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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for VarroMed (EMEA/V/C/002723/0000)

Common name: oxalic acid (as dihydrate)/ formic acid

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

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Introduction

On 16 January 2015, the applicant BeeVital GmbH submitted an application for a marketing authorisation to the European Medicines Agency (The Agency) for VarroMed dispersion for honey bees, through the centralised procedure under Article 3(2)a of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 8 March 2012, as VarroMed contains a new active substance which is not yet authorised as a veterinary medicinal product in the Union. The new active substance consists of a fixed combination of formic acid and oxalic acid dihydrate.

CVMP appointed Stane Srčič as rapporteur and Cornelia Ibrahim as co-rapporteur for the assessment of the application.

The dossier has been submitted in line with the requirements for submissions under Article 13(b) of Directive 2001/82/EC (fixed combination application). The applicant is registered as an SME pursuant to the definition set out in Commission Recommendation 2003/361/EC.

VarroMed is a bee-hive dispersion for honey bees containing formic acid and oxalic acid dihydrate and is available in two pack sizes, a multi-dose bottle (555 ml), and single-dose sachets (15 ml, presented in a multipack of 12 sachets). The withdrawal period for honey is zero days. The recommended indication is: "Treatment of Varroa-mite infestation in bee colonies with and without brood".

On 6 October 2016, the CVMP adopted an opinion and CVMP assessment report.

On 2 February 2017 the European Commission adopted a Commission Decision granting the marketing authorisation for VarroMed.

Scientific advice

The applicant received scientific advice from the CVMP in September 2012 and September 2013. The scientific advice concerned quality aspects, safety aspects (target animal, residues, user, and environment), pre-clinical and clinical studies, and the justification for the fixed combination product. Most aspects of the scientific advice concerned deviations from standard data requirements, in view of the MUMS/limited market status of the product. The CVMP considered that the applicant, in general, followed the advice of the CVMP.

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 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 and this is addressed in section Part 5 – *Conditions or restrictions regarding supply and use* as part of the benefit-risk assessment for VarroMed.

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the system of pharmacovigilance (dated 28 January 2015), 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 reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the dosage form takes place in the EEA. Batch relelase for the EU takes place at Lichtenheldt GmbH, Wahlstedt, Germany. The site has a manufacturing authorisation issued on 6 November 2013 by Landesamt für soziale Dienste, Schleswig Holstein, Germany. An additional site for batch release is at Labor L + S AG, Bad Bocklet-Grossenbrach, Germany, for which GMP compliance was confirmed by the national authority, ZAB-Zentrale Arzneimittelueberwachung, Bayern, Germany.

Manufacture of both active substances, formic acid and oxalic acid dihydrate, takes place in the EU. A GMP declaration for the active substances' manufacturing site was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on a valid GMP certificate available for the active substance site.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and the GMP certification of the manufacturing sites are considered to be in line with legal requirements. The prescription status is considered in the benefit-risk evaluation.

Part 2 - Quality

Composition

VarroMed is presented as dispersion for in-hive use. It is a fixed combination product containing two active substances, formic acid (5 mg/ml) and oxalic acid dihydrate (44 mg/ml).

Other ingredients are: sucrose syrup, citric acid monohydrate, tincture of propolis (20% ethanolic tincture), star anise oil and lemon oil, caramel colour (E105d, colourant), and purified water.

No preservative is included as the dispersion has been demonstrated to be self-preserving, according to the Ph. Eur. General text 5.1.3., due to the two acidic active substances and the resultant low pH of the product (less than 1).

Containers

The product will be presented in two different types of immediate packaging.

The first is a 600 ml multi-dose colourless high density polyethylene (HDPE) plastic bottle containing 555 ml of the product, with a graduated scale on its side, an integral low density polyethylene (LDPE) dropper end and an HDPE tamper-evident screw cap closure. The bottle is supplied in a cardboard box (secondary packaging). The graduations on the bottle are 15 ml (one graduation scale), and their

accuracy and precision have been demonstrated in accordance with the Ph. Eur. monograph 2.2.5. The HDPE and LDPE used for the bottles both comply with the relevant Ph. Eur. (3.1.3.) and EC requirements (Regulation 1935/2004/EC). Appropriate specifications and certificates of analysis have been provided. The choice of the container-closure system has been justified by stability data and is considered suitable for the intended use of the product.

The second type of immediate packaging is single-dose opaque polyethylene terephthalate/aluminium/LDPE (PET/Alu/LDPE) laminated sachets, each containing 15 ml of the product. These are to be supplied in a multipack (cardboard box) of 12 sachets. (Each single-dose sachet contains 75 mg formic acid and 660 mg oxalic acid dihydrate). The sachet material complies with the relevant Ph. Eur. and EU requirements (including Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with foods). Appropriate specifications and certificates of analysis have been provided. The choice of these single-dose sachets has been justified by stability data and is considered suitable for the intended use of the product.

The sachets are opened by means of a perforation. The perforation is inserted during the filling and manufacturing process.

Development pharmaceutics

The product is a bee-hive dispersion, as although the active substances, formic acid and oxalic acid dihydrate are completely dissolved, some excipients are not completely dissolved. The SPC and product information for both the presentations include the instructions to "shake well before use".

The formulation is based on a previously used animal welfare product with a similar formulation.

For the pre-clinical studies and tolerance studies a formulation was used which was very similar to the final formulation, with only minor differences in the quantities of the excipients.

All other studies have been performed with the final formulation. Three industrial scale batches of the product were produced and these were used for stability studies (in both bottles and sachets), for the validation of the analytical methods, and for most of the clinical studies.

Single dose sachets of 15 ml were developed because this is the dose for an average hive (approximately 10000 bees), and these sachets facilitate trickling the dispersion onto the bees in occupied combs. A multi-dose bottle was considered suitable for use in many hives, and also for larger hives (for which a dose of up to 45 ml is recommended). The graduated scale on the bottle facilitates accurate dosing in accordance with the instructions for use in the SPC and other product information.

A study was performed to evaluate the uniformity of volume of delivered doses from multidose container. The results demonstrate the precision of the delivered volume (a single dose volume of 15 ml from a full or half-full bottle, and 45 ml as the maximum dose).

Method of manufacture

The manufacturing process consists of dissolution and/or dispersion of the excipients and the active substances formic acid and oxalic acid dihydrate and then making the dispersion up to volume with purified water. Filling into the primary containers (bottles or sachets) then follows. Some in-process controls are performed. The manufacturing method has been described in sufficient detail and is sufficiently controlled.

Production scale process validation was performed, but will be repeated with commercial scale batches according to the revised validation protocol. This will include the validation of the filling/packaging, which was so far only performed with development batches.

Control of starting materials

Active substances

The product contains two non-pharmacopoeial active substances, formic acid and oxalic acid dihydrate, both of which are produced by the same active substance manufacturer.

Formic acid (FOA)

Formic acid is a colourless liquid with a highly pungent, penetrating odour at room temperature. It is miscible with water.

Formic acid is not described in the Ph. Eur. The information on its manufacture and control is provided according to the active substance master file (ASMF) procedure.

The characterisation of the active substance and its impurities are in accordance with the Guideline on the chemistry of new active substances (CPMP/QWP/130/96-Rev.1). Potential and actual impurities were well discussed with regards to their origin and have been characterised. Impurity limits relevant for veterinary use (e.g. 0.20% for each individual related substance) are applied.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented and are adequately described and validated.

Detailed information on the manufacture of the active substance has been provided in the restricted part of the ASMF and this was considered satisfactory.

No organic solvents and/or catalysts are used during manufacture and therefore no such control is required.

The active substance specification has been set taking into account the Ph. Eur. General monograph Substances for pharmaceutical use (2034) and includes tests for: appearance, identity (colour, HPLC), density and colour/clarity of solution, assay (titration), impurities (HPLC), water content, heavy metals, and residue on ignition (Ph. Eur.). Compendial test methods are utilised. In addition, microbiological controls (total aerobic microbial count (TAMC), total combined yeasts/mould count (TYMC)) are conducted in compliance with Ph. Eur. 5.1.4.

The analytical methods used have been sufficiently adequately described and appropriately validated in accordance with VICH guidelines.

Batch analysis data for three production batches of formic acid are provided. The results are within the proposed specifications and consistent from batch to batch.

Another three batches were analysed and all the results complied with the proposed specifications.

The reference standard used for analytical control complies with the USP monograph.

Formic acid is stored in 20 l HDPE containers. The HDPE conforms with Ph. Eur. monographs 3.1.3. and 3.1.5.

The stability study supports a re-test period of 27 months. This re-test period is applicable when the formic acid is stored in the original packaging at temperatures not higher than 30 °C.

Oxalic acid dihydrate

Oxalic acid dihydrate is available as white crystals and it is soluble in water at 14.3 g/100 ml (25 °C). The structure has been confirmed by ^{1}H NMR, ^{13}C NMR in D_2O and DMSO-*d*6. Elemental analysis was used for its additional confirmation.

Oxalic acid dihydrate is not described in the Ph. Eur. The information on its manufacture and control is provided according to the Active Substance Master File (ASMF) procedure.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on the chemistry of new active substances (CPMP/QWP/130/96-Rev.1). Potential and actual impurities were well discussed with regard to their origin and characterised. Impurity limits relevant for veterinary use (e.g. less than 0.20% for each individual related substance) are applied.

The manufacture of oxalic acid dihydrate is relatively simple. The last step is recrystallisation, prior to drying and packaging. The specifications and control methods for intermediate products, starting materials and reagents have been presented and are adequately described and validated.

Detailed information on the manufacture of the active substance has been provided in the restricted part of the ASMF and these data were considered satisfactory.

No organic solvents and/or catalysts are used during manufacture and therefore no such control is required.

The active substance specification has been set taking into account the Ph. Eur. General monograph substances for pharmaceutical use (2034) and includes tests for: appearance, identity (IR spectrum), assay (titration), impurities (HPLC), water content, heavy metals, and residue on ignition (Ph. Eur.). Compendial test methods are utilised. In addition, microbiological controls (TAMC, TYMC) are conducted in compliance with Ph. Eur. 5.1.4.

The relevant analytical methods used have been adequately described and appropriately validated in accordance with VICH guidelines.

Batch analysis data for three production batches of the oxalic acid dihydrate are provided. The results are within the proposed specifications and consistent from batch to batch.

Excipients

The following excipients are purchased and controlled in compliance with their respective Ph. Eur. monographs: citric acid monohydrate, star anise oil, lemon oil, purified water. The sucrose syrup complies with the monograph in the German DAB. No additional tests are considered necessary.

Tincture of propolis (20% ethanolic tincture) is a non-compendial material. An in-house specification has been provided which includes identity (TLC), purity (Ph. Eur.), assay (Ph. Eur.) and microbiological quality (Ph. Eur.).

The caramel colour (E105d) is a food additive and an in-house specification in line with the requirements of the current Ph. Eur. General monograph substances for pharmaceutical use (2034) has been provided. It includes purity and microbiological quality.

The tests and acceptance criteria in the specifications are considered appropriate to ensure the quality of all the excipients.

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

None of the substances used in the product are of animal origin with the exception of the propolis, which is a bee-derived product. None of the starting materials used for the active ingredients (oxalic acid dihydrate and formic acid) or the finished product are risk materials as defined in the current version of the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01-Rev.3). The product is therefore out of the scope of the relevant Ph. Eur. Monograph and the Note for guidance. TSE-statements for all the raw materials and the final product have been provided by the suppliers and by the manufacturer of the finished product.

Control tests on the finished product

The finished product specification includes appropriate tests for this type of dosage form, that is, appearance, identity, assay, degradation products, and microbial quality. Related substances are only specified for formic acid as there is no related substance of the oxalic acid dihydrate exceeding the identification threshold (0.20%). The proposed test parameters are acceptable.

Descriptions of all the methods used for the control of the finished product and the associated limits are provided. The analytical methods have all been adequately described and suitably validated.

The shelf life specification differs from that used at time of release in that the limits for a specified impurity are wider in the shelf life specification. The limits have been justified.

Batch analysis results for three production scale batches produced at the proposed site of manufacture, both in bottles and sachets, have been provided. All the batches complied with the proposed specifications.

For the specification of the finished product filled into sachets (single-dose containers), the filling volume is specified with both upper and lower limits. The finished product specifications (both release and shelf life) are expressed as extractable volume according to Ph. Eur. 2.9.17. Therefore, the filling volume is specified with an upper and lower limit of 15.0 to 16.6 ml, and this corresponds to 95 to 105% of target fill volume.

The Ph. Eur. 2.9.40. test for the uniformity of dosage units states that ".....the uniformity of dosage units specification is not intended to apply to solutions, suspensions, emulsions or gels in single-dose containers intended for cutaneous administration." and therefore the absence of inclusion of a test and limits for uniformity of dosage units in the finished product specification is justified. In the case of VarroMed the single-dose sachet presentation is administered to a hive of more than 5000 bees, so a very small quantity (µg) should be received by a single bee. Furthermore, the process is sufficiently validated and is controlled by testing of the weight of 100% of the sachets in process, and additionally by the finished product release control (assay, extractable volume, etc).

For the multi-dose bottles (600 ml container size) which are filled with a volume of 555 ml of the dispersion, both the fill volume and uniformity of mass (Ph. Eur. 2.9.27) are controlled.

The CVMP recommends that the bulk process validation is repeated according to the revised protocol with 3 commercial scale batches.

Stability

Formic acid

Stability studies have been performed on samples from three batches stored in HDPE and glass containers. An interaction between formic acid and the container led to out of specification results when stored at 40 °C/75% RH. A new accelerated study was performed under intermediate conditions of 30 °C/65% RH, and according to the guideline on Stability testing of existing active substances and related finished products (EMEA/CVMP/QWP/846/99-Rev.1). After 12 months of this study all parameters were within specification. Data are provided for up to 24 months storage at 25° C/40% RH. According to the guideline on Stability testing of existing active substances and related finished products (EMEA/CVMP/846/99-Rev.1), a re-test period of 27 months is applicable. This re-test period is applicable when formic acid is stored in the original HDPE packaging and at temperatures not higher than 30 °C.

Oxalic acid dihydrate

Stability studies performed were in accordance with the guideline on Stability testing of existing active substances and related finished products (EMEA/CVMP/QWP/846/99-Rev.1). The quality of oxalic acid dihydrate was determined according to the Deutscher Arzneimittel Codex (DAC, German Pharmaceutical Codex) and Ph. Eur. general monographs. Stability data from samples from 3 production batches, packaged in double LDPE bags inside a box (simulating the commercial packaging) and stored at 25 °C/60% RH, confirm the proposed retest period of 24 months when stored at 25 °C.

Finished product

The stability studies were performed in accordance with the guideline on Stability testing of existing active substances and related finished products (EMEA/CVMP/QWP/846/99-Rev.1). The stored samples were tested in accordance with the proposed shelf life specification. The analytical procedures used are stability indicating.

Full testing has only been performed on three production scale batches stored in both types of primary packaging proposed for marketing, that is, single dose sachets and multi-dose bottles.

The batches were stored under long term (25 °C/60% RH and 5 °C) and intermediate conditions (30 °C/65% RH). No batches were stored under accelerated conditions (40 °C/75% RH). The multi-dose bottles were stored upright, but some individual bottles were also stored upside down to investigate prolonged contact of the product with the dropper end of the bottle.

The stability data demonstrate that the product is stable, in both the single dose sachets and the multi-dose bottles, for 24 months under the long term storage conditions of both 25 °C/60% RH and 5 °C. At the intermediate storage condition of 30 °C/65% RH, the product failed to meet the shelf life specification only for the parameter "resuspendability" before the 12 months time point, therefore the product should be stored below 25 °C.

No photostability studies were provided and, therefore, the product should be stored protected from light. A warning is included in the SPC to this effect.

Based on the available stability data, a shelf life of 24 months and storage conditions of "Do not store above 25 °C.", for the bottle only "Keep the bottle tightly closed." and "Keep the bottle/sachets in the outer carton in order to protect from light." as stated in the SPC and other product information are acceptable.

The long term stability study is still in progress and will be continued up to the planned 36 months.

An in-use stability test according to the guideline In-use stability testing of veterinary medicinal products (EMEA/CVMP/424/01) was performed on samples of 3 batches (the same as for shelf life, at the beginning, in the middle and at the end of shelf life) of the product stored in the multi-dose bottles. The results confirmed an in-use shelf life of 30 days is justified and this is included in section 6.3 of the SPC.

Overall conclusions on quality

VarroMed has been classified as a MUMS/limited market product, the requirements for which are specified in the CVMP guideline on quality data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/QWP/128710/2004).

VarroMed is a ready-to-use non-sterile viscous aqueous dispersion for in-hive use. The product is a fixed combination product containing two active substances: formic acid and oxalic acid dihydrate. Both active substances are non-pharmacopoeial and the data for them is provided in ASMFs.

There are two different primary packages: multi-dose HDPE bottles (555 ml), and single dose sachets (15 ml). The secondary packaging used for both is outer cardboard boxes.

The formulation development is described in the dossier. The manufacturing process is a simple standard one using conventional liquid manufacturing techniques. The manufacturing process and its control are described in the dossier.

The specifications proposed for use at release and at the end of shelf life are appropriate to control the quality of the finished product. Analytical methods are appropriately validated.

Dosage form stability studies demonstrate the product to be stable with no adverse trends in any of the parameters investigated, except a failure in resuspendability when the product is stored under intermediate storage temperature conditions. There is sufficient stability data available to support the proposed shelf life of 2 years when stored below 25 °C. In the absence of any photostability studies the product should be protected from light. The proposed 30 day in-use shelf life for the multi-dose bottle is also supported by the data provided. The SPC storage precautions reflect the conclusions of the stability data.

As the manufacturing procedure is a simple standard process and validation data on three industrial scale batches were provided, it is accepted that full scale validation will be performed post-authorisation in accordance with the MUMS Quality guideline. A protocol for the process validation study has been provided and is recommended to submit the data post-authorisation.

The documentation in Part 2 is of sufficient quality with respect to compliance with the relevant VICH and CVMP guidelines.

Based on the review of the data on quality, it can be concluded that the manufacture and control of VarroMed is considered acceptable.

The CVMP recommends that the bulk process validation is repeated according to the revised protocol with 3 commercial scale batches.

Part 3 - Safety

VarroMed contains two active substances, oxalic acid (as the dihydrate, OAD) and formic acid (FOA), both of which are organic acids. Both acids are included in Regulation 37/2010; oxalic acid has a "No MRL required" classification in honey bees, and formic acid has a "No MRL required" classification in all food producing species. A published summary report relating to the safety evaluation performed for the MRL

assessment is available for oxalic acid (EMEA/MRL/891/03, 2003), and cross-reference to the report is made.

Pharmacodynamics

See Part 4.

Pharmacokinetics

Oxalic acid

In humans, oral absorption of oxalic acid is limited (3–20%), but can be increased up to 60% under certain (disease) circumstances. After intravenous administration of small doses of ^{14}C-labelled oxalic acid to humans, oxalic acid was mainly excreted as the parent compound via urine (more than 90%). Plasma elimination half-life was about 2 hours.

In bees, ^{14}C-labelled oxalic acid was absorbed, distributed and metabolised after oral and topical application. Twelve hours after topical application (by trickling), ^{14}C was detected in the haemolymph (peak concentration: 10 µg/g) and in all areas between the honey sac and rectum. In the haemolymph ^{14}C levels decreased to µg/g within 72 hours, and were no longer detectable in the intestine 22 and 31 days post application (EMEA/MRL/891/03). Following topical administration, the tissue distribution of the acid in the different bee organs suggests that some of the acid is ingested.

Sublimation (solid-vapour transformation) of oxalic acid crystals showed that approximately half of the oxalic acid decomposes into carbon dioxide and water, whilst the other half forms fine particles and dust that precipitate in the hive (EMEA/MRL/891/03).

Formic acid

In humans, FOA is readily absorbed through the gastro-intestinal mucosa, skin and lungs, and is largely metabolised. Half-life is between 15 min and 1 h, metabolism takes place in the liver and to a lesser extent in the intestinal mucosa, lungs, kidneys and spleen. FOA is either converted to CO_2 and exhaled or eliminated via urine as unchanged substance. Physiological levels in human urine range from 11.7-60 mg/l. Accumulation in blood can be observed.

The pharmacokinetics of FOA in honey bees have not been studied, and section 5.2 (pharmacokinetics) of the SPC therefore states:"The pharmacokinetics of formic acid in bees are not known."

Toxicology

Single dose toxicity

Oxalic acid

Acute toxicity studies indicate that oxalic acid is of moderate to high toxicity by the oral route in mammalian species. The LD_{50} for pure oxalic acid is predicted to be about 375 mg/kg body weight or about 25 g for a 65 kg human. The oral LD_{50} values determined for rats were 475 mg/kg bw for males and 50–375 mg/kg bw for females. For dogs and cats, the oral toxic doses were 1 g and 200 mg, respectively. After oral administration, the main target organ is the kidney with formation of crystals of calcium oxalate, associated with focal necrosis, mineralisation and impairment of kidney function. However, calcium depletion with sequelae of hypocalcaemia have also been reported. After intravenous

administration of about 40 mg/kg bw to dogs, all animals died shortly after administration. Oxalate binds to blood calcium and induces neurotoxicity and cardiac arrest. Oxalic acid is corrosive to skin and mucous membranes (EMEA/MRL/891/03). In honey bees, sublethal effects of OAD on "division of labour", "olfactory learning" and "longevity" of *A. mellifera* were investigated both in-hive and under laboratory conditions (Schneider et al., Apidologie (2012) 43). After single topical application of 3.5% OAD water solution, corresponding to a dose of 175 µg OAD/bee, a significant decrease in worker bees' activity, nursing behaviour and lifespan were recorded.

Similar effects, i.e. changed feeding behaviour, increased sensitivity to water, changes in flight behaviour and decreased longevity were observed in a laboratory setting after single topical application of 3.5% OAD in sugar water, or 3.5% OAD in combination with a sugar substitute, glycerol 45%. While the exact mode of action remains unknown, the data suggest that effects are not only due to an oral uptake of OAD by bees (due to increased self-grooming) but also to some extent due to dermal absorption. OAD is currently used as single winter treatment, only. The authors conclude that regardless of their findings, the high efficacy of OAD against the mite *V. destructor* still outweighs the possible negative consequences to the honeybee colony and it should remain as one of the main varroacides.

An LD_{50} for OAD in VarroMed (as fixed combination with FOA) of 220 µg OAD/bee from a controlled laboratory study in caged bees (study 2015-01-001) was derived; however, re-calculating the data provided, the CVMP considered this dose to be 195 µg OAD/bee.

Pathological findings after topical application of 10% OAD were observed in different internal organs of honey bees. After 24 h, there were severe alterations in the ventricular epithelial layer, while degeneration of the rectal epithelium was clearly seen by 48 h post application. Irreversible lesions appeared at 48 h in different bee organs with increased cellular damage after 72 h. This indicates that the effect of oxalic acid continues after initial contact causing permanent lesions in digestive and excretory organs. OAD concentrations of 20% in 50% sugar solution led to acute mortalities of more than 60%.

Formic acid

Acute toxicity of FOA is highest after inhalation (LD_{50} 7.4 mg/l/4h in the rat), whereas oral or parenteral toxicity are low and moderate, respectively. The lowest LD_{50} of 145 mg/kg was seen in mice after intravenous application. *In vitro* investigations with rat and mouse embryo cultures revealed increased mortality after exposure to concentrations of 11.8 mmol/l and more, due to reduction in pH.

FOA is reported to be moderately toxic to aquatic animals.

Repeat dose toxicity

Oxalic acid

Repeated dose toxicity of oxalic acid in laboratory rats (studies up to 70 days in duration) was evaluated as part of the MRL procedure. The main target organ of toxicity was the kidney, however due to deficiencies in the studies it was not possible to establish a NOEL.

Formic acid

According to "VICH GL18" on Residual solvents, FOA is a solvent with low toxic potential and has been categorized as Class 3, it may be regarded as less toxic and of low risk to target animal and human consumer health.

Published data indicate that after repeated administration of FOA doses of 8.2, 10.3, 90, 160, and 360 mg/kg bw/day in the drinking water for 27 weeks to groups of 6 rats, no toxic effects were observed up to doses of 160 mg/kg bw/day. Administration of 0.2% Ca-formate in the drinking water to 10 Wistar rats for 3 years (corresponding to 150-200 mg/kg bw/day) or of 1% Na-formate to Wistar rats for 1 year (corresponding to 730 mg/kg bw/day) did not lead to toxic effects.

Two and 13 weeks NTP studies in rats and mice exposed to vapours of FOA (up to 62 mg/m^3, 32 ppm) produced a NOAEL of 31 mg/m^3 (16 ppm) based on minor effects (increased liver and kidney weights and minimal degeneration of nasal olfactory epithelium).

Following a repeated dose study of two years in rats the NOAEL was reported as 1.2% formic acid in the diet. No teratogenic or carcinogenic effects have been found. However, FOA produced mutagenic results in a *Drosophila* genotoxicity test system.

Tolerance in the target species of animal

See part 4.

Reproductive toxicity, including developmental toxicity

Oxalic acid

Reproductive toxicity of oxalic acid was evaluated as part of the MRL procedure in 2003 (EMEA/MRL/891/03).

Based on a 2-generation reproductive toxicity test in mice, oxalic acid is considered to be a weak reproductive toxicant, with effects seen on number of litters, number of live pups, live pup weight, prostate gland weight, relative kidney weight and quality of sperm following dietary exposure at a level corresponding to approximately 275 mg/kg bw/day.

In a non-standard pilot developmental toxicity study in rats, high doses of oxalic acid (136 mg/kg bw) induced kidney toxicity in pups. In a second non-standard study in rats no effects were seen at doses of up to 205 mg/kg bw. No NOEL could be derived from these non-standard studies.

Formic acid

In a 13 week inhalation study investigations were carried out on sperm morphology and vaginal cytology with groups of 10 rats and mice exposed to FOA concentrations of 0, 8, 32 and 128 ml/m^3. In male animals no relevant effects on sperm motility, sperm concentration or on testis or epididymis weights were found. In female animals, the oestrus cycle was not affected.

In an *in vitro* study with rat embryo cultures severe embryotoxicity was observed at FOA concentrations of 18.66 mmol/l and more (pH 6.94). The NOEC is given as 3.74 mmol/l.

In vivo administration of FOA in the drinking water to Wistar rats over 5 generations at a concentration of 0.2%, and over 2 generations at concentrations of 0.4% (corresponding to 150-200 mg/kg bw/day) yielded no evidence of effects on fertility or the development of the embryos.

Genotoxicity

Oxalic acid

Oxalic acid produced negative results in Ames tests and in an *in vitro* chromosome aberration test in Chinese hamster lung cells. While a weakly positive result was seen in a chromosome aberration test with plant root meristem cells, the relevance of this for mammals is not demonstrated. No *in vivo* genotoxicity data are available. In view of the lack of positive effects in standard *in vitro* tests and the absence of relevant findings in the chronic toxicity study, oxalic acid is not considered to be genotoxic.

Formic acid

Valid *in vitro* genotoxicity studies including several mutagenicity tests in Salmonella, an SOS chromotest with *Escherichia coli* and a test for sister chromatid exchange with V79 cells yielded negative results. A chromosomal aberration test with Chinese hamster ovary (CHO) cells revealed clastogenic effects only when the pH value of the exposure medium was reduced (pH below 6). A weak positive result was observed in a reverse mutation test with *E. coli* carried out in 1951, but only with a high bacterial density with constant substance concentration. A test for X chromosomal recessive lethal mutations with *Drosophila melanogaster* carried out in 1964 yielded positive results after inhalation exposure and administration with the food.

Taking all the available studies into consideration, there is, however, no convincing evidence that FOA is mutagenic. This is consistent with the position expressed in NTP Technical Report 1992.

Carcinogenicity

Oxalic acid

A non-standard two-year carcinogenicity study in rats revealed no evidence of carcinogenicity for oxalic acid (EMEA/MRL/891/03).

Formic acid

Up to the highest tested concentration of 300 µg/ml, FOA had no effects on metabolic cooperation in V79 cells. Thus, this study yielded no evidence of a tumour-promoting effect of FOA.

Painting the mouse ear with 8% FOA twice a week for 50 days revealed no evidence of histological or histomorphometric changes, unlike with the positive controls croton oil and Tween 60. In chronic toxicity studies which, however, do not meet present-day standards, the incidence of tumours was not increased in rats.

Studies of other effects

Observations in humans

Oxalic acid

Oxalic acid is an endogenous substance in plants and mammals, occurring naturally in honey, and is present in the human diet. Dietary intake is on average 50 mg/day and the natural oxalic acid levels in human tissues are in the range of 0.6–4 mg/kg. In humans, high oral doses of oxalic acid have led to severe poisoning and death, with reported fatal doses ranging from 3–30 g/person. Kidney impairment

and failure is seen as a sequel to the precipitation of insoluble calcium oxalate crystals which block and destroy renal tubules.

Oxalic acid is irritating to skin, eye and the respiratory tract. Its dermal toxicity is low. No deaths were reported following the topical application of 20 g/kg bw oxalic acid to 3 rabbits (EMEA/MRL/891/03).

Formic acid

FOA is a natural intermediate and final product in microbes, plants and in animal metabolism, occurring naturally in honey. In humans, FOA is present in blood at concentrations of up to 4.8 mg/100 ml. VarroMed contains approximately 0.5% FOA. Pure FOA is a strong acid which, depending on the concentration, has irritative to caustic effects on mucous membranes, eyes and skin. FOA is volatile, and inhaling vapours might cause irritation of eyes and nose with sore throat, cough, chest tightness and headache.

Cases of humans accidentally exposed to both substances underline the locally irritating properties of oxalate and FOA on skin and eyes. Furthermore, irritating (2-10%) and corrosive effects (> 10%) on mucous membranes via inhalation and oral ingestion (e.g. ulceration, coughing, vomiting, hematemesis) and systemic effects are described (e.g. muscular irritability, tetany, convulsions; shock, oliguria, anuria, haematuria, albuminuria; cardiac irregularities and circulatory collapse). It should additionally be noted, that FOA is dermally well absorbed and has the potential to induce sensitization.

User safety

A user safety assessment according to current guidance (Guideline on user safety for pharmaceutical veterinary medicinal products, EMA/CVMP/543/03-Rev.1) has been performed. The beekeeper is identified as the person most likely to be exposed to the veterinary medicinal product. The risk of accidental ingestion is considered negligible for adults, when the product is administered according to the SPC.

Although FOA is volatile and has, therefore, a potential of inhalation toxicity, this route of exposure is considered negligible, since the acid is dissolved/diluted in the product. OAD is not volatile and there is no potential of inhalation toxicity.

Possible routes of exposure are dermal and/or ocular due to spillage, and oral and/or ocular through hand-to-mouth/hand-to-eye contact. The main risk of the product is based on the known potential of irritation and/or corrosion of both organic acids depending on their concentrations.

For FOA, the irritation/corrosive potential is considered not relevant due to its low concentration in the product (0.5%). However, the concentration of oxalic acid (4.4%) is considered to cause irritative effects during accidental exposure (e.g. conjunctivitis, corneal damage; gangrenous ulcerations of skin). Therefore, suitable warning phrases are included into the SPC.

Taking all into account, the following warning phrases have been included in the SPC and package leaflet accordingly:

- This veterinary medicinal product is irritating to the skin and eyes. Avoid contact with the skin, eyes and mucous membranes. In case of accidental spillage onto skin, wash the affected areas immediately with running water. In case of accidental spillage into the eye(s), flush the eye(s) immediately with clear running water for 10 minutes.
- Children should not come into contact with this veterinary medicinal product. Accidental ingestion may cause adverse reactions.

- Personal protective equipment consisting of protective clothing, acid-resistant gloves and glasses should be worn when handling the veterinary medicinal product. Change heavily contaminated clothes as soon as possible and wash before re-use.
- People with known sensitivity to formic acid or oxalic acid should administer the veterinary medicinal product with caution.
- Do not eat, drink or smoke while using the product.

Since the product can be stored at home, the risk of accidental oral exposure of children cannot be excluded; however, the concentrations of the acids in the product are such that the product is not considered to represent a greater risk to children than many normal household products routinely stored in the home. A warning to store the product away from children is included in the SPC and product literature.

Environmental risk assessment

An environmental risk assessment was submitted. According to the CVMP revised guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38 (EMEA/CVMP/ERA/418282/2005-Rev.1) the environmental risk assessment can stop in Phase I as both active substances are naturally occurring substances. No further assessment is deemed necessary.

FOA is reported to be moderately toxic to aquatic animals; however, at the presented concentration, it is not considered that special disposal warnings are necessary.

VarroMed is not expected to pose a risk to the environment when used according to the SPC.

Residue studies

MRLs

The active substances in VarroMed, oxalic acid dihydrate and formic acid, are allowed substances as described in table 1 of the annex to Commission Regulation (EU) No 37/2010:

Pharmacologically active substance	Marker residue	Animal species	MRL	Target tissues	Other provisions	Therapeutic classification
Oxalic acid	N/A	Bees	No MRL required	N/A	NO ENTRY	Anti-infectious agents
Formic acid	N/A	All food producing species	No MRL required	N/A	NO ENTRY	NO ENTRY

Excipients include star anise oil and lemon oil which were demonstrated to be equivalent to Anisi aetheroleum (see EMEA/MRL/413/98-final) and Citri aetheroleum (EMEA/MRL/407/98-final), which are included in table 1 of the Annex to Regulation (EU) No 37/2010 with "No MRL required" classification.

All other excipients listed in section 6.1 (tincture of propolis (20% ethanolic tincture), ethanol, citric acid monohydrate (E 330), caramel colour (E 150d), and purified water) of the SPC 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 or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

Pharmacokinetics

No pharmacokinetic studies have been performed with the proposed product. Oxalic acid is a natural constituent of honey and is found normally in the range of 1–800 mg/kg, depending on the botanical source of the honey. There are no data in the literature about the distribution of oxalic acid into the wax.

Depletion of residues

No residue depletion studies have been performed with the proposed product.

Withdrawal periods

Organic acids are known to cause residues in honey harvested after treatment. The proposed product is a new combination of two organic acids and, therefore, no information on possible residue concentrations after treatment is available from literature or any other sources. Both, FOA and oxalic acid, have "no MRL required" classifications. This classification is based on the assumption that the substances are not administered during honey flow; the proposed use of the product is consistent with this.

Nevertheless, the following worst case estimation of consumer exposure to residues demonstrates that even if the product were used during honey flow, the additional intake of oxalic and FOA residues would not represent a risk for the consumer:

Use of the highest dose of 225 ml (45 ml in 5 consecutive treatments, 44 mg OAD per ml product) would result in a total amount of 9900 mg OAD (corresponding to 7065 mg oxalic acid) per bee hive. As no reference value for consumer safety (ADI; upper tolerable intake) is available for oxalic acid, this was compared to the reference value as set in Council Directive 2001/110/EC. Fifty milliequivalents acid per kg honey is equal to 3151 mg/kg (ppm) when expressed as oxalic acid dihydrate (corresponding to 2250 mg oxalic acid per kg). Even if only 4 kg honey would be harvested from a bee hive treated at that dose, the honey would be marketable. In practice, even in weak bee hives the amount of first harvest of honey is expected to be much greater than 4kg, leading to a greater degree of dilution.

The concentration of FOA in the product is much lower (5 mg/ml). Treatment at the highest intended dose would result in 1125 mg FOA per bee hive. The reference value of 50 milliequivalents acid per kg honey is equal to 2300 mg/kg (ppm) when expressed as FOA. So, distribution in only 3 kg honey would be sufficient to result in honey which complies with the threshold value set in Council Directive 2001/110/EC. Also comparison with the ADI of 3 mg/kg bw (corresponding to 180 mg/person) leads to the conclusion that there is no risk for the consumer: Even if the total amount of FOA would be in one kg of honey only, intake of 20 g honey (the amount included in the CVMP's standard food basket) would lead to an exposure of 22.5 mg FOA only, which is far below the ADI.

As the amount of harvested honey is expected to be much higher than the figures used in the calculations above, it is concluded that acidity of honey from treated bee hives would be within the same range as seen in honey from untreated bee hives. Therefore, the requirements outlined by in Council Directive 2001/110/EU have been taken into account.

A zero day withdrawal period is considered appropriate.

Conclusion on the safety and residues documentation

Both active substances of VarroMed have been known in veterinary medicines for a long time, and are included in Regulation 37/2010; oxalic acid has a "No MRL required" classification in honey bees, and FOA

has a "No MRL required" classification in all food producing species. Both the active substances and all excipients are either naturally present in foods or are accepted for use in foods.

Pharmacodynamics

Oxalic acid has no identified pharmacological or therapeutic properties in mammalian species. The mode of action against Varroa mites is not well understood, but direct contact or ingestion of oxalic acid by the mite is required. The acaricidal effect is attributed partly to a sensitivity of the mites to acid pH.

FOA has irritative to caustic effects on mucosal membranes, eyes and skin in mammalian species; this effect is concentration-dependant and occurs following either direct contact or when inhaled as a vapour. The mode of action against Varroa mites is not well understood, but may result from its corrosive properties. Following the use of VarroMed, a delayed low-to-moderate acaricidal activity was shown for up to 144 hours after administration under laboratory conditions.

VarroMed also seems to increase the grooming behaviour of bees, resulting in mechanical removal of adult mites attached to their body.

Pharmacokinetics

Oxalic acid: In bees, ^{14}C-labeled oxalic acid was absorbed, distributed and metabolised after oral and topical application.

FOA: The pharmacokinetics of FOA in honey bees has not been studied.

Toxicology

OAD is of moderate to high acute toxicity by the oral route in mammalian species, and irritating to skin and mucous membranes. Repeated dose toxicity in laboratory rats, reproductive toxicity, genotoxicity and carcinogenicity were all evaluated by the CVMP as part of the MRL evaluation (EMEA/MRL/891/03). The CVMP considered oxalic acid at high concentrations to be a weak reproductive toxicant, but not to be genotoxic or carcinogenic. In honey bees, pathological findings after topical application of an overdose (10%) of oxalic acid were observed in different internal organs of honey bees; adverse effects of OAD in water solution at 175 µg/ bee showed decrease in worker bees' activity, nursing behaviour and lifespan.

In high concentrations FOA was found to be highly embryotoxic *in vitro*, but no effects were observed during *in vivo* developmental studies over 2 and 5 generations. There is no convincing evidence that FOA is mutagenic or carcinogenic.

User safety:
The beekeeper is identified as the person most likely to be exposed to the veterinary medicinal product. Possible routes of exposure are dermal, ocular. Although FOA is volatile, relevant exposure via inhalation is considered not relevant, since the formulation is a solution and the amount of FOA is low. Since oxalic acid in particular can be irritating (as reported in Reg. 1272/2008) to the skin, eyes, and mucous membranes at the concentration given in the product, appropriate warnings are included in the SPC and product literature, in order to minimise the possibility of adverse effects on the user.

Environmental safety:
VarroMed is not expected to pose a risk to the environment when used according to the SPC.

Consumer safety:
The data support the proposed withdrawal period of zero days for honey.

Part 4 – Efficacy

VarroMed was initially proposed to be used for the diagnosis of *Varroa destructor* infestation in honey bee colonies, and for the treatment of Varroa infestations in hives without brood (i.e. winter treatment) and also in the presence of brood before or after nectar-flow (i.e. spring, late summer/autumn treatment). The proposed treatment dose is a single application of 15–45 ml/hive depending on colony strength; however, for treatment in spring and late summer/autumn repeated administrations are recommended (3–5 applications at 6-day intervals) in order to remove mites that appear at eclosion of the brood cells.

In support of the efficacy, two laboratory studies (dose finding, justification of the fixed combination), three pre-clinical (dose confirmation) studies were performed. Pre-clinical studies were conducted in both maritime (Celle, Germany) and continental climate (Vienna, Austria; Bucharest, Romania).

All clinical studies were well designed, taking into consideration the CVMP Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA/CVMP/EWP/459883/2008).

Pharmacodynamics

The active ingredients are two naturally occurring organic acids, oxalic acid and FOA, which are used as antiparasitics against varroosis (*Varroa destructor*) in honey bees. At present, little is known about their mechanisms of action in the target species, honey bees, or the target pathogen, Varroa mites.

Oxalic acid dihydrate (OAD)

OAD has no identified pharmacological or therapeutic properties in mammalian species. It is a constituent of plants where its physiological role is not precisely known. It may also function as a pH regulator and might have antioxidant properties (EMEA/MRL/891/03).

In honey bees, no pharmacodynamic data on oxalic acid are available, and the mode of action of oxalic acid against Varroa mites is not well understood. It is assumed that oxalic acid acts via direct contact with mites or ingestion of haemolymph containing oxalic acid. The acaricidal effect is attributed partly to a sensitivity of the mites to acid pH (0.9–1 depending on the concentration). Oxalic acid is believed to immobilize calcium, thus impairing the calcium-potassium ratio in mite tissues.

Formic acid (FOA)

FOA has irritative to caustic effects on mucosal membranes, eyes and skin; this effect is concentration-dependant and occurs following either direct contact or when inhaled as a vapour.

The mode of action of FOA in honey bees or on mites has not been fully elucidated. The available data suggest that impairment of *Varroa destructor* may result from local effects that are due to the corrosive action of FOA vapours. In addition, absorbed FOA may cause acidosis and may impair the mite's energy supply through inhibition of the mitochondrial respiratory chain resulting in a neuro-excitatory effect on arthropod neurons.

FOA when used in-hive in high concentrations as vapour (e.g. 60% solution via evaporation), has been shown to be active against adult mites on the honey bees, and to kill mite nymphs within capped brood cells. In addition, variable activity against male and female adult mites under the brood cap has been shown, which may have consequences for mite reproduction since mating and fertilisation take place within cells. Evaporation rates are temperature dependant, and in-hive vapour concentrations might therefore be variable.

The mechanism of action of FOA at lower concentrations following direct contact (e.g. when administered by trickling) is unknown. Under laboratory conditions, FOA at a concentration of 0.5% (VarroMed) did not show immediate acaricidal efficacy after trickling (i.e. within 24 hours); but data indicated a delayed variable acaricidal activity for up to 144 hours after administration (see section on justification of the fixed combination).

Development of resistance

Data submitted provide scarce knowledge about the mechanisms of action of FOA and oxalic acid against Varroa mites. The proposed treatment regimen for this fixed combination product could have the potential for enhancing the development of resistance of Varroa mites, taking into account that oxalic acid might be administered up to 9 times per year, and that administration via trickling will generally not lead to an even distribution of the substance, i.e. subinhibitory concentrations on individually infested bees are to be expected, which both may promote resistance development.

However, both substances (as monosubstances) are already used in bees for years, and so far resistance against any of the two substances has not been reported in the literature. VarroMed is, therefore, unlikely to pose a risk in regard to the development of resistance to *Varroa destructor.*

Pharmacokinetics

See Part 3.

Justification of the fixed combination

The applicant justified the fixed combination of oxalic acid and formic acid with a superior acaricidal effect, and better tolerance when compared to the use of the active substances alone.

However, superior acaricidal activity could not be demonstrated when compared to OAD alone (cage study 2012-01-005, and pilot efficacy study), and insufficient data were provided to show an improvement in the tolerance of the product compared to OAD alone. The study (2012-01-005) was not suitable to demonstrate the benefit of the fixed combination, due to short observation time (restricted to immediate acaricidal effect).

In addition a laboratory dose determination study (2015-01-001) was provided in caged bees comparing the acaricidal effectiveness and bee tolerance of the fixed combination product with that of FOA or OAD alone at 4 different dose levels (i.e. approximate doses of 0.8, 1.5, 3.0 and 4.5 mg/ml FOA and 6, 13, 25 and 39 mg/ml OAD, respectively), including a negative (infected, untreated) control. The concentrations used reflect doses of approximately 3.8 (A), 7.7 (B), 15 (C) and 22 (D) µg FOA, and 30 (A), 65 (B), 125 (C) and 195 (D) µg OAD per bee (5 µl dispersion), i.e. groups B and C would reflect approximately the lower and higher dose within the recommended dose range of VarroMed.

Bees were cooled down to 0-2 °C prior to treatment. Immobile bees were treated with a single topical dose of 5 µl of the test formulation, and then infested with mites (10 mites per cage with 10 bees) once, either on day 0 (day of treatment), or 24, 48 or 72 h after treatment, i.e. a total of 4 cages were used with one cage per concentration per infestation time point. This test series was repeated once. Thus, a total of 2 (repetitions) × 5 (formulations) × 4 (concentrations) × 4 (infestation time points) = 160 test cages with 10 bees each were used; each test cage was accompanied by a control cage with 10 untreated infested bees. After treatment the bee cages were transferred to an incubator and kept at 33±2 °C and humidity between 55% and 65%. The bees were observed one hour after transfer for feeding status and behaviour. Further controls were performed at 24 hour intervals, up to 144 h after treatment. Dead mites (acaricidal

effect) and dead bees (tolerance) were recorded; feeding status and behaviour was only recorded in case of abnormalities. The following results were obtained:

Oxalic acid alone showed acute mite mortality of almost 100% at 24 h post-application at all test concentrations except the lowest concentration tested. Delayed acaricidal activity at 144 h post application was 10-50% at the test groups B, C.
Bee mortality at these two concentrations was up to 75% from 48 h post-application onwards. At the highest tested dose (D), bee mortality was 70-100%.

Formic acid alone did not exhibit any meaningful acute acaricidal effect at any concentration tested. However, delayed acaricidal activity at 144 hours after treatment was noted. This effect was variable ranging from 15% to 45% (average 30%) and from 20 to 60% (average 39%), for concentrations B and C, respectively.

No bee mortality was observed. At the highest tested dose (D) bee mortality was 5%.

The fixed combination (VarroMed) treatment resulted in immediate acaricidal efficacy, which lasted longer (delayed effect) than oxalic acid treatment alone.

At the lowest tested concentration (A), acute acaricidal efficacy after 24 h post application was 35%. Delayed acaricidal activity varied from 48 – 144 h post appliccation from 10-25%. No bee mortality was observed at this concentration.

At the lower recommended treatment dose B (65 OAD + 7.7 µg FOA/bee), mite mortality was 90% at 24 h; variable mite mortality at 144h post-application was observed after delayed infestations in the range of 25–55% ("delayed effect").
Bee mortality was in the range of 0–40%.

At the highest recommended treatment dose (C, 125 OAD + 15 µg FOA/bee) and the highest tested dose (D, 195 OAD + 25 µg FOA/bee) mite mortality was 90–100% from 24 hours and delayed acaricidal activity after delayed infestation at 144 hours p.appl. was in the range of 30–60% (C, D). However, bee mortality in group C was considerably lower (0-35%) than in the highest tested group (D, 5-75%) 144 hours p.appl.

The *placebo group* (dispersion without active substances) did not show any relevant acaricidal effect and did not have any impact on the tolerance of the bees.

The CVMP noted that the new study showed some deviations from the CVMP "*Guideline on veterinary medicinal products controlling Varroa destructor parasitosis in bees*" (EMA/CVMP/EWP/459883/2008) as only 2 (and not 3 as recommended for dose finding) cages with 10 bees each were used per formulation/dose/infestation time. However, the applicant justified performing the test with lower numbers of cages, since the recommendations in the guideline are not intended to be used for confirming a fixed combination, and studies undertaken following the guideline recommendations would have resulted in a huge number of tests (more than 480 test plus control cages).

The Committee agreed that results of this laboratory study (2015-01-001) showed an immediate acaricidal effect (at 24 h) of the fixed combination, and also indicate a delayed acaricidal effect of up to 60% at 144 h for all tested doses. The clinical benefit of the delayed acaricidal activity was not demonstrated in clinical studies, the only field study comparing the proposed fixed combination product with each of the mono-substances failed to show a clinical benefit resulting from the variable delayed acaricidal activity of FOA in addition to the immediate acaricidal activity of oxalic acid. However, the applicant showed a clinical benefit of this fixed combination in comparison with literature data of the two well-established active substances according to the CVMP guideline on pharmaceutical fixed combination

products (EMEA/CVMP/83804/2005, point 6.1.2). While the CVMP noted that comparison with literature data is difficult, considering the different study conditions (e.g. region, season, infestation pressure) and study protocols, the approach by the applicant for this minor species was considered acceptable, and the CVMP agreed that the data provided indicated an improved efficacy of the fixed combination when compared to the use of the single substances alone.

Tolerance, measured in bee mortality, was better after administration of the combination than OAD alone. A dose of 65 µg OAD/bee (i.e. the lower limit of the recommended dose range of VarroMed) was equally well tolerated in the fixed combination and OAD alone. However, at higher doses e.g. 125 µg OAD/bee (upper limit of the recommended dose range) a clear improvement in tolerance was seen for the fixed combination. The estimated LD_{50} values are 195 µg OAD/bee in the fixed combination compared to 125 µg OAD/bee for OAD alone (in placebo). The CVMP, therefore, considered that the laboratory data showed an improved tolerance of the fixed combination compared to the use of OAD alone.

The CVMP noted some deficiencies in the data provided; however, taking into account that the application is considered for a minor species, the CVMP on the basis of the laboratory test considered the fixed combination justified in view of improved tolerance and efficacy.

Dose justification

A laboratory study (2012-01-005) to investigate the efficacy of a dispersion containing different compositions and dosages of the active substances against the bee mite *Varroa destructor* and the tolerance of bees has been performed in Germany. In these studies, caged infested honey bees (n=10 per cage, 3 cages per test) received a dispersion of 5 µl/bee; bee and Varroa mite mortality was determined after 24, 48 and 72 hours.

The amount of 5 µl per bee was derived from calculations from the pre-clinical studies, where 15 ml VarroMed were administered per hive/colony (broodless) to a 10 000 bee colony (estimated colony strength approx. 4 weeks before treatment). For efficacy, an amount of 1.5 µl per bee can be calculated, but assuming that during the administration via trickling on occupied bee spaces single bees may receive considerably higher concentrations, the more than 3-fold concentration of 5 µl was chosen for testing. The CVMP agreed with this approach.

In this study, four different doses (33, 66, 132, or 220 µg OAD/bee combined with 3.75, 7.5, 15, and 28 µg formic acid/bee, respectively) were administered to caged infested bees. The ratio of approximately 8 parts of OAD:1 part of FOA was derived from practical treatment experiences.

For the fixed combination, acaricidal efficacy above 90% was achieved at 48 h after administration, from doses of 66 µg OAD and 7.5 µg FOA per bee, and more. Similarly, acaricidal activity of OAD alone exceeded the threshold of 90% at 24 h after administration, from doses corresponding to 66 µg OAD. Acaricidal activity was nil for placebo (vehicle) and FOA alone.

Bee mortality 72 hours after administration of the fixed combination and OAD alone at the two lower doses tested (31.8 µg or 66.3 µg OAD/bee, 3.6 µg or 7.3 µg FOA/bee) was comparable and near to 0%. At the maximum tested dose (195 µg OAD/bee, 21.6 µg FOA/bee) bee mortality was 20% for OAD alone compared to 30% for the proposed fixed combination. Bee mortality after administration of FOA alone was nil.

Similar results regarding immediate acaricidal efficacy were obtained from a second laboratory study in caged bees after single administration of comparable test formulations and concentrations (2015-01-001, see section dose justifications). Regarding tolerance, the proposed combination was

better tolerated by bees than OAD alone, with an LD_{50} of 125 µg/bee for OAD alone and 195 µg OAD/bee when used in the fixed combination product.

From these results a single treatment dose of 66 µg OAD and 7.3 µg FOA/bee was derived, and further tested under field conditions. The CVMP agreed to this approach.

In winter, a single treatment is recommended (15 ml per hive). A single dose will depend on the colony size, i.e. 15, 30 or 45 ml for colonies up to approximately 12,000 bees, 12,000 - 30,000 bees, or more than 30,000 bees per hive, respectively. Very small hives (less than 5000 bees) should not be treated.

In the presence of brood, mites might be present in capped brood cells where they cannot be reached by single treatment. Repeated treatment every 6 days is therefore recommended in the presence of brood (spring and autumn treatment), depending on the extent of mite infestations indicated by mite fall:
In spring, treatment should be conducted at the start of the season when colony size is increasing and the natural mite fall is more than 1 mite per day. The treatment should be repeated twice more, if more than 10 mites are detected on the floorboard within 6 days after the first treatment (maximum of 3 treatments).

In autumn, treatment should be conducted in late summer/early autumn when colony size is decreasing, and the natural mite fall is more than 4 mites per day. The treatment should be repeated twice, 6 days apart (i.e. 3 administrations). The treatment should be repeated twice more (that is to a maximum of 5 treatments), if more than 150 mites (colonies from the second year) or more than 90 mites (nucleus colonies in the first year) are detected on the floorboard within 6 days after the third administration

The CVMP noted that the treatment regimens recommended for spring and autumn, and the thresholds used to trigger further treatment have not all been investigated under field conditions. However, since this repeated treatment approach is well-established for Varroa control, the CVMP accepted the data for this product intended for use in a minor species.

Since the product has only been tested in bee hive types that can be opened from the top, the use of this product is currently restricted to these types of hives.

Target animal tolerance

Target animal tolerance following single and repeated administration of the proposed fixed combination product was investigated in a field study (2012-01-004) over three periods (winter – autumn – winter) from November 2012 until March 2014 in an apiary at the Institut für Bienenkunde in Celle (Germany) in line with the CVMP Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA/CVMP/EWP/459883/2008), and supported by results from other pilot studies.

Period I: (Reported also as pilot efficacy study, 2012-01-001, maritime climate). Winter treatment of broodless colonies of *A. mellifera carnica* (approximately 5000–11000 bees/colony) with a single administration of the recommended dose of 15 ml/hive of the test product (number of colonies=5), a placebo (vehicle, n=5) or a negative control. The final formulation intended for marketing was not used in this study. (The difference to the final product was an excipient-emulsifier macrogolglycerol hydroxysteatate, which is not considered to have any impact on the product and was therefore acceptable). Colony estimation was performed 2 weeks before treatment and in spring. The mean colony size after overwintering was comparable in all groups. One placebo colony died during the study.

Period II: (Reported also as part of the field study, 2013-02-003) Late summer/autumn treatment of colonies (approximately 16000–27 000 bees/colony) with brood was performed either with the test product (n=10) or with a positive control (flumethrin, n=6). VarroMed was administered at a dose of 30 or 45 ml/hive 4 times at 6-day intervals. Colony estimation was performed 2 weeks before treatment and

1 and 5 weeks after treatment. The treatment was performed after a warm and dry day with still warm evening. Honeybee mortality (dead honeybees per day) in the test group increased from approximately 11.4 to 20.6 during the treatment, whilst the mortality in the control group (flumethrin) decreased from approximately 11.1 to 5.7.

Period III: (Reported as part of field study, 2013-02-007). Winter treatment of broodless colonies (4000-31000 bees/colony) with either the test product (n=10) or a negative control (no treatment, n=6). VarroMed was administered once at a dose of 15 or 30 ml (average colonies) or 30 or 45 ml/hive (strong colonies). Colony estimation was performed 2 weeks before treatment and in spring. In the test group, the decline in honey bee numbers after treatment was greater than before treatment, and greater than compared to the control; however, the mean colony strength after overwintering was comparable in all groups.

Worker bees:

The CVMP noted that during and after treatment with VarroMed, mortality of worker bee mortality was increased. Tolerance data derived from other pilot efficacy studies (2012-01-002, 2013-02-002) and the field studies following spring or winter treatment (2013-02-005, 2013-02-006, 2014-02-003, 2014-02-001) also indicate an increase in bee mortality during and after treatment with the test article. After winter treatment this might have a negative effect on the colony development in spring (2012-01-002, Vienna). A negative long-term effect of oxalic acid has also been described in published literature indicating that oxalic acid administered in autumn by trickling, might weaken a colony with a corresponding negative effect (weak bee colony) in the following spring. Nevertheless, the authors also stated, that regardless of the findings of their study, the high efficacy of oxalic acid as a single winter treatment against *Varroa destructor* would still outweigh the possible negative consequences to the honeybee colony.

Toxic effects of oxalic acid at sublethal doses on bees are known from literature (Schneider et al., Apidologie (2012) 43, 218-225), indicating that treatment of newly emerged worker bees with 175 µg OAD per bee resulted in a significant decrease in worker activity, nursing behaviour and longevity. The CVMP expressed concerns that the maximum recommended treatment dose in the dosing range for VarroMed (132 µg OAD/bee) is near the sublethal dose of 175 µg/bee, and that under practical conditions individual bees might be exposed to higher doses than the recommended one. However, the CVMP noted that the applicant provided data demonstrating a factor 1.6 improvement in the LD_{50} of the fixed combination compared to OAD alone in placebo (2015-01-001). The estimated LD_{50} values are 195 µg OAD/bee in the fixed combination. Nevertheless, a warning was added to the SPC and product information that the dose should be carefully adjusted to the colony size in line with a dosing table, in order to avoid overdosing.

In period II of the tolerance field study worker bees with protruding proboscis observed in colonies treated with the test article could be seen; which might be due to enhanced toxicity of the product at high temperatures/insufficient water sources (bees might lick the suspension as water supply). A warning was therefore added to the SPC to ensure that treated bees should have sufficient access to drinking water.

Queens:

In line with scientific advice, the CVMP accepted the omission of specific studies in queens, considering that it would be conclusive enough when it is confirmed that the bee colony over the normal lifespan of a queen (i.e. 2 years) is showing normal viability. During the studies (see above), no impact on queens was observed in any of the periods, and queens still layed fertile eggs in late summer/autumn and spring. However, long term tolerance in queens over 2 years and the maximum number of recommended treatments was not investigated, i.e. spring, autumn and winter treatment with up to the maximum

recommended number of 9 administrations per year. The course of treatment of hives (queens) during the observed seasons (winter 2012/13 – autumn 2013 – winter 2013/14) covered only a time span of 18 consecutive months with a total of six VarroMed treatments, only.

The CVMP noted that the course of treatment was not entirely in line with the scientific advice of the CVMP (2013), where 2-year data were requested. However, CVMP accepted that as the swarming of queens and mating only takes place in spring, data over 18 months which covered two mating seasons would therefore address queen tolerance over 2 years. Since the 18-months data did not indicate a negative impact on queen tolerance, the absence of further data was accepted. However, a warning has been included in the SPC and product information that long term tolerance of VarroMed has only been tested over 18 months, and that a negative impact of VarroMed on queens or colony development after longer treatment periods cannot be excluded. Bee keepers are also advised to check regularly that the queen is present, but avoid disturbing the hives in the days following treatment.

Conclusions:

VarroMed was generally well tolerated following a single dose (15 ml) during winter treatment. However, repeated treatment in autumn and spring was associated with increased mortality of worker bees during and after treatment, in some instances with negative impact on colony development. This effect was, however, also noted following administration of comparator products.

Long term tolerance has only been tested over 18 months, and a negative impact of VarroMed on queens or colony development after longer treatment periods cannot be excluded; appropriate warnings are included in the SPC and product literature.

Clinical field trials

Dose confirmation (winter treatment)

Three dose confirmation studies were carried out each using a single dose of the test product for winter treatment in maritime climate in Germany (study 2012-01-001) and continental climate in Austria (study 2012-01-002) and Romania (study 2012-01-003). Studies followed the recommendations of the scientific advice given by the CVMP in 2013. There were, however, no preclinical studies in mediterranean climate conditions.

In the tests at least 15 (5 test, 5 placebo, 5 negative control) colonies were included. The temperature and relative humidity outside and inside the hives were monitored daily. All three studies used the western honey bee (*Apis mellifera carnica*) although in the study protocol of the preclinical studies the Italian bee (*Apis mellifera ligustica)* was also mentioned. The colony strength was evaluated by the Liebefeld estimation method and the presence of the queen before and after treatment was confirmed. The rate of mite mortality before and after treatment was determined.

To measure residual mite fall, a single follow-up treatment with coumaphos was conducted approximately 1 week after the last VarroMed treatment. Mite mortality was counted on floor boards (mesh-fitted try) which were protected from ants and earwigs for 4 weeks prior to treatment, during treatment and one week after the treatment. Bee mortality was determined by counting dead bees at the flight entrance and in front of the hive every 2 days during treatment period in Gary bee traps. Flight activities of bees were observed during the trial and no abnormalities have been recorded due to VarroMed application.

In study 2012-01-001 (Germany) the mean acaricidal efficacy of the proposed product was 84.6% on average (76.5–90.9%), compared to 22.9% (7.5–38.1%) for placebo and 23.4% (8.9–35.3%) for the control (no treatment). In study 2012-01-002 (Austria) the acaricidal efficacy following single

administration of 15 ml/hive proved to be only 64.5% (33.3–100%). In study 2012-01-003 (Romania) the acaricidal activity of the test product was compared to each active substance alone after single administration at the recommended dose, and to placebo (vehicle) and control (no treatment), respectively. The results of the study show that the acaricidal efficacy following single administration of 15 ml VarroMed/colony was nearly identical with 88.7% (68–100%) efficacy compared to 88.5% (69-98%) efficacy for OAD alone. The acaricidal efficacy of FOA alone was nil.The delayed acaricidal effect of the fixed combination of VarroMed could not be investigated in this study as it requires brood to be present in the hive.

The mean colony strength after overwintering of all colony groups was comparable. Every colony group had one colony with noticeable lower amounts of honey bees. However, the amount of 15 ml VarroMed may be not enough for colonies with 10000 or more bees, since the efficacy in two stronger colonies (study 2012-01-001) of 9–11.300 bees tested (76–83%) was slightly less than the acaricidal efficacy in three smaller colonies of 5–7500 bees (83–91%).

The follow-up treatment with coumaphos followed the user instructions according to its diagnosis indication (1 application for diagnosis of varroosis) but not the instructions of coumpahos for the treatment of varroosis (2 applications).

Based on the field studies, a more suitable dosing regimen adjusted to colony size was identified, leading to single application of VarroMed at a dose range of 66 µg OAD and 7.5 µg FOA/bee up to 132 µg OAD and 15 µg FOA/bee. This is considered acceptable.

There were no differences in bee mortality between the groups and VarroMed did not induce abnormality in behaviour after single administration of 15 ml VarroMed per colony. The single dose of 15 ml/hive appeared to be well tolerated. Direct trickling onto the bees, however, may result in considerably higher individual doses of more than 5 µl VarroMed/bee, which might increase bee mortality. Care should, therefore, be taken to administer VarroMed evenly over the bees in winter cluster.

Clinical studies

Field studies investigating the efficacy and safety of VarroMed in the treatment of varroosis in spring, autumn or winter were performed in three different climate zones; i.e. continental climate (Stuttgart, Vienna, Austria,), maritime climate (Celle, Germany) and mediterranean climate (Marchamalo, Spain).

In the continental and maritime climate studies, the Carniolan honey bee, *Apis mellifica carnica*, was used, while in the Mediterranean climate studies, the Spanish bee, *Apis mellifera iberiensis,* was used. In each location 20 test and 12 control groups (flumethrin) with *Varroa destructor* infestation were enrolled. Infestation level was variable (low/moderate/high, 0–7.3%) in winter, spring or autumn treatment. The colony sizes were approximately 5000–30000 bees.

Varroa mite and honey bee mortality were monitored twice per week for 4 weeks during the treatment period and thereafter until one week after the last treatment. Varroa mites were counted on the floor boards of each hive, dead honey bees were counted on flight entrance and in front of the hive, in Gary bee traps and on floor boards. The follow-up treatment with coumaphos was administered only once (although two applications at 7-day intervals are prescribed for treatment). In line with the CVMP "*Guideline on veterinary medicinal products controlling Varroa destructor parasitosis in bees*" (EMA/CVMP/EWP/459883/2008), the Liebefeld method was used to estimate the colony strength. In case of clinical signs of colony losses or weak colonies, presence of other diseases (such as nosemosis, foulbrood and others) was investigated.

VarroMed was applied at a dose of 15 or 30 ml for average colonies and for strong colonies at a dose of 30 or 45 ml. Average and strong colonies were not defined in the study protocol. In general, 3-5 applications at 6-day intervals were recommended for spring and autumn treatments, i.e. in the presence of brood. According to the study protocol treatment in spring and autumn should be discontinued once the mite mortality of the whole test group decreased for two following treatment intervals. Treatment was performed by trickling the dispersion on bees in occupied bee spaces in the hive. Bee colonies were treated primarily late evening without flight-activity to facilitate good distribution of the product among the bees within the hive during the dark period.

Treatment in the absence of brood (winter treatment)

Three pilot efficacy studies (see preclinical part) and four field studies were performed under continental, maritime and mediterranean conditions without brood present in the bee colonies. The field studies were carried out in three locations (Germany: Stuttgart, study 2013-02-006, and Celle, study 2013-02-007; Austria: Vienna, study 2013-02-006 and Spain: Marchamalo, study 2013-02-008). VarroMed was administered once for winter treatment.

In these studies infestation level with Varroa mites was low, moderate or high showing variable mean efficacy rates from 72.4 to 97.5% depending on the study conditions.

For winter treatment a low efficacy level of 73.8% was seen in one study with infestation level up to 7.3% under continental climate (Germany), but higher efficacy levels were seen in other studies in maritime (89.4%) and continental (97.5%) and under mediterranean climate (90.5%).

On average, in all winter treatments the efficacy was 87.6%.

It is noted that in the clinical studies the follow up treatment was postponed from 7-10 days to 16 to 20 days, and that follow-up treatment with coumaphos was administered once as recommended in the product literature for diagnosis of *Varroa*.

Based on the study results showing a mean efficacy range of 72.4% to 97.5% with a mean of 87.6%, the CVMP considered that the data were adequate to demonstrate efficacy of a single use of VarroMed for winter treatment.

Treatment in the presence of brood

Autumn treatment

Three studies (2013-02-002, 2013-02-003, 2013-02-004) were conducted after the last nectar-flow and without honey-supers, and with decreasing brood and colony strength. The studies were carried out in three climate zones: continental (Germany: Stuttgart, study 2013-02-002), maritime (Celle, Germany, study 2013-02-003) and mediterranean (Marchamalo, Spain, 2013-02-004).

In the first continental study (Stuttgart, 2013-02-002), efficacy was on average 84.5%. The test product was administered 7 times at 4-day intervals . In the treatment group as well as in the positive control group treated with flumethrin additional measures (brood removal, lactic acid (spraying) and FOA fumigation were necessary because of a high overall infestation level (0.4–7.7% per colony). The deviation from the recommended VarroMed treatment concept (more applications and shorter treatment intervals) showed negative effects on the colony development inducing higher bee mortality, especially in smaller colonies. One small colony died probably due to the overdose. Thus, based on these study results, the safety margin for VarroMed following repeated administrations is considered to be low.

The maritime study (Celle, 2013-02-003) showed adequate overall efficacy (average 95.7%) after the maximum treatment recommendation (5x) at low to moderate infestation level (0–2.3% per colony). Directly after treatments the decline of colony size was greater in test groups compared to control groups. One tested hive (no. 158) died during the study without apparent abnormalities/diseases.

The third Mediterranean study (Marchamalo, 2013-02-004) showed insufficient efficacy (average 75.2%) compared to the control (flumethrin: efficacy 90%) after five subsequent treatments. This trial location had the lowest mite infestation levels compared to all other sites. In more than 50% of colonies (12 of 20) less than 50 mites/hive were detected in total (including follow-up treatment) per colony. This is atypical for a situation in autumn and was not expected beforehand. It is therefore likely that the low number of mites in the colony at the start of the study provides a reason for the somewhat lower efficacy.

In the scientific advice EMA/CVMP/SAWP/268210/2013, CVMP acknowledged that if used as part of an integrated Varroa control concept, lower efficacy levels of 80% and 90% than the one recommended by the CVMP Guideline (above 90%) could be acceptable. Based on this criterion, 2 out of 3 studies showed adequate efficacy levels of VarroMed following repeated administration, although it was noted that only 1 out of the 3 studies followed the recommended posology.

The CVMP noted that the recommended treatment schedule for autumn treatment, i.e. 3-5 applications depending on a low mite fall (threshold of 150 mites or more for colonies, and of 90 mites or more for nucleus colonies) has not been used in the above field studies. However, autumn treatment is performed after last honey harvest and the goal of autumn treatment is to keep the infestation level below 3%, i.e. threshold of 300 mites per colony of average size (10.000 bees) and 180 mites per colony (nucleus colony, approximately 6.000 bees). A retrospective data analysis of all studies was provided and the dose regimen used with the goal to attain a maximum mite reduction with a minimum of tretaments. Until winter treatment, mites still replicate and in the worst scenario mites level could be doubled (multiplication factor of 1.16 (Kraus and Page, 1995). Therefore, not to exceed 300 or 180 mites per colony, the colonies should contain less than half of the threshold, i.e. 150 or 90 mites, respectively, after autumn treatment. Based on this, VarroMed should be applied at least 3 times in 6-day intervals. A 4th and 5th treatment should only be carried out wenn more than 150 / 90 mites are found after the last treatment.

Spring treatment

Spring treatments were performed before first nectar flow, with brood present in the hive and increasing bee populations. The locations were the same as for the winter treatment 2013/2014, i.e. two in continental climate (Germany, study 2014-02-001; and Austria, study 2014-02-002) and one in mediterranean climate (Spain, study 2014-02-003). The study design was the same as for autumn treatment.

The efficacy of the product was high (99.7%) in continental climate (Germany) with investation levels of 0.0-1.14% per colony. Total mite falls were 2-32 mites after 5 subsequent VarroMed treatments.

A second study, under continental conditions (Austria, 2014-02-002) also showed a low natural mite fall at the beginning of the study (D-31: mean 2.7 mites, D-7: 1.3). Low infestation level was confirmed after the 5th treatment cycle, where 0–19 mites were counted over the entire treatment period (arithmetic mean 6.5 mites/hive). Mean percent efficacy was 85.4 % (0–100%). The calculated efficacy was lower in the mediterranean climate (85.1%) after 4 VarroMed treatments, however, colonies were not very heavily infested before treatment (0.0-1% per colony).

The CVMP noted that the requested infestation level of 300 to 3000 mites according to the CVMP "*Guideline on veterinary medicinal products controlling Varroa destructor parasitosis in bees*"

(EMA/CVMP/EWP/459883/2008) was not observed in theses studies, and it was questioned whether repeated treatment cycles with VarroMed were really necessary under these conditions. However, the Committee recognised that while high infestation levels of 3 - 6% are generally tolerated in autumn much lower mite levels are considered harmful in early spring due to the exponential multiplication of the mites until the summer. The low infestation rates were, therefore, acceptable in agreement with current scientific views.

The CVMP noted that treatment with VarroMed was not conducted according to the recommended posology, i.e. first treatment with a dose of 15-45 ml per hive at the start of the season with increasing colony population and when the natural mite fall is more than 1 mite per day. Repeated treatment, if more than 10 mites were detected on the floorboard within 6 days after 1st treatment (maximum of 3 treatments).

Retrospective data analysis was performed for all locations for the spring treatments taking into account colonies with mite-fall (6-day period) after the first application above the threshold (>10 mites). The remaining mite infestation levels after 3 applications were calculated for each hive based on the total number of mites observed in all treatments including the follow-up treatment. The results indicated that 3 treatments were sufficient to reduce the mite levels to residual infection levels below 1% in all but one case (which showed a reduction from a very high level of 11 to 1.36%).

Overall, in 2 out of 3 studies the efficacy of VarroMed was on average 85% when the mite infestation rate was low before treatment. This deviation from the CVMP "*Guideline on veterinary medicinal products controlling Varroa destructor parasitosis in bees*" (EMA/CVMP/EWP/459883/2008) was acknowledged in the scientific advice.

Diagnosis

The applicant initially proposed the use of VarroMed also for diagnosis of Varroa infestation. However, in the absence of adequate data supporting this indication, the proposed indication was withdrawn during the assessment of the application.

Overall conclusions on efficacy

Pharmacodynamics:

Both active substances in VarroMed, OAD and formic acid, are naturally occurring organic acids with acaricidal effects; however, little is known about the mechanisms of action in the target species, honey bees, or the target pathogen, Varroa mites. It is assumed that oxalic acid acts via direct contact with mites or ingestion of haemolymph containing oxalic acid. The acaricidal effect is attributed partly to a sensitivity of the mites to acid pH. As regards to formic acid, impairment of *Varroa destructor* may result from local effects that are due to the corrosive action of FOA vapours. In addition, absorbed FOA may cause acidosis and may impair the mite's energy supply through inhibition of the mitochondrial respiratory chain resulting in a neuro-excitatory effect on arthropod neurons.

Resistance:

Both substances (as monosubstances) are already used in bees for years, and so far, resistance against any of the two substances has not been reported. VarroMed is therefore unlikely to pose a risk in regard to the development of resistance to *Varroa destructor.*

Justification of the fixed combination

The fixed combination of oxalic acid and formic acid with a superior acaricidal effect, and better tolerance when compared to the use of OAD alone was justified. A laboratory trial in caged bees confirmed that treatment with the fixed combination resulted in immediate acaricidal efficacy in the same magnitude than oxalic acid alone, but lasting longer (delayed effect) than oxalic acid treatment alone; also, improved tolerance of the fixed combination compared to use of the single substances was shown in this trial and in comparison to literature data. The CVMP on the basis of the laboratory test considered the fixed combination justified in view of improved tolerance and efficacy.

Dose justification

The proposed dose is based on the results of two laboratory studies in caged bees, evaluating acaricidal efficacy and tolerance of the fixed combination at different dosages with the monosubstances and placebo. Immediate acaricidal efficacy of more than 90% was observed from a dose of 65 µg OAD and 7.7 µg FOA/bee 24-48 hours post application, with low bee mortality. From this data a treatment dose of 15 ml/colony, corresponding to 66 µg OAD and 7.5 µg FOA/ bee was derived.

Target animal safety:

Toxic effects are mostly attributed to the oxalic acid component in the fixed combination.

At the recommended treatment dose with VarroMed (containing 65–126 µg OAD/bee), VarroMed was generally well tolerated following a single dose during winter treatment. However, increased mortality of worker bees was seen during and after repeated treatment, in some instances with negative impact on colony development. This effect was however also noted with comparator products, and is also reflected in the product literature.

Long term tolerance of VarroMed in queens has been demonstrated over two mating seasons; however, data were only provided over 18 months (and not 24 months as requested by scientific advice). Appropriate information is included in the SPC.

The margin of safety of the proposed fixed combination appears to be low. At approximately 1.5 x overdose (195 µg OAD and 25 µg FOA/bee) increased bee mortality was observed in a laboratory study in caged bees. Toxic effects of OAD at sublethal doses (175 OAD µg/bee) are also known from literature (reduced worker bees' activity, nursing behaviour and lifespan). A warning is therefore included in the product literature to carefully follow dosing instructions to avoid overdosing.

Dose confirmation:

Three dose confirmation studies were carried out in maritime climate (Germany), and continental climate (Austria, Romania) using a single dose (15 ml) of VarroMed per hive (winter treatment). The efficacy differed in these trials: in Germany 84.57% (76.47–90.9%), in Romania 88.75% (68.02–100%) and in Austria 64.49% (33.33–100%).

In no case, an efficacy threshold of preferably more than 90% was achieved, as requested by the CVMP Guideline (EMA/CVMP/EWP/459883/2008). However, the CVMP agreed in a scientific avice (2013) that efficacy between 80–90% could be accepted when an integrated Varroa control concept is in use. Based on these data, a more suitable dosing regimen adjusted to colony size was derived, leading to single application of Varromed at a dose range of 15-45 ml Varromed/colony, corresponding to a dose range of approximately 66 µg OAD & 7.5 µg FOA/bee up to 132 µg OAD & 15 µg FOA/bee, which is considered acceptable.

Clinical field studies:

A number of field studies were provided, performed in winter, autumn and spring, and in two different climate zones i.e. continental (Germany, Austria), and mediterranean (Spain) climate.

Winter treatment: The efficacy of VarroMed following single administration in winter to colonies without brood and variable infestation rates at study begin proved to be variable from 72.4% to 97.5% depending on the study conditions. The overall efficacy was 87.6% on average, which is below the recommended threshold of 90%. However, the CVMP agreed in a scientific advice (2013) that efficacy between 80-90% could be accepted when an integrated Varroa control concept is in use, and agreed that the data demonstrated efficacy of a single dose of VarroMed for winter treatment. Varromed was well tolerated in these trials.

Autumn treatment: In total, 3 autumn studies were carried out at different geographical regions and showed the following efficacies: 95.7% after 4-5 treatments at 6 days intervals (Germany), 84.5% after 7 treatments at 4-days intervals and concomitant treatments (Germany), and 75.2% after 5 administrations at 6 days intervals (Spain). As agreed in the scientific advice, if used as part of an integrated Varroa control concept, efficacy levels above 80% could be acceptable to demonstrate efficacy, and 2 out of the 3 studies showed the efficacy of Varromed following repeated administration. The CVMP noted that the recommended treatment schedule for autumn treatment, i.e. 3-5 applications depending on mite fall (threshold of 150 mites or more for colonies, and of 90 mites or more for nucleus colonies) was used only in 1 out of the 3 above field studies; however, a retrospective data analysis of all studies and the dose regimen used was provided; and, taking into account that the application is considered for a minor species, the CVMP considered the data sufficient to conclude on the efficacy of the proposed dosing regimen.

Spring treatment: Efficacy of VarroMed for the treatment of colonies with brood in spring requiring repeated administrations at 6-days intervals was investigated in 3 field studies (2014-02-003, 2014-02-002 and 2014-02-001) under continental and mediterranean climate conditions. After 4-5 treatments with the recommended dose at 6-days intervals, the efficacy of the product was variable with an average of 92.4% (99.7 % in Germany, 85.4% in Austria, and 85.09 % in Spain). Low infestation rates were observed in all three field studies and may be the result of good beekeeping practice. Increased bee mortality during and after treatment was observed with impact on colony development in 2 out of 3 studies.

The recommended treatment schedule of 1 or 3 treatments depending on a threshold of 10 mites or more for post treatment mite fall has not been investigated in any of the studies.

A retrospective data analysis was submitted for all locations for the spring treatments only taking into account colonies with mite-fall (6-day period) after the first application above the threshold (>10 mites). The remaining mite infestation levels after 3 applications were calculated for each hive based on the total number of mites observed in all treatments including the follow-up treatment. The results indicated that 3 treatments are sufficient to reduce the mite levels to residual infection levels below 1% in all but one case (which showed a reduction from a very high level of 11% to 1.36%).

Diagnosis

The use of VarroMed also for diagnosis of *Varroa* infestation was initially proposed. However, in the absence of adequate data supporting this indication, the proposed indication was withdrawn.

Part 5 – Benefit-risk assessment

Introduction

VarroMed is a bee-hive dispersion for honey bees, containing as active substances a fixed combination of oxalic acid dihydrate and formic acid. VarroMed is available in two pack sizes, a multi-dose bottle, and a multipack of 12 single-dose sachets. The proposed withdrawal period for honey is zero days.

The proposed indication is "Treatment of varroosis (*Varroa destructor)* in honey bee colonies with and without brood". The proposed dose is 15–45 ml (depending on colony size), which is repeated 3–5 times, every 6 days, in case of hives containing brood.

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 under Article 13(b) of Directive 2001/82/EC (fixed combination application).

Benefit assessment

Direct therapeutic benefit

The benefit of VarroMed is its efficacy in the treatment of varroosis (*Varroa destructor)* in honey bee colonies with and without brood.

The fixed combination of formic acid and oxalic acid dihydrate has been satisfactorily justified in a laboratory trial, showing extended duration of efficacy ("delayed effect") and improved tolerance when compared to oxalic acid dihydrate alone.

Efficacy of the product in the treatment of varroosis in honey bees was confirmed in a large number of laboratory/field studies in different European climate conditions in winter (i.e. broodless period) and in spring and autumn (i.e. presence of brood), at the proposed dose of 15- 45 ml depending on colony size.

For winter treatment, a single dose showed acaricidal efficacy with an average of 88% (72 - 98% depending on colony size and infestation level). The efficacy for winter treatment is therefore acceptable, provided that the product is used as part of an integrated Varroa control programme.

In the presence of brood (i.e. late summer/autumn or spring treatment), treatment is to be repeated 3–5 times, every 6 days, in order to cover 1–2 mite life cycles. In spring treatments with very low to low mite infestation level, the efficacy was variable with an average of 92.4% (85.1–99.7%). In autumn treatments with decreasing brood the efficacy level was likewise variable with an average of 85% (75.2–95.7%). These levels of efficacy were acceptable provided that the product is used as part of an integrated Varroa control programme.

The fixed combination is justified.

Additional benefits

An additional benefit of the product is considered to be the ease of administration by beekeepers, as the product is ready-to-use and requires no further preparation.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Safety:

Measures to manage the risks identified below are included in the risk management section.

Risks for the target animal:

At the recommended treatment dose, a single dose of VarroMed as winter treatment is generally well tolerated. However, clinical trials showed that repeated treatment (autumn and spring) was associated with increased mortality of worker bees during and after treatment, in some instances with negative impact on colony development. However, this effect is comparable to treatment with comparator products, and is reflected in the product literature.

Long term tolerance of Varromed in queens has been demonstrated during a period of 18 months.

The design of the final product and presentations are suitable to ensure accurate dosing under practical conditions.

Risk for the user:

The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations. Suitable safety advice is included in the SPC and other product literature.

Risk for the environment:

The product is not expected to pose a risk to the environment, when used according to the SPC recommendations.

Risk for the consumer:

The withdrawal period for honey is zero days.

Risk management or mitigation measures

Appropriate information has been included in the SPC and other product information to inform on the potential risks of this product relevant to the target animal and the user; and to provide advice on how to prevent or reduce these risks.

The withdrawal period is set at zero days for honey.

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 CVMP considered the request against all the criteria prescribed in Article 2 of Commission Directive 2006/130/EC and considered that:

- 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 characteritics does not refer to contraindications related to other veterinary medicinal products commonly used without prescription;
- Neither the veterinary medicinal product nor any other product containing the same active substance has to the knowledge of the CVMP previously been the subject of frequent serious adverse reaction reporting;
- The veterinary medicinal product is not subject 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 therefore considered that the product complies with all the criteria prescribed in Article 2 of Commission Directive 2006/130/EC and that therefore the application for exemption from the requirement for the veterinary medicinal product to be dispensed only against veterinary prescription is acceptable.

Evaluation of the benefit-risk balance

The overall benefit-risk is deemed positive.

The product has been shown to be efficacious the treatment of varroosis (*Varroa destructor)* in honey bee colonies with and without brood.

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users, the environment and consumers, when used as recommended. Appropriate precautionary measures, including withdrawal period, have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of VarroMed is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.

Divergent position on a CVMP opinion on the granting of a marketing authorisation for VarroMed (EMEA/V/C/002723/0000)

The undersigned wish to express a divergent position to the CVMP Opinion on this application for a marketing authorisation, for the reasons outlined below:

Justification of the fixed combination

In the opinion of the undersigned, the fixed combination of oxalic acid dihydrate (OAD, 44mg/ml) and formic acid (FOA, 5mg/ml) is not scientifically justified. The postulated superior acaricidal efficacy and improved tolerance when compared to the use of the active substances alone as claimed by the applicant cannot be derived from the preclinical and clinical data provided.

- Superior acaricidal efficacy

In two laboratory studies in caged bees (2012-01.005, 2015-01-001) high immediate acaricidal efficacy of 90% or more was demonstrated for the fixed combination product which is attributed solely to OAD.

In study 2015-01-001 potential delayed acaricidal effects were investigated following repeated mite infestations at 0, 24, 48 and 72 hours after administration of the fixed combination or the substances alone. The results suggest a variable delayed acaricidal effect of the fixed combination as well as of OAD and FOA alone. Due to the limited number of bees (2 cages with 10 bees each per test item/concentration/mite infestation time point the data are not robust enough to assess the variability, and thus the precision of the results; consequently, any observed effect might be due to randomness. In conclusion, data do not confirm the postulated delayed acaricidal effect resulting from FOA in the fixed combination compared to the substances alone.

- Improved bee tolerance

Based on the bee mortalities at the different concentrations/doses tested in study 2015-01-001 the applicant roughly estimated an LD_{50} of 195µg OAD/bee for the fixed combination (highest dose tested), compared to LD_{50} of 132µg OAD/bee for OAD in placebo solution, and derived a margin of safety of 1.6. The undersigned are of the opinion that the comparison of LD_{50} values is inadequate to conclude a clear improvement of tolerance of the fixed combination and, on the contrary, data indicate that the margin of safety of both the fixed combination and OAD alone in bees is rather low. Furthermore, it is noted that the results of this laboratory study are in contrast to study 2012-01-005 where higher bee mortality was observed after application of the fixed combination at the highest dose tested compared to OAD alone (30% *vs.* 20%). Hence, the data obtained in these laboratory studies are considered inconclusive. Schneider et al. (2012) observed sublethal effects on worker bees (decreased activity, nursing behaviour, reduced life span) after topical administration of 5µl 3.5% OAD sugar solution which corresponds to a dose of 175µg/bee, the average dosage a single bee would receive at colony treatment using trickling of 3.5% OAD. The undersigned are of the opinion that under practical field conditions direct trickling of Varromed (containing 4.4% OAD) onto the bees likely results in comparable individual dose volumes. In conclusion, the tolerance of the fixed combination is to be considered in the range of that known for OAD alone.

The postulated clinical benefit resulting from the fixed combination of OAD and FOA, i.e. the enhanced acaricidal activity and improved safety, has not been confirmed under field conditions. Superiority of the proposed fixed combination compared to the substances alone was investigated in only one pilot field study including a total of 25 bee colonies. (2012-01-003). After single administration of the fixed combination at the recommended dosage (15ml/colony) efficacy was on average 88.75% compared to 88.51% for OAD alone, 12.6% for FOA alone, 25% for placebo (Varromed-vehicle), and 7% (no treatment). Bee mortality was not evaluated in this study.

The comparison with literature data that suggest lower efficacy rates of OAD following repeated administration to brood right colonies when compared to Varromed is inadequate to support the postulated superiority of Varromed because the studies reported in the literature have been conducted under different conditions and treatment regimens. Furthermore, it is to be noted that the repeated treatment approach for Varroa control using OAD is not well established in Europe because veterinary medicinal products containing OAD are approved only for single treatment of broodless colonies (winter treatment). Moreover, published data indicate that repeated administration of OAD in broodright colonies is associated with negative effects on brood and bees (Rosenkranz et al., 2010).

Target animal tolerance

The undersigned are of the opinion that the tolerance of Varromed in bees after repeated treatments has not been sufficiently proven. In the clinical field studies, increased bee mortality was observed during and after treatment (4-5 applications at 6-days intervals) with impact on colony development in 2 of 3 spring studies. Increased bee mortality during and after Varromed treatment compared to baseline was documented in all three autumn studies following 5 applications in 6-days intervals or more. In one of these studies (2013-02-003, Stuttgart, DE) small colonies died likely due to overdose.

Safety following long term use including the usual life span of a queen (2 years) has not been demonstrated as advised by CVMP's advices (EMA/CVMP/SAWP/451269/2012; EMA/CVMP/SAWP/268210/2013). There is only one study with subsequent treatments of colonies in winter (1 application) – autumn (4 applications) - winter (1 application) for approximately 12 months. It is to be noted that the maximum recommended treatment of 9 applications of Varromed/year has not been investigated.

Efficacy

The undersigned are of the opinion that the efficacy of Varromed in the treatment of varroosis in spring and autumn has not been substantiated by scientifically sound data.

Spring treatment

Efficacy rates in spring studies were on average 89.4% (2014-02-003, Marchamalo, Spain), 98.2% (2014-02-001, Stuttgart, DE), and 89.4% (2014-02-002, Vienna, A). However, in all of these studies the mite infestation before treatment initiation was low (almost below 1 mite/day) and, hence, far below the infestation rate of 300-3000 mites/colony recommended in the Guideline on Veterinary Medicinal Products controlling Varroa destructor Parasitosis in Bees (CVMP/EWP/459883/2008/2010). The undersigned consider these infestation rates in all three spring studies too low to obtain reliable results. Moreover, the necessity of treatment appears questionable under these conditions.

Despite these low infestation rates, VarroMed was administered 4 or 5 times at 6-days intervals in all three studies. This does not comply to the recommended dosing regimen for spring, i.e. one treatment if natural mite fall is 1 mite per day, and two further treatments if more than 10 mites are detected on the floorboard within 6 days after the first treatment (maximum 3 treatments). No reliable efficacy values can

be derived from these studies regarding this recommended dosing regimen. A retrospective analysis of the data to support the revised treatment schedule including mite threshold levels for treatment is considered inacceptable as it is only explorative and not verified by data.

Autumn treatment

Three studies were carried out with mean efficacy rates of 95.7% (2013-02-003, Celle, DE), 84.5% (2013-02-002, Stuttgart, DE), and 76.5% (2013-02-004, Marchamalo, ES). Only two of these studies provide efficacy rates between 80 and 90% in line with CVMP's scientific advice. However, in one of these two studies (2013-02-002, Stuttgart, DE) sufficient efficacy was only achieved after 7 treatments at 4-days intervals and concomitant treatments including brood removal, lactic acid spraying and formic acid evaporation were necessary, due to high infestation levels of 0.4% -7.69% prior to treatment initiation. It can be assumed that under such study conditions showing an adequate infestation level according to the guideline, it is unlikely that adequate efficacy rates could be achieved with only 3-5 treatments with Varromed as recommended by the applicant. Prolonged treatment with Varromed was associated with negative effect on colony development.

The recommended treatment schedule, i.e. treatment initiation based on a threshold of more than 4 mites/day, and more than 3 treatments if more than 150 mites (colonies from the second year) or more than 90 mites (nucleus colonies in the first year) are detected on the floorboard within 6 days after the third administration has not been followed in any of these studies. A retrospective analysis of the data to support the revised treatment schedule including mite threshold levels for treatment is considered inacceptable as it is only explorative and not verified by data.

Conclusion

The undersigned are of the opinion that the benefit resulting from additive acaricidal activity and improved tolerance of the fixed combination of OAD and FOA has not been sufficiently justified. Efficacy in the treatment of varroosis after repeated administrations in spring and in autumn has not been sufficiently proven, and serious concerns remain regarding negative effects on bees and colony development after repeated use of Varromed. Consequently, the benefit-risk balance is considered unfavourable for both spring and autumn treatment.

As regards the claimed winter treatment for Varromed, efficacy rates of the product were almost in the range of 80%-90% accepted by CVMP's scientific advice. Following single administration Varromed was well tolerated and no adverse effects were detected on colony development in the following spring. Although the combination of OAD and FOA is not substantiated by meaningful data, the undersigned are of the opinion that the benefit-risk balance is favourable for this indication. In conclusion, marketing authorisation is only justified on condition that the clinical indication, treatment of varroosis, is limited to single winter treatment of varroosis in honey bees.

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