully resolved.

The stability studies on the finished product were conducted in accordance with VICH guidelines and the data provided might have supported a shelf life of 3 years when stored below 25 °C, and stored in the original container (due to a lack of any photostability data). The veterinary medicinal product would have needed to be for immediate use only since there was a lack of adequate in-use stability data.

Some further quality information would also have been recommended to be provided post-authorisation. In general the application takes into account current rules and guidelines. However, several outstanding quality issues remained that would have needed to be resolved prior to a marketing authorisation being granted.

Part 3 – Safety

The active substance of HopGuard Gold is a purified semi-solid extract from *Humulus lupulus* containing approximately 48% of beta acids as potassium salts (also referred to in this report as Potassium Beta Resin or KBR), which is a new active substance not authorised for use in a veterinary medicinal product in the EU before. The active substance is also known as purified hop extract; and is included in Commission Regulation (EU) No 37/2010 under the name of "Purified semi-solid extract from *Humulus lupulus L*. containing approximately 48% of beta acids (as potassium salts)". The active principles in the purified hop extract are the hop beta acids lupulone (35 to 70%), colupulone (20 to 55%) and adlupulone (10 to 15%). A full safety file in accordance with Article 12(3)(j) has been provided.

The application makes reference to the previous assessment of the CVMP in the context of the application for the establishment of maximum residues limits for purified semi-solid extract from *Humulus lupulus* containing 48% of beta acids (as potassium salts) in honey (procedure No: EMEA/V/MRL/003923/FULL/0001); and the scientific advice to the applicant (procedure No: EMA/CVMP/SAWP/52845/2013) relating to quality, safety and MRL issues for the development of this veterinary medicinal product.

Safety documentation

Pharmacodynamics

See part 4 for information on pharmacodynamic effects relevant to the intended therapeutic effect.

Hops are reported to have a number of pharmacological effects, many of which have been associated with particular hop components. However, the extraction and purification steps for HopGuard Gold

result in an extract that is almost free from alpha acids, essential oils and flavonoids, and contains mostly hop beta acids (approximately 45%–65%, i.e. lupulone, colupulone and adlupulone).

Hop beta acids have been associated with antibacterial, sedative and ectoparasitic properties. Data are not available to allow identification of no effect levels for pharmacological effects in mammals associated with hop beta acids. In addition, the active ingredient includes a significant amount of unidentified components and no information is available on potential pharmacological effects of these. The absence of these data was accepted by the CVMP for the approval for the MRL for purified hop extract as it was considered that ingestion of honey would result in only a relatively small addition to the overall consumer intake of beta acids and other resins extracted from hops and in view of the fact that hops have been cultivated and used by humans for hundreds of years.

Pharmacokinetics

See part 4.

Toxicological studies

No data are available on the toxicology of the final extract, purified hop extract. However, some limited data are available on the toxicology of hop beta acids and particularly lupulone. 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.

Overview of toxicology

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 600 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 or of other resins present in the active substance. The absence of this data was accepted by the CVMP for the approval for the MRL for purified hop extract as it was considered that ingestion of honey would result in only a relatively small addition to the overall consumer intake of beta acids and other resins extracted from hops and in view of the fact that hops have been cultivated and used as flavouring agents by humans for hundreds of years. It is also noted that 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 are considered to be non-toxic and safe with no significant adverse effects."

Studies of other effects

Data on the ocular irritancy of the HopGuard Gold product are not available. However, a study evaluating the ocular irritancy of purified hop extract was provided. Purified hop extract is classified as a severe eye irritant.

Studies on the skin irritation and skin sensitization potential of the HopGuard Gold product were not provided. However, based on the high pH of the formulation and the fact that the product is considered a severe eye irritant, it is concluded that the product can be classified also as a skin irritant, and no further studies may be required. Dermal allergic reactions are the only relevant scenario. Although the likelihood of allergic reactions is considered to be quite low, it is possible to include an additional warning regarding possible allergies.

Excipients

The excipients present in the HopGuard Gold are well known constituents of human and veterinary medicines and unlikely to present a hazard.

User safety

The applicant has presented a user safety risk assessment which has been conducted following the sections outlined in the CVMP Guideline on user safety for pharmaceutical veterinary medicinal products (EMEA/CVMP/543/03-Rev.1).

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely are those of dermal and ocular exposure.

Regarding the dermal exposure, the product is considered to cause skin irritation and to be a skin sensitiser; however, the possible risk to the user is sufficiently mitigated by the proposed warnings in the product literature. In relation to ocular exposure, the eye irritation study demonstrated that purified hop extract is a severe eye irritant. As a consequence, it is recommended that precautionary statements shall be placed in the product information.

As a result of the user safety assessment the following advice to users/warnings for the user should be included in the product literature:

- The veterinary medicinal product can cause irritation of the skin and eyes. Avoid direct contact with skin, eyes and mucous membranes.
- People with known hypersensitivity to hop acids or any of the other ingredients should avoid contact with the veterinary medicinal product.
- Personal protective equipment consisting of impermeable gloves and the usual beekeeper protection equipment should be worn when handling the veterinary medicinal product. Wash contaminated clothes after use.
- In case of accidental contact with skin, wash the affected area thoroughly with soap and water.
- In case of accidental contact with the eye(s), rinse the eyes thoroughly with copious amounts of clean running water and seek medical advice.
- Do not eat, drink or smoke while using the product.
- Wash hands after use.

Environmental risk assessment

A Phase I environmental risk assessment (ERA) was provided according to the decision tree from the VICH guideline GL6. The environmental risk assessment can stop in Phase I and no Phase II assessment is required because the active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. The veterinary medicinal product will not come into contact with water or soil.

The SPC includes the following risk mitigation measures for HopGuard Gold: “Any unused veterinary medicinal product or waste material derived from such veterinary medicinal product should be disposed of in accordance with local requirements. Do not contaminate ponds, waterways or ditches with strips or empty packaging”.

The use of HopGuard Gold according to the SPC is not expected to pose a risk for the environment. Nevertheless, considering that the active substance has acaricidal activity, it is pertinent to include a specific warning in the SPC to avoid the accidental contamination of surface water and potential unwanted impacts on non-target organisms.

Residues documentation

MRLs

The MRL status of the constituents of HopGuard Gold is as follows:

Pharmacologically active substance	Marker residue	Animal species	MRL	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 against ectoparasites

The excipients propylene glycol and polysorbate 60 are allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required when used as in this product.

The cardboard strip does not have an MRL status. The chance that this excipient will be present in the honey collected from hives treated with HopGuard Gold is very small. The supplier certified that the strip is food grade and is compatible with aqueous and fatty food.

Analytical method

Not applicable since numerical MRLs have not been established for any of the constituents of HopGuard Gold.

Residue studies

Pharmacokinetics

No studies investigating the pharmacokinetics of purified hop extract in honey bees were provided.

Depletion of residues

Three studies have been conducted with the final formulation of HopGuard Gold in honeybees. The studies covered different treatment protocols:

1. 1 application of 2 strips, for a 28 day period
2. 2 applications of 2 strips, first application for 14 days, second application for 14 days
3. 2 applications of 2 strips, first application for 21 days, second application for 21 days

Samples of honey and wax were collected from different parts of the hive. No residues above the limit of detection of the method were reported in any of the samples (LOD=0.41 mg/kg). The stability of the samples was not studied during the validation of the analytical method.

Another three residue depletion studies were presented applying the commercial product strips to the brood chambers of hives during the application for establishment of an MRL for the active substance. 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.

The analytical method used in all the residue depletion studies was the same. The accepted LOQ for this method is 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 demonstrate the absence of hop beta acids. However, as indicated earlier, the CVMP concluded, in its MRL evaluation, that the level of consumer intake of beta acids and other resins extracted from hops could be accepted.

Withdrawal periods

A "No MRL required" status was granted for the active substance of HopGuard Gold based on its low toxicity profile, the small additional exposure likely to occur as a result of its use in the treatment of bees and its common use in the food industry as flavouring agents and in herbal medicines. It is not expected that its use in honey production will significantly increase the overall consumer intake of hop-derived residues. Therefore, a withdrawal period of zero days in honey is adequate and is not considered to pose a risk for the consumers.

Overall conclusions on the safety and residues documentation

Safety:
Honey bees are considered a minor species and the availability of veterinary medicines for honey bees is very limited. This was taken into account for the evaluation of the safety of HopGuard Gold and its active substance, purified semi-solid extract from *Humulus lupulus L*. containing approximately 48% of beta acids (as potassium salts).

There is very little information regarding the pharmacological and toxicological properties of the purified hop extract. However, the lack of information is considered acceptable in this case. The toxicity of the components of the purified hop extract can be considered to be very low. Hops have been cultivated and used by humans for hundreds of years and are used as flavouring agents, most notably in the brewing process, where they contribute a bitter taste to beer. Hops are considered to be non-toxic and safe with no significant adverse effects reported by the European Medicines Agency's Committee on Herbal Medicinal Products (HMPC).

Regarding the user safety, the only routes of exposure considered are topical and ocular following hand to eye contact, and exposure would be to the whole product. In relation to ocular exposure, the

eye irritation study demonstrated that the potassium beta resin is a severe eye irritant. As a consequence, precautionary statements shall be included in the product information. In relation to skin exposure, no skin irritation and skin sensitization studies of the HopGuard Gold product are presented. Based on the high pH of the formulation and the fact that the product is considered a severe eye irritant, it is concluded that the product can be classified also as a skin irritant, and no further studies may be required. Appropriate warnings for the user have been included in the product literature and some minor amendments have been proposed. In light of the low toxicity profile of the active substance and the unlikeliness of dermal contact if the amended user safety warnings are followed, it is considered that the possible risk to the user can be sufficiently mitigated by the warnings of the product literature.

With respect to the environmental risk, the active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Based on the data provided, the use of HopGuard Gold is not expected to pose a risk for the environment when used according to the SPC.

Residues:
The applicant has provided three new residue depletion studies to prove the absence of residues in the honey treated with HopGuard Gold. No residues above the limit of detection of the method were reported in any of the samples from the studies (LOD=0.41 mg/kg). The results from these studies are similar to those found in previous studies, which were assessed by the CVMP during the application for the establishment of MRLs for purified semi-solid extract of *Humulus lupulus L.* containing approximately 48% of beta acids (as potassium salts). While the analytical method used in all of these studies has not been validated in compliance with the standards recommended in the current guidelines, this method was accepted with its limitations by the CVMP during the assessment of the MRLs for potassium beta resin as the method is not required for monitoring of residues.

A “No MRL required” status was recommended for the active substance of HopGuard Gold by the CVMP.

It is concluded that exposure to honey produced in hives treated with HopGuard Gold is not considered to present a risk for the consumers. Therefore, a withdrawal period of zero days in honey can be established for the product HopGuard Gold.

Part 4 – Efficacy

Pharmacodynamics

See also part 3.

The active substances of the product are hop beta acids, e.g. lupulone, colupulone or adlupulone. Both hop alpha and beta acids are weak organic acids found in the lupulin glands of the cones of the female hop plant, *Humulus lupulus L.*; however, only hop beta acids were found to have a potential effect on *Varroa* mites.

The mode of action of hop beta acids against *Varroa* mites has not been fully characterized, but direct contact between hop beta acids and the mite is required. At the present, little is known about the mechanisms of action of hop beta acids.

HopGuard Gold are cardboard strips impregnated with a viscous liquid containing 16% of hop beta acids. Since hop beta acids are not volatile, bees have to come into direct contact with the liquid.

A systemic effect of hop beta acids in *Apis mellifera carnica* honey bees is unlikely according to literature data. Traces of hop beta acids (below 1%) were recovered in the haemolymph of *Apis mellifera carnica* after topical application, possibly by penetrating through the bee cuticle but the contents are too low to establish a systemic effect against *Varroa* mites.

Available data suggest that hop beta acids exert their action on the mites following topical exposure by an as yet not fully characterised mechanism. Three possible mechanisms are proposed for the action of hop beta acids on *Varroa destructor* mites in the bee hive, namely:

Repellency: it is thought that the mites are repelled from the parts of the hive that are treated with HopGuard Gold, i.e. the brood chambers.

Oviposition: the presence of hop beta acids reduces the amount of mite eggs deposited in treated areas.

Anti-feedant: a naturally occurring substance in certain plants that adversely affects insects which eat them. This has been described in literature where fewer two-spotted spider mite adults and eggs were found in hop plants treated with hop beta acids compared with controls.

Given the differences between the biochemistry of insects and mammals, none of the proposed pharmacodynamic effects is expected to be replicated in man.

Development of resistance

From the data provided, limited information about the mechanisms of action of hop beta acids is available. However, hop beta acids are a new chemical entity, which have not been used in veterinary medicine before, and so far, resistance against hop beta acids has not been reported in the literature. Although the risk of resistance development with regard to the use of this product has not been specifically investigated, the use of HopGuard Gold is currently not expected to pose a risk in regard to resistance development.

Pharmacokinetics

No pharmacokinetic studies in honey bees have been performed. The target for the hop beta acids are mites in the phoretic phase of their life cycle (i.e. mites on top of the bees, outside the capped cells). The exposure of bees to the active substance is considered to be only topical, as a systemic effect of hop beta acids in the bee is negligible (see above).

The absence of pharmacokinetic studies in honey bees is therefore considered acceptable (see also scientific advice).

Dose justification

The applicant justified the dose and posology based on bibliographic references, and results from laboratory and field studies.

Two laboratory dose determination studies were submitted investigating different levels of active substance administered to caged bees either applied topically directly or via contact to the cardboard strip. The second study also investigated the effects of presence or absence of sugar in the

formulation. The tests were performed in line with the CVMP Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA/CVMP/EWP/459883/2008).

The first study included several tests investigating the tolerance and efficacy of different commercially available formulations containing hop beta acids in caged bees. *Apis mellifera carnica* and *Varroa destructor* were recruited from an apiary in Germany. Bees were treated topically by trickling of hop beta acids in solution or via contact to a cardboard strip. Hop beta acids in solution were administered to the treatment groups either using the same amount (5 µl per bee) but different concentrations (0.125 – 3%, group 1), or using increasing amounts (0.5 – 1.3 µl per bee) at a concentration of approximately 16% (group 2). For the strips (group 3; old formulation), differently sized cardboard strips per cage were used. The tests were conducted with a least 30 bees per concentration; and the efficacy determination was based on Varroa mite mortality at 24, 48 and 72 hours.

Group 1: Tolerance was tested at doses equivalent to 6, 12.5, 25, 37.5, 50, and 150 µg hop beta acids per bee. Bee mortality in the treated bees was low with 1.67, 1.67, 5.00, 5.00, 5.00 and 5.00%, respectively. Bee mortality in the placebo control group was 1.67%. Also, efficacy was tested at doses corresponding to 6, 9, 12.5, 25, 37.5, and 50 µg hop beta acids/bee. Mite mortality in treated bees was 50.00, 43.33, 73.33, 90.00, 93.33 and 93.33%, respectively. Mite mortality in the control group was 33.33%.
Group 2: Tolerance was tested at doses equivalent to 80, 100, 150 and 200 µg hop beta acids/bee. Bee mortality in the treated bees was high with 63.3, 58.33, 80.00, and 88.33%, respectively. Bee mortality in the placebo control group was 5%.
Group 3: Tolerance was tested at doses equivalent to 9.82, 12.5, 15.18 and 20.52 µg/bee. Bee mortality in the treated bees was low with 1.00, 2.00, 1.00 and 0.67%, respectively. Bee mortality in the placebo control group was 0.40%.

In this study, the minimum effective dose of the active substance directly applied to bees using the solution (group 1) that achieved 90% mite mortality was 25 µg/bee, and doses of up to 150 µg/bee of potassium beta acids were well tolerated by bees. However, the dose range in the study to determine the efficacy (6-50 µg/bee) did not correspond to the dosages used to determine the safety in the different tests provided using the card board strips (9.82-20.52 µg/bee), and the efficacy of the minimum effective dose (25 µg/bee) was not tested with the bee-hive strips.

In the second study , the maximum tolerable concentration of hop beta acids in honey bees and the efficacy against *V. destructor* after dermal application was investigated in the presence or absence of sugar. *Apis mellifera carnica* and *Varroa destructor* were recruited from honey bee colonies of an apiary located in Berlin (Germany). The laboratory and field tests were not conducted with the final product, but with a commercially (in the USA) available formulation of hop acids, containing approximately 10% hop beta acids.

Tolerance of hop beta acids was tested on *A. m. carnica* under laboratory conditions. Bees were treated individually by dermal application (trickling of 5 µl solution onto the abdomen) of the test solution in different concentrations. The test was conducted in two different ways: (1) with a solution containing sugar syrup (50%), or (2) with a solution without sugar syrup (distilled water or 0.1% KOH). The pH value was not altered and stayed at solution pH 10.3.

Bee mortality was monitored for 3 days in intervals of 24 hours. The tolerance tests were conducted with at least 30 bees per concentration (10 bees per cage, 3 cages) and one replicate (minimum number of tested bees = 60 per concentration) at an average temperature of 22 °C and relative humidity 63%. In addition, in order to simulate conditions in the field, where bees on the upper part of the combs are exposed to higher dosages than bees in other hive locations, additional laboratory

tests were performed with only 10% of bees per cage being treated (“acceptor bees”). These bees are expected to distribute the product via direct contact to the untreated "companion bees". Bees received different concentrations of hop beta acids (1.00–5.00%), corresponding to a dose of hop beta acids of 50–250 µg/bee.

Results showed that administration of hop beta acids at the lowest tested dose in sugar solution (1%, i.e. 50 µg/bee) resulted in a significantly higher bee mortality compared to control bees (26.7%). The dermal application of hop beta acids at pH 10 in distilled water was well tolerated by the bees in concentrations up to 4% (200 µg/bee), whilst dosages of 250 µg diluted in distilled water were less well tolerated, in particular by acceptor bees when they were placed in cages with low numbers of companion bees. The cage tests with acceptor bees show that higher concentrations (up to 5%) can be tolerated especially when larger numbers of companion bees are present. Duration of treatment was extrapolated from the posology used for another bee-hive strip with the same active ingredients but different excipients (not authorised in the EU, but used in the USA). In the absence of brood, a single treatment was considered sufficient. However, in the presence of brood (spring, summer, autumn treatment), treatment should be repeated, once, after 21–28 days, as treatment does not reach mites in capped brood cells.

In addition to these studies, field studies were performed in order to assess the efficacy and safety of HopGuard Gold with the proposed dose of 1-2 bee-hive strips per colony under field conditions (see Field trials).

Conclusions:

In conclusion, the dose justification is mostly based on bibliographic references and laboratory studies. A minimum effective dose of 25 µg/bee and maximum tolerable dosages of up to 150 µg/bee were established when administered directly to caged bees. However, an extrapolation of these doses to the recommended dose in terms of number of strips and dosing interval of the product has not been adequately justified. In addition, the minimum effective dose and the maximum tolerated dose were not tested with the bee-hive strips, and also insufficient data are available from field studies, to confirm the safety and efficacy of the proposed dose and dosing interval under field conditions.

In the absence of adequate efficacy and safety data from field studies, the proposed posology for HopGuard Gold is currently not considered adequately supported.

Dose confirmation studies

See Field trials.

Target animal tolerance

Target animal tolerance was studied in two laboratory studies, described above, and in field studies (see field trials).

In addition, the applicant submitted a small scale field tolerance study, which according to the applicant was GCP-compliant and performed in compliance with the CVMP Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA/CVMP/EWP/459883/2008). This study examined the tolerance of HopGuard Gold in healthy honeybee colonies (*Apis mellifera macedonica*) in Greece during summer (in the presence of brood) over 45 days. Colonies were grouped according to the treatment they were receiving, to get a similar mean strength and mite infestation. No data have been provided to support this statement and it is still unknown by which means homogeneity was achieved and randomization was performed. Each group consisted of six

colonies. The length of treatment was 28 days and included two applications of two strips, at 14-day intervals, at D1 and D14, i.e. a shorter treatment interval as the proposed one of 21-28 days for summer treatment.

HopGuard Gold was administered at different doses as follows:
Group 1 (n=6): 2 applications of 4 strips (4 strips per application was applied) of HopGuard Gold, i.e. 2 x recommended treatment dose (RTD).
Group 2 (n=6): 2 applications of 2 strips (2 strips per application was applied) of HopGuard Gold, i.e. 1 x RTD.
Group 3 (n=6): 4 blank cardboard strips, replaced at a 14 day interval for a 28 day period (negative control).

General behaviour: The applicant stated that no changes were observed in any case and in any parameter monitored (e.g. flight activity, foraging, agitation, erratic movements, egg-laying, and aggressiveness). However, no data has been provided to support this statement and so no conclusions can be drawn of the affectation of the general behaviour.

Worker bees: According to the applicant, the application of HopGuard Gold at both doses (1 x and 2x RTD) resulted in significant higher bee losses when compared to control. Increased mortality in honeybees was only noted within the first 24-48 hours post application, and affected mainly the bees that had been in direct contact with the liquid dripping off the bee-hive strips at the time of application (wet bees). Adult bee losses (worker bees) were recorded using an under-basket placed in front of the entrance of each colony (a gary trap) and the number of dead bees were recorded on days -3, 3, 5, 14 and 28. After 48 hours, bee mortality (measured in numbers of dead worker bees in front of the experimental colonies) returned to the same level as the control colonies. None of these statements have been adequately supported with raw data in line with the principles outlined in VICH GL 9.

Drones: The applicant states that at 2x RTD (4 strips of HopGuard Gold), mortality in drones was significantly higher in treated colonies than in control hives. No significant differences in drone mortality counts were observed between the normal dose (two strips) and control. The available data to justify this statement is scarce and not in line with the principles outlined in VICH GL 9.

Queens: The applicant states that no queens were lost in colonies which received the normal dose (1xRTD) of HopGuard Gold or in the control colonies and the laying of the queens was not affected. However, in the overdosed group (2xRTD) two out of the six queens were found dead, fully wet in traps, within the first 24 hours post application. None of these statements have been adequately supported with raw data in line with the principles outlined in VICH GL 9 and the explanation provided about the queen loses in the overdosed group is considered unsatisfactory.

Brood: According to the applicant, results showed that the brood area in all hives increased during treatment with HopGuard Gold. Colonies which received 2xRTD presented an average increase of 7.42%, while colonies receiving the 1xRTD increased their brood area by an average of 97.76%. Two out of the six queens in the 2xRTD were lost during the trial, thus explaining the low brood increase in the batch of double dosing. Control colonies presented an average increase of their brood area by 54.79%. All these statements have not been adequately supported with raw data in line with the principles outlined in VICH GL 9.

Colony strength: The applicant states that development of control colonies was significantly higher compared to development of the colonies where double dosing (2xRTD) of the product was used, whilst no significant differences were observed between control colonies and colonies receiving a normal dose (1xRTD). All these statements have not been adequately supported with raw data in line with the principles outlined in VICH GL 9. In the light of the shortcomings identified, solid safety

conclusions cannot be drawn from this study. The applicant has not provided the requested missing information in line with the principles outlined in VICH GL 9 on Good Clinical Practice, and outstanding issues still remain unclarified.

In addition to the above described small scale target animal safety study, tolerance was also investigated in the field studies (see *Clinical field trials* below), and further studies, which were however only considered as supportive and presented the same shortcomings as mentioned above (e.g. cannot be considered GCP-compliant).

One study tested different formulations in order to optimise the delivery and acceptability of the active substance, using an earlier strip presentation containing a hop beta acid solution with a mean efficacy greater than 90%. This initial formulation was well-accepted by the colony and caused no deleterious effects on the queen or the colony. Bee mortality was described of 7.16%, although there is no statistically significant difference in absolute number of bees. There was, however, no adequate information on adverse effects of the treatment on colony queens or on reproduction.

The reproductive capacity and longevity of queens were assessed in another small scale field study. To evaluate the reproductive capacity of queens, five colonies over a two year period were treated with hop beta acids (not the final product formulation) during two winters; the control colonies received oxalic acid. After treating colonies consecutively for two years with hop beta acids there was no indication of adverse effects on colony over-wintering and the development of treated colonies in the following spring. There were no significant differences found in the brood area between the groups. There were no losses of queen or weaker reproduction capacity of queens. Colonies were lost but it could be attributed to normal winter losses which vary between 25 and 30%.

Conclusions:

Although the applicant stated that the administration of the product at the recommended dose could be expected to be safe for the colonies, higher mortality in adult bees was noted in experimental colonies compared to untreated controls, within the first 24–48 hours post application, mainly affecting those bees that had been in direct contact with the liquid dripping from the bee-hive strips at the time of application. After a period of 48 hours, mortality levels of worker bees (measured as dead bees in front of experimental colonies), returned to the same level as the control colonies. Field studies did not confirm the target animal safety of the application.

Pivotal information in line with the principles outlined in VICH GL 9 and the CVMP Varroa guideline are still missing and further clarification is needed on a number of issues raised in regard to the target animal safety (worker bees, drones and queens) of the application; therefore, no final conclusions can be drawn on target animal safety.

Clinical field trials

Dose confirmation / Clinical studies

A number of field studies (5 declared as GCP) were provided investigating the efficacy and tolerance of HopGuard Gold in the treatment of varroosis, in the presence or absence of brood. According to the applicant these studies were conducted in compliance with VICH GL 9 (Good Clinical Practice).

Studies in the presence of brood:

Seven field studies were provided, which were performed in the presence of brood in summer or autumn; two field studies undertaken in the EU (Greece and Italy), and five supportive studies undertaken in the USA, Canada and South America.

One EU field study was conducted in Greece in autumn, investigating the efficacy of Hopguard Gold when administered at a single dose or once repeated at different dosing intervals, including the proposed dosing interval of 28 days.

The single dose (2 strips) was administered once in 8 colonies, and left in place for a 28 day period. Repeated use was tested with different dosing intervals with 2 applications of 2 strips. One group received the first application of 2 strips for 28 days, and treatment was repeated (i.e. 2 new strips were administered) for another 28 days. In another (third) group, treatment intervals lasted 14 days each. A fourth group (control) was only treated with placebo (1 application of 2 blank strips, for a 28 day period). Follow-up treatment was performed using consecutive treatments with thymol, fluvalinate and oxalic acid.

Results showed efficacy levels of 78.84% in the group treated once. Repeated treatment resulted in efficacy levels of 91.95% (at fixed 28 days interval) and 87.12% (14 day interval), compared to 10.14% (control). However, the efficacy levels of 91.95% with the posology of 2x2 at 28 days (which is not the claimed posology of 2x2 at 21-28 days) cannot be considered for an efficacy assessment taking into account all the shortcomings mentioned above (e.g. study not in line with the principles outlined in VICH GL9 and in the CVMP Varroa guideline). In brief:

- No original field data capture forms, no randomisation or blinding methods, no monitoring of adverse events, theoretically colony strength evaluation (using Fries *et al.,* 1991), no inspection data (e.g. amount of brood, queen presence or absence, flight activity during the study, amount of bee bread and honey), no weather data, efficacy assessment not clearly defined, short natural mite fall monitoring period (e.g. day -5, day -3 and day 0). Also, the follow-up treatment was not in line with the Varroa guideline.
- The applicant confirmed that safety was not an evaluated parameter. Safety assessment is based on statistical comparison (LSD Fisher test) of colony strength estimation values after the test and before the follow-up treatment (day not clearly defined) which is not in accordance with the recommendations of the CVMP Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA/CVMP/EWP/459883/2008, "Varroa Guideline"). Furthermore no brood, queen or worker bee safety assessment is performed.
- Test group n = 8; control group n = 4.

Another EU field study was conducted in Italy during the summer testing HopGuard Gold at overdoses. One group received the proposed posology of 2 x 2 strips, but at shorter dosing intervals than the proposed 28 days, i.e. at 14 day intervals. A group was left untreated (control). The follow-up treatment proposed in the protocol (two applications of thymol at one week interval followed by Tau-fluvalinate during three weeks) does not match with the field practice (two applications of so-called “tampone” followed by two applications of oxalic acid).

Results showed efficacy levels of 74.14% in the group treated with the 2 x 2 posology (14 days intervals), compared to 62.56% in the overdose group (3 x 2 strips at 7 day intervals). Mite mortality in the untreated control group was 5.51%. Tested posology is different from the claimed one.

Efficacy levels of 74.14% when overdosing cannot be considered for an efficacy assessment. Taking into account all the shortcomings mentioned above (e.g. study data not in line with the principles

outlined in VICH GL9 and in the CVMP Varroa guideline), no conclusions on safety assessment can be drawn either. Furthermore, statistical comparison on bee mortality counts using the data provided by the applicant shows significant differences between groups (ANOVA; p-value = 0.00004). Overall, the studies indicated that in the presence of brood, the best efficacy results (≥90%) were achieved when HopGuard Gold was administered twice per hive (2 strips per administration), at intervals of 28 days between treatments (2 x 2 strips). However, the proposed recommended treatment interval for HopGuard Gold in the presence of brood is 21-28 days and no further justification has been provided regarding the recommended posology and dosing interval of the product. Also, the follow-up treatment was not in line with the Varroa guideline.

Furthermore, brood safety, queen presence and worker bee mortality and behaviour at this dose have not been clearly supported with adequate raw data. Conclusions on the safety of the product at the recommended posology cannot be drawn.

Studies in the absence of brood:

In the absence of brood (winter treatment) three field studies were provided, conducted in different European countries.

The first study, conducted in Italy**,** used the proposed single application of 1 strip of HopGuard Gold for a 14 day period, after which time the strips were removed from the hive. The study was conducted without a control group. Therefore, no conclusions can be drawn from the efficacy results obtained (96%).

A second field study was conducted in Greece**,** using three different posologies: One group (7 colonies) received a single strip for a 14 day period, a second group (6 colonies) received two strips for a 14 day period, and a third (control) group (6 colonies) was treated with a placebo. Efficacy results showed a mite mortality rate of 87.4% in group 1, 95% in group 2, and 9.2% in the untreated control group. These efficacy levels cannot be considered for an efficacy assessment taking into account all the shortcomings mentioned above (e.g. study data not in line with the principles outlined in VICH GL9 and in the CVMP Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA/CVMP/EWP/459883/2008). In brief:

- No original field data capture forms, no randomisation or blinding methods, no monitoring of adverse events, no inspection data (e.g. queen presence or absence, flight activity during the study, amount of bee bread and honey), efficacy assessment days not clearly defined, short natural mite fall monitoring period (e.g. day -4 and day -1), the follow-up treatment proposed in the protocol (two applications of oxalic acid) does not match with the field practice (one application of oxalic acid).
- No data on colony strength. The applicant assumes “a theoretical number between 8000 and 11000 bees”.
- No safety assessment. The applicant confirmed that safety was not an evaluated parameter whilst monitoring the mite fall for the efficacy evaluation.
- Test group n = 6; control group n = 6.

A third field study, conducted in Italy (Winter), used a different posology than the proposed one in the SPC. Treatment (2 strips) was repeated once after 14 days (with a single strip) for another 14 days (group 1), or twice, at dosing intervals of 7 days (group 2). A third (control) group was treated with placebo (untreated cardboard strip, left in place for 28 days). For follow-up treatment, amitraz and as a second control application oxalic acid were used.

During the course of the trial, the protocol for the 14-day group changed due to increased bee mortality, so that the second application consisted of one strip only for 14 days. Efficacy results showed a mite mortality rate of 95% in group 1, 96% in group 2, and 9.9% in the untreated control group.

However, as mentioned above, the study was not presented in line with the principles outlined in VICH GL9 and in the CVMP Varroa GL, and pivotal data were missing. Although no detailed safety assessment was performed, it was concluded by the study authors that "*the amount of product overdosed some colonies and had an effect on the bee population. … The trial should be repeated using a treatment regime more modified for wintering colonies.*"

Overall, relevant information on the study design and results of the studies is still missing and/or has not been adequately supported by data in line with the principles outlined in VICH GL 9 or the requirements outlined in the CVMP Varroa guideline, as requested by the CVMP. There is no homogenous design of the studies: each of them have a different protocol and were tested using different posologies, different data are collected in each case (e.g. strength, dead bees, weather data), and different procedures (e.g. colony strength evaluation method) are performed. Taking into account all these issues, none of the studies can be considered pivotal. Furthermore, no conclusions can be drawn as to the safety of the product in any of the field studies assessed.

In the case of the two studies performed in Greece, which used the proposed posology in the SPC, and showed adequate efficacy results, various outstanding issues were not adequately addressed in regard to the efficacy assessment, and no conclusions on the target animal safety assessment could be drawn.

Conclusions:

In the absence of pivotal data, no conclusions can be drawn on the efficacy and tolerance of HopGuard Gold in the treatment of varroosis in presence or absence of brood.

The applicant's dose proposal as stated in the SPC (e.g. "1 strip per 5 frames covered with bees in each brood chamber") is not supported appropriately by field data. Conclusions on the efficacy and safety of the field studies data cannot be drawn due to a number of outstanding issues. Different dosing regimens (e.g. number of strips per administration, duration of treatment i.e. presence of the strips in the hive, and frequency of administration(s)) were used in field studies to clarify the recommended posology of the product, which is a major concern. The applicant did not clarify and justify the dose of the product depending on the colony size (small, medium or large colonies), type of beehive and mite infestation levels in the field studies. It is important to note that the field studies conducted in third countries could only be considered as supportive information as regional climates and beekeeping practices including hive types and bee breeds could differ from those of the EU whereas studies performed in Europe cannot be considered pivotal studies to confirm the efficacy and safety of the product in the light of the outstanding issues still identified.

According to the applicant, it appeared that HopGuard Gold was well-tolerated in all the field studies. However, relevant information in line with the principles outlined in VICH GL 9, as requested by the CVMP is still missing to support this claim (e.g. original field data capture forms to support the long-term evaluation of bee mortality, colony behaviour, colony reproduction and honey production). Safety of the product in the target species is still a major concern.

Conclusions cannot be drawn on the efficacy and safety of the product.

Overall conclusion on efficacy

Pharmacodynamics:

The mode of action of hop beta acids against *Varroa* mites has not been fully characterized, but direct contact between hop beta acids and the mite is required. At the present, little is known about the mechanisms of action of hop beta acids but the available data suggest hop beta acids might have oviposition-deterrence and repellence to mites as well as lethal effect on mites by physical contact. In addition a possible anti-feedant effect in mites has also been described.

Resistance:

From the provided data at present, little is known about the mechanisms of action of hop beta acids. However, so far, resistance against hop beta acids has not been reported in the literature.

Pharmacokinetics:

No pharmacokinetic studies in honeybees have been performed, which is acceptable.

Dose determination:

Dose justification was based on bibliographic references, laboratory and field studies. However, although a minimum effective dose of 25 µg/bee was proposed by the applicant and doses of up to 150 µg/bee were well tolerated by bees under laboratory studies, the recommended dose in terms of number of strips and dosing interval of the product has not been adequately justified. The outstanding issues identified by the CVMP have not been adequately addressed. Therefore, the justification of the proposed dose is still dependant on the field studies.

Tolerance:

In the TAS study (small scale study), HopGuard Gold was administered using two different posologies (e.g. 1x RTD and 2x RTD). No conclusions can be drawn from this study as relevant information in line with the principles outlined in VICH GL 9, as requested by the CVMP, is still missing to support the applicant's statements.

Administration of the product at the recommended dose (1x RTD, but at a shorter treatment interval than proposed in the SPC) seemed well tolerated by queen bees and drones as stated by the applicant, although higher mortality of worker bees was observed during application when compared to control colonies.

The applicant also stated that the administration of two applications of the double dosage (2x RTD with a 14 day interval) produced a higher number of dead honeys bees, and two dead queens out of six, within the first 24 hours post application; also, mortality in drones was significantly higher in treated colonies than in control hives. Data on the influence of treatment on brood is inconclusive.

Conclusions on target animal safety cannot be drawn as relevant information in line with the principles outlined in VICH GL 9 and the CVMP Varroa guideline is still missing to support the applicant's statements. Outstanding issues are still pending to be solved.

Efficacy:

The results from the 2 EU field studies showed that in the presence of brood (summer, autumn treatment) best efficacy results (≥90%) were obtained when HopGuard Gold was administered twice (2 strips per administration), with a treatment interval of 28 days (2+2). However, relevant information in line with the principles outlined in VICH GL 9 and the CVMP Varroa guideline has not been provided, and the efficacy of the product in colonies with large amount of brood is questioned.

The results from 3 EU field studies in winter conditions (broodless period) were provided, but relevant information in line with the principles outlined in VICH GL 9 and the CVMP Varroa guideline is still missing to support the applicant's efficacy claim.

Furthermore, no safety conclusions can be drawn from any of the field studies (neither in the presence of brood nor in the broodless period).

Different dosing regimens (e.g. number of strips per administration, duration of treatment i.e. presence of the strips in the hive, and frequency of administration(s)) were used in field studies differing from the recommended posology of the product, which is a major concern when assessing the efficacy and safety of the product. In addition, based on the data presented by the applicant, the recommended dose, treatment frequency, efficacy and safety are not adequately justified. Therefore, the claimed posology is currently not supported.

In the absence of pivotal data, no conclusions can be drawn on the efficacy of HopGuard Gold in the treatment of varroosis in presence or absence of brood.

Part 5 – Benefit-risk assessment

Introduction

HopGuard Gold bee-hive strips contain a purified hop extract (purified semi-solid extract from *Humulus lupulus L*. containing approximately 48% of hop beta acids (as the potassium salts)) as the active substance, and are presented in bags of two packs sizes, containing either 12 or 24 bee-hive strips. The proposed withdrawal period for honey is zero days.

The applicant applied for the following indication: Treatment of varroosis due to *Varroa destructor*.

The proposed dose is the in-hive use of up to two bee-hive strips per hive in the brood chamber (1 strip per 5 frames), repeated after 21–28 days (in the presence of brood); the maximum number of applications per hive should not be more than 12 strips per year. During the broodless period a single application is proposed of 1–2 strips, for a duration of 14 days.

The eligibility to the centralised procedure was agreed upon by the CVMP on 8 November 2012 as hop beta acids are considered a new active substance, which was not authorised as a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004.

The product has been classified as MUMS/limited market, and, therefore, reduced data requirements apply, and these have been considered in the assessment.

The application was submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

Benefit assessment

Direct therapeutic benefit

The benefit of HopGuard Gold would have been its efficacy in the treatment of varroosis due to *Varroa destructor* in honey bee colonies with or without brood.

However, the dose of HopGuard Gold strips has not yet been adequately justified based on the data presented, which is a major concern. The efficacy of the product in the treatment of varroosis due to

Varroa destructor was tested in caged bees and in field studies in different seasons (summer, autumn and winter, i.e. in the presence and absence of brood). Although some of these studies indicate satisfactory efficacy results (≥90%) in the presence of brood and in broodless periods, pivotal information in line with the principles outlined in VICH GL 9 on Good Clinical Practice and the CVMP Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA/CVMP/EWP/459883/2008) is still missing. Therefore, it is considered that the proposed posology and dosing interval have not been adequately justified, which remains a major concern.

The CVMP, therefore, considers that, at present, the data provided are inadequate to support an acceptable level of efficacy for the proposed indication at the proposed dose, and that the benefit of the product has therefore not been demonstrated.

Additional benefits

The product increases the range of available treatment possibilities for varroosis in bees and would provide a new treatment possibility for a minor species.

Risk assessment

Quality:

The finished product is a veterinary medicinal product of herbal origin. Although the formulation, manufacture and proposed specifications are reasonably well described at the time of withdrawal of the application it was not proven that a product of consistent quality would always be produced as there were outstanding issues which would need to be satisfactorily resolved prior to a marketing authorisation being granted. The assessment of the quality of the product therefore remains incomplete whilst there are outstanding issues remaining.

Safety:

Risks for the target animal:

Although the applicant stated that the administration of the product at the recommended dose could be expected to be safe for the colonies, higher mortality in adult bees was noted in experimental colonies compared to untreated control, within the first 24–48 hours post application, mainly affecting those bees that had been in direct contact with the liquid dripping from the bee-hive strips at the time of application. After a period of 48 hours, mortality levels of worker bees (measured as dead bees in front of experimental colonies), returned to the same level as the control colonies. Field studies did not confirm the target animal safety of the application. Pivotal information in line with the principles outlined in VICH GL 9 and the CVMP Varroa guideline are still missing and further clarification is needed on a number of issues raised in regard to the target animal safety of the application; therefore, no final conclusions can be drawn on target animal safety.

Risk for the user:

Appropriate warnings for the user have been included in the product literature and some minor amendments have been proposed. Given the low toxicity profile of the active substance and the unlikeliness of dermal contact if the amended user safety warnings are followed, it is considered that the possible risk to the user can be sufficiently mitigated by the warnings in the product literature.

Risk for the environment:

HopGuard Gold is not expected to pose a risk for the environment when used according to the SPC

recommendations. Standard advice on waste disposal is included in the SPC.

Risk for the consumer:

Hop beta acids have been evaluated previously in respect to the safety of residues and are included in table I of the Annex to Regulation (EU) No 37/2010 with a "no MRL required" classification for honey. HopGuard Gold is not expected to pose a risk to the consumer of honey derived from treated hives when it is used according to the SPC recommendations. The residue studies available did not show residues of concern in the honey following treatment, and the withdrawal period is set at 0 days.

Special risks:

The use of HopGuard Gold is currently not expected to pose a risk in regard to resistance development.

Risk management or mitigation measures

Risk management or mitigation measures including warnings in the SPC and product information would need to be considered pending additional information from the applicant.

Conditions or restrictions regarding supply and use:

The applicant applied for exemption from the requirement for the veterinary medicinal product to be dispensed only against veterinary prescription by reference to Article 2 of Commission Directive 2006/130/EC. The criteria prescribed in Article 2 of Commission Directive 2006/130/EC are:

- The administration of the veterinary medicinal product is restricted to a formulation requiring no particular knowledge or skill in using the product (apart from the specific knowledge present and reasonably expected by a bee keeper).
- The veterinary medicinal product is not expected to present an apparent direct or indirect risk, even if administered incorrectly, to the animal or animals treated, to the person administering the product or to the environment.
- The summary of product characteristics does not contain any warnings of potential serious side effects deriving from its correct use.
- Neither the veterinary medicinal product nor any other product containing the same active substance has previously been the subject of frequent serious adverse reactions reporting.
- The summary of product characteristics does not refer to contraindications related to other veterinary medicinal products commonly used without prescription; the veterinary medicinal product is not expected to be subjected to special storage conditions.
- Consumer safety: Potential residues in honey obtained from treated honey bees are not expected to constitute a risk to the consumer even where the veterinary medicinal product is used incorrectly.
- There is no clear evidence to suggest that incorrect use would lead to an increased risk to human or animal health due to the development of antimicrobial resistance.

The CVMP considered that the assessment of the application would need to confirm that the product complies with all the above criteria. However, in view of remaining unresolved issues, no decision on this request was made.

Evaluation of the benefit-risk balance

In the presence of outstanding major and other concerns, no conclusions can be reached on the

benefit-risk balance.

The applicant applied for the following indication: Treatment of varroosis due to *Varroa destructor*. In the presence of outstanding major and other concerns, no conclusions can currently be reached on the benefit-risk balance of the application.

The product presents an acceptable risk for users, the environment and consumers, when used as recommended. However, the proposed dose and efficacy at the proposed indication, as well as target animal safety remain undemonstrated. The therapeutic benefit therefore remains inconclusive as the efficacy has not been substantiated. In addition, the assessment of the quality of the product remains incomplete as the resolution of some issues is still outstanding.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) considers that the application for HopGuard Gold is not approvable at the present time since "major objections" (and other concerns) were identified which preclude a recommendation for marketing authorisation. No conclusions can currently be reached on the benefit-risk balance of the application.