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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Oxybee (EMEA/V/C/004296/0000)

International non-proprietary name: oxalic acid dihydrate

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

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Introduction

The applicant Dany Bienenwohl GmbH submitted on 28 September 2016 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Oxybee powder and solution for bee-hive dispersion for honey bees, through the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004 (significant innovation or interest of animal health at Community level).

The eligibility to the centralised procedure was agreed upon by the CVMP on 10 September 2015 as the applicant showed that the product would be in the interest of animal health at the Community level.

The applicant applied for the following indication: For the treatment of varroosis (*Varroa destructor*) of honey bees (*Apis mellifera*) in brood-free colonies.

The active substance of Oxybee is oxalic acid dihydrate (OAD), an antiparasitic substance used in honey bees for treatment of varroosis. The target species is honey bees.

Oxybee is a powder and solution for bee-hive dispersion, which when mixed contains 39.4 mg OAD/ml. The product is available in two pack sizes. The smaller is a 500 ml bottle containing 17.5 g OAD (in 375 g of solution) which is packaged with one 125 g sachet of (flavoured) sucrose. The larger pack size consists of a 1000 ml bottle containing 35 g OAD (in 750 g of solution) packaged with two 125 g sachets of (flavoured) sucrose. Prior to use, the sucrose powder is added to the solution (containing the active substance) in the bottle, and then mixed, in order to achieve the final bee-hive dispersion (which contains 35 g OAD per kg of dispersion, equivalent to 25 mg/ml oxalic acid anhydrous).

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

The rapporteur appointed is Johan Schefferlie and the co-rapporteur is Katarina Štraus.

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

On 7 September 2017, the CVMP adopted an opinion and CVMP assessment report.

On 1 February 2018, the European Commission adopted a Commission Decision granting the marketing authorisation for Oxybee.

Scientific advice

The applicant received scientific advice from the CVMP on 6 November 2015. The scientific advice pertained to the quality part of the dossier. The CVMP considered that the applicant, in general, followed the scientific advice.

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. The CVMP considered the request and agreed to it, provided that the assessment of the application confirms that the product complies with all the criteria prescribed in Article 2 of Commission Directive 2006/130/EC. This is addressed in Part 5 – *Conditions or restrictions regarding supply and use* as part of the benefit-risk assessment for Oxybee.

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the system of pharmacovigilance (dated 18 August 2016), 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, quality control and release of the active substance, oxalic acid dihydrate, takes place in the EU. The sites have GMP certificates issued by the relevant authorities confirming GMP compliance. A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the batch release site. The declaration was based on a GMP certificate available for the active substance manufacturing site.

Manufacture, packaging (primary and secondary) and quality control of the finished product is performed in the EU. Batch release of the dosage form takes place at Wirtschaftsgenossenschaft Deutscher Tierärzte e.G., Garbsen, Germany. The site has a GMP certificate for the manufacture of the veterinary medicinal products issued by the German authorities (Staatliches Gewerbeaufsichtsamt, Hannover, Germany), which confirms GMP compliance.

Overall conclusions on administrative particulars

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

The GMP status of both the active substance and finished product manufacturing sites has been satisfactorily established and are in line with legal requirements.

Part 2 - Quality

Composition

The product Oxybee powder and solution for bee-hive dispersion is presented as a bottle containing a solution of oxalic acid dihydrate (OAD) and either one or two sachets of flavoured sucrose, depending on the pack size. Before first use the sucrose powder is added to the solution in order to form the dispersion.

When mixed as directed, Oxybee bee-hive dispersion contains 39.4 mg/ml OAD as the active substance.

The OAD solution contains the active substance, oxalic acid dihydrate, together with the excipients citric acid monohydrate (stabilizer), glycerol 85% (co-solvent and viscosity modifier) and purified water (solvent).

The sachet of powder contains sucrose (viscosity enhancer), anise oil (aromatic substance) and eucalyptus oil (aromatic substance).

All the excipients are listed in section 6.1 of the SPC.

Containers

The OAD solution is presented in bottles of 500 ml or 1000 ml containing 444.25 ml or 888.5 ml of OAD dispersion, respectively, made from colourless high density polyethylene (HDPE) with child-proof screw caps made from HDPE/polypropylene (PP).

One HDPE bottle (500 ml, 1000 ml) is supplied with one or two sachets (respectively) of the flavoured sucrose powder in a cardboard box (secondary packaging).

The contents of the flavoured sucrose powder sachet(s) is/are added to the contents of the appropriate sized bottle of the oxalic acid solution. The oxalic acid solution bottle is therefore used for the (final) bee-hive dispersion.

The flavoured sucrose powder is presented in sealed sachets containing 125 g of flavoured sucrose. The sachets are made of four-layered foil (clay coated paper [outer side], low density polyethylene (LDPE), aluminium (Al), LDPE [inner side]). The sachets are opened by tearing along a perforated strip which is inserted during the filling and manufacturing process.

The primary packaging materials used for the bottles, screw caps and sachets all comply with the relevant European Pharmacopoeia (Ph. Eur.) and EU requirements. Appropriate specifications and certificates of analysis have been provided. The choice of the container-closure systems has been validated by stability data and is adequate for the intended use of the product.

Development pharmaceutics

When the product is mixed ready for use, Oxybee is a (non-sterile) aqueous dispersion for in-hive use in the honey bee colony.

The packaging components chosen for the finished product have been justified by stability data.

The excipients (citric acid monohydrate, glycerol (85%), purified water, sucrose, anise oil and eucalyptus oil) are well-known and considered of suitable quality and used in appropriate concentrations.

There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SPC. Compatibility of the chosen excipients with the active substance was demonstrated by stability testing.

Oxybee is a non-sterile dispersion for in-hive use which includes no preservative. Because of the high concentrations of OAD in both the solution and the mixed/ready-to-use bee-hive dispersion, the pH is very low and therefore microbiological contamination would be very unlikely. Monitoring the microbiological quality during stability studies resulted in total aerobic microbial count (TAMC) and total yeast/mould count (TYMC) values and also the absence of *S. aureus* and

P. aeruginosa, consistent with the requirements of the Ph. Eur 5.14, veterinary medicinal products for cutaneous use.

The formulation used during the clinical studies is the same as that intended for marketing.

Method of manufacture and process controls

Manufacture of both the OAD solution and the flavoured sucrose powder is described in detail.

For the OAD solution, the manufacturing process is simple and consists of dissolution of the different components. The final solution is filled into the bottles which are then closed, labelled and packaged.

The flavoured sucrose powder is manufactured by mixing and blending the different components. The final powder mixture is then filled into the pre-labelled sachets. All relevant and necessary in-process controls are included.

Process validation was performed with two production scale batches, each consisting of OAD solution and sucrose powder, manufactured by the dosage form manufacturer according to a pre-defined validation plan. The batches of OAD solution are full production scale size. Although the batch sizes of the flavoured sucrose powder are only 92% of full scale production size, this is considered acceptable given the simplicity of the manufacturing method.

The critical steps in the manufacture of the OAD solution, the flavoured sucrose powder and the final product were identified and addressed. It was demonstrated that the manufacturing process is capable of producing the finished product to the intended quality and in a reproducible manner.

Evaluation of the outcome of process validation was performed on two batches separately using standard statistical calculations. The quality of the validation batches corresponds to the specifications set and demonstrates the reproducibility of the manufacturing process. However, in accordance with Guideline on quality data requirements for veterinary medicinal products for MUMS/limited market (EMA/CVMP/128710/2004-Rev.2), the applicant commits to conduct process validation studies on full scale batches post authorisation and inform EMA if problems are encountered during validation of the process at the full scale, and present a proposed action. It was demonstrated that the defined process is capable of producing a homogenous product that complies with the product specifications.

Control of starting materials

Active substance

Oxalic acid dihydrate (OAD) exhibits no stereoisomerism. The potential for polymorphism is not relevant for this product as the OAD is in solution.

There is no monograph for OAD in the Ph. Eur., however OAD (Acidum oxalicum) is described in the HAB (Homöopathisches Arzneibuch (HAB), German Homeopathic Pharmacopoeia) and the DAC (Deutscher Arzneimittel Codex (DAC), German Medicines Codex). The respective monographs are provided. OAD is tested according to the current DAC and HAB requirements both at time of release of the active pharmaceutical ingredient (API) by the API manufacturer and then to full specification immediately before use by the manufacturer of the product.

The active pharmaceutical ingredient starting material (API-SM) is primarily used for technical purposes, it is currently not commercially available for medicinal products in a pharmaceutical grade in full compliance with GMP. However, Volume 4 of Good Manufacturing Practice Part II: *Basic Requirements for Active Substances used as Starting Material* states that in the case of

ectoparasiticides for veterinary use, other standards than these guidelines that ensure that the material is of appropriate quality, may be used.

A class 1C metal is used in the manufacture of the API-SM. Three batches of API-SM and one validation batch of the final active substance were tested with a suitable validated method. Although there is no applicable veterinary guideline, according to the CHMP Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents (EMEA/CHMP/SWP/4446/2000) the oral exposure limit for class-1C metals is 25 ppm. The results demonstrate that the class 1C metal is only found in very low and safe concentrations.

Production of oxalic acid dihydrate (API-GMP Grade)

The active substance and its impurities have been adequately characterised and the OAD chemical structure confirmed by three different spectroscopic techniques.

The characterisation of the active substance and its impurities are in accordance with the EU draft Guideline on the chemistry of new active substances (veterinary) (EMA/CVMP/QWP/49477/2017). Potential and actual impurities were well discussed with regards to their origin and characterised.

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), related substances (GC), water content, heavy metals, sulfate, chlorides and sulfated ash. Ph. Eur. and HAB compendial test methods are utilised except for related substances. Acceptable impurity limits are applied. The related substances are determined by a non-pharmacopoeial but validated method (GC). In addition, microbiological controls (TAMC, TYMC) are conducted in compliance with Ph. Eur. 2.6.12 and 2.6.13.

The relevant analytical methods used have been adequately described and where necessary appropriately validated in accordance with VICH guidelines. Batch analysis data for three production batches of the oxalic acid dihydrate are provided and are within the proposed specifications and consistent from batch to batch.

The final active substance (OAD, GMP grade) is packaged into bags placed in a drum. The plastic material used in the primary packaging in contact with the active substance is in compliance with Directive 10/2011/EC and Directive 1183/2012/EC.

No formal stability data for the active substance has been provided, however the applicant states that the active substance will be tested to full specification immediately before use in the manufacture of the finished product. This is acceptable in accordance with the Guideline on Quality data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/QWP/128710/2004).

Excipients

All the excipients in Oxybee are well known pharmaceutical ingredients and their quality is compliant with the respective Ph. Eur. standards. Certificates of analysis are enclosed for all excipients.

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

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

Control tests on the finished product

Finished product specifications are provided for use at release for the OAD solution, the flavoured sucrose powder and the mixed/ready-to-use bee-hive dispersion.

The flavoured sucrose powder is checked for appearance (visual), odour (organoleptic) and loss on drying (Ph. Eur. method 2.2.32). Validation of these analytical procedures is not considered necessary. The shelf life specification of the flavoured sucrose powder is the same as its release specification and this is justified.

For the oxalic acid dihydrate solution, the analytical procedures used for the determination of appearance, degree of colouration of the solution, identity and microbiological quality are Ph. Eur. methods. For the determination of identity of the active substance two methods, colour reaction (HAB) and an in-house HPLC method, are used, in line with the recommendations in VICH Guideline GL39. For the assay of oxalic acid dihydrate an in-house HPLC method is used, which is appropriately validated.

For the mixed/ready-to-use dispersion, the applicant uses the same validated HPLC method as for the OAD assay, as well as the same analytical procedures for the determination of appearance, degree of colouration of the dispersion, determination of identity of the active substance and microbiological quality. Furthermore, pH, relative density and dry residue of extracts are determined according to Ph. Eur. methods.

The proposed release specification parameters and their values are acceptable.

All the batches complied with the proposed specifications. For release testing of the product, two pilot batches of the OAD solution and the flavoured sucrose powder were used. From each batch, both the final pack sizes, 375 g OAD (in a 500 ml bottle) and 750 g OAD (in a 1000 ml bottle) were prepared. The provision of batch analysis data on only two pilot batches is in line with the CVMP Guideline on quality data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/QWP/128710/2004).

Stability

Active substance

No formal stability studies were conducted. The active substance is tested immediately before manufacture of the finished product in accordance with its full specification. This is in accordance with the CVMP Guideline on Quality data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/QWP/128710/2004).

Finished product

The in-use shelf life (stability) specification for the ready-to-use dispersion (after mixing) has been proposed and justified. Impurities are assayed with an appropriately validated HPLC method.

No stability studies were performed with inverted bottles. This is justified, since the screw cap is made from the same material (HDPE) as the bottle and therefore no effect on the stability of the product due to its storage orientation is expected.

No light stability studies were performed. This is also justified, since in the CVMP Guideline on quality data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/QWP/128710/2004-Rev.1) it is stated that photostability data is not required as long as the product is provided in a carton (or other suitable protective packaging) and is labelled "Protect

from light". Section 6.4 of the SPC (and other relevant sections of the product information) include the warning "Keep the bottle and sachet in the outer carton in order to protect from light", and the lack of any light stability studies is therefore acceptable. Furthermore, the sucrose powder and the OAD solution are packed protected from light while the mixed/ready-to-use product has to be stored in the refrigerator which also protects the product from light.

For the stability studies two pilot scale batches and two commercial scale batches were manufactured by the finished product manufacturer.

For the two pilot scale batches, the OAD solution was filled into both bottle sizes in their intended packaging. Likewise for the two commercial scale batches. Stability studies in accordance with the CVMP Guideline on Stability testing: stability testing of existing active substances and related finished products (EMEA/CVMP/QWP/846/99-Rev.1), were then performed using both real time stability studies at 25 °C/60% RH (long term conditions) and at 40 °C/75% RH (accelerated conditions) on these four batches (2 pilot scale; 2 production scale) of the finished product.

In-use stability studies were performed on the two pilot batches (both package sizes) and on the two commercial batches of mixed and ready-to-use finished product (dispersion), all stored, as recommended, refrigerated at 2 °C – 8 °C.

The proposed parameters, limits and methods used for the shelf life specifications are acceptable. Regarding the limits proposed for the content of OAD in the OAD solution and in the mixed/ready-to-use dispersion, the limits proposed for use during shelf life are identical to those proposed for release. The applicant has investigated any degradation products of oxalic acid dihydrate in the OAD solution using the HPLC assay test method and setting relevant limits that are considered as qualified.

For the determination of degradation products the applicant refers to the HPLC method for assay. Based on the available stability results, which demonstrate that both the flavoured sucrose powder in their sachet(s), and the unopened bottles with the OAD solution are stable for up to 24 months at 25 °C/60% RH and for up to 6 months at 40 °C/75% RH, a shelf life of 24 months, without any special storage precautions, is claimed for the product as marketed.

Based on the available stability data for the mixed/ready-to-use dispersion an in-use shelf life of 1 year when stored in a refrigerator (2 °C – 8 °C) is claimed. On the SPC and other product information, it is therefore stated: "After mixing the bee-hive dispersion: Store in a refrigerator (2 °C – 8 °C)."

In addition, according to the CVMP Guideline on In-use stability testing (EMEA/CVMP/424/01), an in-use stability test has been conducted on two pilot scale batches of the mixed/ready-to-use dispersion at the end of their in-use shelf life. Test results covering 3 months are provided. The data provided further substantiates the claimed 1 year in-use shelf life. The applicant has provided an assurance that the studies will be finalised and that data will be provided immediately to the EMA if (potentially) outside specification results occur at the end of the claimed in-use shelf life (with proposed action).

Overall conclusions on quality

Information on the development, manufacture and control of the active substance and the two finished product components (the bottle containing the OAD solution and the sachet(s) containing the flavoured sucrose powder) and the mixed/ready-to-use dispersion 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. The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical aspects

relevant to the performance of the product have been investigated and are controlled. Information has been presented to give reassurance on TSE safety.

The control tests applied to the active substance and the finished product are appropriate. The analytical methods are well described and their validation data confirm their suitability. The active substance is tested immediately before manufacture of the finished product in accordance with its full specification. Stability studies on the finished product have been performed according to VICH guidelines. The claimed shelf-life of 2 years (no special storage precautions) for the product as marketed and the in–use shelf life of 1 year (refrigerated) for the mixed/ready-to-use product (bee-hive dispersion) have been justified.

Recommendations:

The applicant is recommended to conduct process validation studies on full scale batches post authorisation, and inform EMA if problems are encountered during validation of the process at the full scale, and present a proposed action.

The applicant is recommended to finalise the in-use stability testing on the two pilot batches of the mixed/ready-to-use dispersion at the end of their shelf-life to substantiate the claimed 1 year in-use shelf life. Data will be provided immediately to the EMA if outside specification results occur at the end of the claimed in-use shelf life (and a proposed action presented).

Part 3 – Safety

Oxybee contains oxalic acid dihydrate as active substance, which is a strong organic acid. Oxalic acid, for use in honey bees, is included in Regulation 37/2010 with a "No MRL required" classification. A published summary report relating to the safety evaluation performed for the MRL assessment is available for oxalic acid (EMEA/MRL/891/03-FINAL, 2003) and cross-reference to the report is made.

Safety documentation

Pharmacodynamics

Oxalic acid dihydrate (OAD) is a strong organic dicarboxylic acid (pKa 1.25 and 4.14) in the form of a dihydrate. The mode of action of OAD against Varroa mites is not yet well understood, but direct contact between oxalic acid and the mite is required. Its pharmacodynamic action is mainly attributed to its low pH. In mammalian species OAD has no identified pharmacological or therapeutic properties.

See Part 4 for more information on pharmacodynamics in honey bees.

Pharmacokinetics

In mammals oxalic acid is an end product of metabolism of natural components of some amino acids, glycolate and ascorbic acid. Endogenous sources constitute approximately 30–70% of the oxalic acid excreted daily via urine (about 20–30 mg) in humans.

Absorption:

In mammals absorption of oxalic acid after oral intake is largely dependent on pH, presence of calcium (and magnesium) ions and presence of oxalate-degrading bacteria in the gut.

The absorption of oxalic acid after oral administration in humans is between 3–20%, but under certain circumstances (i.e. in certain diseases that cause fat malabsorption, facilitating calcium binding to fatty

acids and thereby enhancing oxalate absorption) this can be increased up to 60%. In rodents, the absorption of oxalic acid after oral administration is less than 30%.

Plasma/Tissue Distribution:

In human tissues natural oxalic acid levels are in the range of 0.6 to 4 mg/kg in blood, kidney, liver, muscle, brain and bone, with the highest concentrations measured in the kidney and the lowest in the brain. In mammals overdoses can lead to calcium oxalate deposits in tissues and notably formation of kidney stones.

Metabolism:

In mammals the substance is mainly excreted as the parent compound. Degradation appears to be by oxalate degrading bacteria in the gut.

Excretion:

After intravenous administration of small doses oxalic acid is mainly excreted via the urine as the parent compound (>90%), with small amounts excreted via faeces. Faecal excretion mainly results from dietary oxalic acid intake. Plasma elimination half-life is about 2 hours in rodents.

See Part 4 for information on pharmacokinetics in honey bees.

Toxicological studies

Single dose toxicity

Acute oral toxicity studies of oxalic acid were carried out in rats, dogs and cats and indicate that oxalic acid is of moderate toxicity by the oral route in mammalian species. Toxic doses ranged from 375 mg/kg for female rats (LD_{50}), 475 mg/kg for male rats (LD_{50}), 1 g for dogs and 200 mg for cats. The mean lethal oral dose for an adult human is reported to be 15–30 g oxalic acid. However, doses of 5 g have been reported to be fatal.

Symptoms of acute poisoning may include severe gastrointestinal pain and distress, bleaching of mucous membranes, cardiovascular collapse, neuro-muscular symptoms, kidney damage and death.

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. Calcium depletion has also been reported. Oxalate binds to blood calcium and induces neurotoxicity and cardiac arrest.

Repeat dose toxicity

Long-Evans rats were fed diets with oxalic acid at levels equivalent to 0, 2 and 5 g/kg bw/day for 70 days. Body weight decreased in both dose level groups and renal toxicity was found. The ingestion of oxalic acid appeared to be associated with inhibition of gonadal, adrenal and thyroid activity.

Following subcutaneous injections of 25, 50 and 75 mg/kg bw/day for up to 3 weeks in rats, haematuria with increased urinary white blood cells, epithelia and casts was observed. Mild nephrotoxicity was found to be caused by tubular obstruction, from the lowest administered dose.

A NOEL could not be established. Repeated dose toxicity studies in a second species are not available and no long-term repeated dose toxicity studies were reported.

Tolerance in the target species of animal

See part 4.

Reproductive toxicity

Study of the effect on reproduction

Reproductive toxicity of oxalic acid was assessed in CD-1 mice in a fertility study which included a range finding module. Due to lack of conspicuous effects in the first generation only the highest dose (275 mg/kg bw/day) and control group were continued for production of the second generation. The substance did not induce overt teratogenic effects or postnatal toxicity. Oxalic acid appeared to interfere with spermatogenesis. It was not possible to derive a NOEL since data from the lower dose level groups were not recorded for the second generation.

Study of developmental toxicity

Two teratogenicity studies from public literature are reported, one in rats and one in sheep. Neither study used valid protocols covering embryotoxic and teratogenic effects.

The teratogenicity studies in rats and sheep show no gross malformations, but at high doses tubular malformation (rat pups) or renal oxalosis (lambs) was observed. A NOEL could not be derived from these studies. No final conclusion on teratogenic or embryotoxic effects can be drawn.

Genotoxicity

There are only limited data from non-GLP studies and published reports to assess the genotoxic potential of oxalic acid.

In the Ames test oxalic acid dihydrate was clearly negative in the *Salmonella typhimurium* assay.

The data from a chromosome aberration test *in vitro* on mammalian Chinese hamster lung fibroblasts indicate oxalic acid is not a clastogenic compound.

There was a weak positive result in a chromosome aberration test with plant root meristem cells but the relevance of this effect for mammals is unknown and the study was poorly documented.

Based on the above studies it is concluded that oxalic acid dihydrate is not genotoxic.

Carcinogenicity

In Osborne-Mendel male and female rats administered oxalic acid at mean daily doses of 50 to 600 mg/kg/ bw/day in feed for 2 years no evidence of carcinogenicity was found.

Although this study did not meet the criteria set out in current guidelines, based on the test results in combination with the negative results in the mutagenicity tests, it can be assumed that oxalic acid is devoid of carcinogenic properties.

Studies of other effects

No specific studies on the immunotoxicity of oxalic acid were provided. This is acceptable because no indications of such effects were observed in toxicology or pharmacodynamics studies.

Oxalic acid is moderately irritant to skin and severely irritating to the eyes, the respiratory and gastrointestinal tract. The dermal toxicity is low with an LD_{50} of 20,000 mg/kg bw determined in the rabbit.

In the intestine of most animal species, including humans, anaerobic bacteria (for example *Oxalobacter formigenes*) are present and metabolise oxalic acid.

Oxalic acid overdosage may induce hypocalcaemia with subsequent neurological signs (peripheral and CNS effects). In susceptible subpopulations (humans with severely compromised kidney functions or primary hyperoxaluria) hypocalcaemia-induced neurological sequelae are to be expected even after moderate oxalic acid intake.

Excipients

The excipients in Oxybee are purified water, sucrose, glycerol 85%, citric acid monohydrate, anise oil and eucalyptus oil. All of the excipients are compounds found in normal foodstuffs, and not of human health concern at the concentrations presented.

User safety

The applicant has presented a user safety risk assessment which has been conducted largely in accordance with CVMP Guideline (EMEA/CVMP/543/03-Rev.1).

The main potential routes of accidental contact with the product have been considered and are concluded to be dermal and ocular. Users are expected to be beekeepers, who might be exposed when preparing/mixing the sugar powder with the solution, or when administering the final dispersion. In order to minimise the possibility of oral ingestion by children, the product is presented in child-resistant packaging.

Oxybee has a very low pH of 1, and oxalic acid is irritating to skin, eyes and the respiratory tract. It is considered likely that adverse events will occur as a result of dermal or ocular contact with this product, even at small quantities. Therefore, warning sentences should be in place, and the user should wear protective equipment (consisting of protective clothing, acid-proof gloves and safety glasses) while handling the product.

While oxalic acid is a weak reproductive toxicant, it is not expected that the user will be exposed in such a way that embryotoxic or reprotoxic effects will occur after dermal exposure. Therefore, no warnings regarding reproductive toxicity are considered necessary.

As a result of the user safety assessment the following advice /warnings for the user are considered appropriate:

- This veterinary medicinal product is highly acidic and could have irritating and corrosive effects on the skin, eyes and mucous membranes.
  Avoid oral exposure, including hand-to-mouth contact. Avoid direct skin and eye contact, as well as hand-to-eye contact.
- Personal protective equipment consisting of protective clothing, acid-proof gloves and safety glasses should be worn.
  Wash hands and exposed skin with soap and plenty of water immediately. Do not eat, drink or smoke whilst handling and applying the veterinary medicinal product.
  Remove contaminated clothing immediately. Used dosing devices and empty containers should be disposed of immediately and appropriately.

- In case of accidental ingestion, clean mouth with water and drink water or milk, but do not induce vomiting. In case of eye contact, immediately rinse the eye thoroughly with water (remove contact lenses first). Seek medical advice immediately and show the package leaflet or the label to the physician.

Environmental risk assessment

A Phase I environmental risk assessment (ERA) was provided in line with VICH guideline GL6 on environmental impact assessment (EIAS) for veterinary medicinal products – phase I (CVMP/VICH/592/98-FINAL). The environmental risk assessment can stop in Phase I as the active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment.

Oxybee is not expected to pose a risk for the environment, when used according to the SPC. The SPC includes the following risk mitigation measures for Oxybee:
"*Any unused veterinary medicinal product or waste material derived from such veterinary medicinal product should be disposed of in accordance with local requirements. Oxybee should not enter water courses as this may be dangerous for fish and other aquatic organisms*."

Residues documentation

MRLs

The active substance in Oxybee is an allowed substance 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	Not applicable	Bees	No MRL required	Not applicable	No entry	Anti-infectious agent

The excipients listed in section 6.1 of the SPC are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

Residue studies

Pharmacokinetics

No pharmacokinetic study has been performed with the proposed product. However, from literature it can be concluded that oxalic acid is absorbed, distributed and metabolised after oral and topical administration to bees. The major residue to be expected in honey will be oxalic acid.

Depletion of residues

No residue depletion studies performed with the proposed product have been provided. However, several literature studies were provided with the MRL application for oxalic acid (OA). These studies were conducted with concentrations of OA comparable to those used for the proposed product.

Investigations were mainly conducted as field trials and were not performed under GLP conditions. Nevertheless, the investigations covered a realistic spectrum of treatment conditions and gave a useful overview of the possible residues of OA in honey (sum of free acid plus oxalates) as they would occur under conditions of good beekeeping practice. Taking these data into account, it is concluded that elevated residues/residue levels outside the range of natural variability are not expected when the product is used according to good beekeeping practice for the treatment of varroosis of honey bees in brood-free colonies. This conclusion would apply even if the product were administered during spring or summer. However, according to the product information the proposed product is to be used in the brood free period, (5–6 ml per seam, corresponding to approximately 0.2 g OAD or 0.15 g OA), when harvesting of honey is already completed. No significant residues of OA are therefore expected in honey.

Withdrawal periods

The natural content of OA in honey ranges from 1–800 mg/kg. Published studies conducted with concentrations of OA comparable to those used for the proposed product indicate that no elevated residue levels and/or residues in honey outside the natural variability are expected when OA is used for the treatment of varroosis of honey bees in brood-free colonies in accordance with the product information. In light of this, and considering the "No MRL required" status of OA, a withdrawal period of 0 days for honey is justified for use of the product in line with the recommendations in the product information ("not to be used during honey flow").

Overall conclusions on the safety and residues documentation

Pharmacology:

Oxalic acid is a ubiquitous substance in mammalian tissues and plants and both the active substance and the excipients are compounds found in normal foodstuffs. In mammals oxalic acid is an end product of metabolism of natural components of some amino acids, glycolate and ascorbic acid.

Endogenous sources constitute approximately 30–70% of the oxalic acid excreted daily via urine in humans. Faecal excretion mainly results from dietary oxalic acid intake, the major source being plant derived foods. Absorption is low (2–5% in normal humans) and strongly influenced by pH, presence of calcium and oxalate-degrading bacteria in the gut.

Toxicology:

Oxalic acid is moderately to highly toxic after a single exposure via the oral route. The symptoms of acute toxicity in humans may be divided into those caused by a local corrosive action and those resulting from absorption and excretion of the soluble oxalate. Symptoms may include severe gastrointestinal pain and distress, bleaching of mucous membranes, cardiovascular collapse, neuro-muscular symptoms, kidney damage and death.

The main target organ after repeated exposure is the kidney. No NOEL is established for oral toxicity. Repeated dose toxicity studies in rats were performed, oral doses of 2 to 5 g/kg bw/day for 70 days resulted in moderate nephrotoxicity, while subcutaneous injections of 25 to 75 mg/kg bw/day for up to three weeks caused mild nephrotoxicity.

Oxalic acid has been shown to be a weak reproductive toxicant. At significant doses embryotoxic and developmental effects may occur, but no NOEL could be established.

The product has a very low pH. It is corrosive to skin, and irritating to eyes and the gastrointestinal tract.

User safety:

A user safety assessment in line with the relevant guidance document has been presented. User exposure of skin or eyes to small amounts (droplets) or by oral ingestion of Oxybee may cause serious harm. Therefore, warning sentences and risk mitigation measures are required.

Based on the assessment presented the product does not pose an unacceptable risk to the user when used in accordance with the SPC. The appropriate warnings for the user have been included in the product literature.

Environmental safety:

An appropriate environmental risk assessment was provided. The product is not expected to pose a risk for the environment when used according to the SPC.

Residues:

The applicant presented published studies that evaluated the risk of residues of OA in honey at concentrations comparable to those used for the proposed product. The natural content of OA in honey ranges from 1–800 mg/kg. The literature data indicated that no elevated residue levels and/or residues outside the natural variability are expected when oxalic acid is used for the treatment of varroosis of honey bees in brood-free colonies in accordance with the product information.
A withdrawal period of zero days for honey is justified for use of the product in line with the recommendations in the product information.

Part 4 – Efficacy

Oxybee is indicated for the single treatment of varroosis (*Varroa destructor)* of honey bees (*Apis mellifera)* in brood-free colonies. The proposed dose of 5–6 ml of the mixed/ready-to-use bee-hive dispersion per occupied seam (the gap between two brood frames) is to be trickled onto the bees using a suitable dosing device (e.g. disposable syringe).

Pharmacodynamics

Oxalic acid (OA) is a well-established miticide, used for control of *Varroa destructor* infestations in honey bee colonies. At present little is known about the mechanisms of action of oxalic acid dihydrate (OAD) in the target species, honey bees, or the target pathogen, Varroa mites. However, the pharmacodynamic action of OA is mainly attributed to its low pH (1.3).

Honey bees:

In honey bees treatment with OAD appears to cause damage to bee organs, mainly the midgut and rectum, and may alter bee behaviour and longevity (see target animal safety).

Varroa mites:

The mechanism of action of OA against *V. destructor* is not completely understood. It is assumed that oxalic acid acts via direct contact with mites or by ingestion of haemolymph containing oxalic acid. The acaricidal effect of OA is mainly attributed to its low pH (1.3). Oxalic acid is believed to immobilize calcium, thus impairing the calcium-potassium ratio in mite tissues.

The applicant provided published laboratory studies describing the effect of OA on mites:

In a study by Aliano *et al*. (2006), glass vials were coated with OA and mites collected from adult worker bees were inserted into the vials and incubated for 24 hrs. An LD_{50} of 0.26 µg OA/cm^2 mite surface was found in this study. The data suggest that direct contact is necessary for OA to exert its

lethal effects.

In a study by Milani (2001), the calculated LD_{50} of mites ranged from 0.68 to 1.90 µg OA/cm^2. Addition of glucose or glycerol to the OA was shown to increase mortality of mites, this was likely being caused by the formation of small droplets (increased hygroscopicity). The effect appeared to be higher against mites collected from adult bees compared to mites collected from brood.

In regard to the other ingredients in the formulation, the addition of glucose in combination with glycerol was developed in order to increase the effectiveness.

Development of resistance

Solutions of OAD are widely used for the control of *V. destructor* in Europe and other regions. To date, there have been no reports of resistance of mites against OA. Oxybee is therefore unlikely to pose a risk in regard to the development of resistance to *Varroa destructor.*

Pharmacokinetics

Honey bees:

Limited information is available on the pharmacokinetics of the active substance, OAD, in honey bees.

In one study in bees, after trickling of sugar solution containing ^{14}C-labeled OA, a maximum contamination of adult bees was reached in the first four days, which decreased 10-fold after one week, and 60-fold two weeks after treatment. Radioactive CO_2 was detected in samples of hive air, indicating metabolism of OA by the bees.

In another study, following topical administration of an aqueous solution of OA to honey bees, the oxalic concentration in internal organs of bees increased and a maximum appeared within 4–6 hours after treatment. It was concluded that following topical application, OA penetrates bee keratin and reaches internal structures, which can explain its toxic effect on bees.

In another study, oral administration of a 2% solution of OA in aqueous 50% sucrose solution gave the highest values for OA in haemolymph, 12 hours after trickling of ^{14}C-labelled OAD to bees. In the haemolymph, ^{14}C-activity decreased to a level of 2 µg/g within 71 hours and was no longer detectable in the intestines 22 and 31 days post application.

In conclusion, little is known about the pharmacokinetics of the active substance, oxalic acid dihydrate, in honey bees. It does however appear that the substance is at least partially absorbed and metabolised after topical and oral application.

Distribution within the hive:

Within the hive, after trickling bees with 3.5% OAD in sugar solution, it could be shown that direct bee-to-bee contact, but not trophallaxis is required for distribution of OA within the hive (Alliano and Ellis, 2008).

OA does not penetrate capped brood cells, and therefore doesn't reach mites within the capped cells. The use of Oxybee should therefore be restricted to a time when the colony is broodless.

Dose determination

No dose determination or dose finding studies were performed, but the applicant justified the proposed dose of 5-6 ml dispersion per occupied seam with references to a wide range of recently published literature.

Efficacy of OAD was evaluated using different concentrations of OAD (between 2 and 6%) with or without adding different amounts of sugar, and using different methods of administration as well as various climate conditions. Trickling a solution containing 3–3.5% OAD was reported to have acceptable tolerance and efficacy levels of 90–95% against *V. destructor* in cool climates when brood was not present, and therefore the applicant proposed this dose to be used for the dose confirmation studies.

Target animal tolerance

Target animal tolerance was demonstrated by the results of two field studies conducted in 2012 and 2013 in Germany and in 2015 and 2016 in Hungary. Both studies were performed under GCP as controlled, blinded, randomised critical tests, and tested the recommended dose as well as an overdose (1.5 x RTD). For study design, see "field trials" below.

The results of the field studies showed that Oxybee was well tolerated at the recommended single dose of 5–6 ml per occupied seam, applied once during winter time in the broodless period. Overdoses of 20% and 50% in volume resulted in higher bee mortality and lower colony size in spring, although colony survival and brood area in spring were not affected. No differences were noticed on winter survival of colonies between groups treated with the recommended dose and placebo groups. An appropriate warning against the use of higher doses than recommended is therefore included in the SPC.

Overall, the performance of colonies treated with Oxybee at the recommended dose was not significantly different from the placebo colonies and the results thus support the safety of the product.

In addition, results of toxicity tests and published literature were used to support the tolerance of oxalic acid dihydrate in honey bees:

Based on the results of two of the three toxicity studies reported, the LD_{50} of OAD by topical application in bees is approximately in the range of 530–550 µg/bee, which is about 2–3 times higher than the average recommended dose/bee for trickling treatment with 3.5% OAD solutions. Treatment with OAD appears to cause damage to bee organs, mainly the midgut and rectum, and may alter bee behaviour and longevity.

A study by Prandin *et al.* (2001) showed that in home-made solutions of OA and sucrose (4.2%/50%) stored for a prolonged period (16 months) at room temperature, significant amounts of hydroxymethylfurfural (HMF) may develop, which are toxic to bees, causing ulceration of the digestive system. Levels of over 150 mg/l HMF in sugar solution may cause increased mortality (Jachimowicz and El Sherbiny, 1975). However, it is not likely that HMF will cause a problem in the toxicity and safety of Oxybee, because the OA/sucrose dispersion is to be freshly prepared prior to use.

Worker bees

In a study published in 2012 by Schneider, sublethal effects of the treatment with OA on behaviour and longevity of worker bees were investigated. 150 newly emerged worker bees were treated with 3.5% OA solution by topical application (dosage 175 µg/bee) and introduced into a colony. Behavioural observations were carried out and the longevity of every worker bee was recorded. Results were compared with the findings in a control group treated with water. Regarding behaviour, treated bees showed statistically significant increased self-grooming, higher tendency to inactivity and reduced nursing behaviour. Median survival time for treated bees was 3 days shorter (13 days) than that of control bees (16 days).

Aliano *et al*. (2006) demonstrated low acute toxicity of OAD to honey bees and high acute toxicity to mites. In this study, bees dosed 2000, 1600, 800, or 400 µg of OA per bee died after 72 h. Bees

subjected to 100 µg of OA per bee survived more than 72 h, and had mortality similar to the control treatment.

Hive development

Literature data indicate that a single treatment with OAD solution was in most cases well tolerated by the bees in concentrations up to 4.6% (approximately 3 µl per bee), although the treated colonies showed a significant weakening during winter compared with controls. Studies with a higher concentration of 5% described increased bee mortality in autumn, bad overwintering (reduced colony strength) and impaired spring development of treated colonies.

Repeated autumn or summer treatments by trickling were poorly tolerated by bees in the Central European regions. However, differences in the tolerance were noted in different climatic areas. Whereas in Southern Europe OAD concentrations up to 7% seem to be tolerated even if bee colonies were treated three times, tolerated treatment of bees in Northern Europe is only described up to 6% OAD, but with reduced colony size after overwintering.

Brood

A toxicological study where in-hive brood treatment was carried out showed that 3% OA in sucrose solution did not significantly affect bee brood or adult bees. When sucrose solutions with different concentrations of OA (3%, 3.7% or 4.5%) were fed to worker bees *ad libitum*, only the highest concentrated solution (4.5% OA / 31.3% sucrose) caused death of bees, whereas consumption of the lower concentrations led only to epithelial cell death in the midgut. Apart from the midgut, it was demonstrated that other internal organs in larvae such as the salivary glands show varying degrees of morphological cellular alteration and increased levels of cell death after treatment with OA.

Queens

No specific data were provided regarding reproductive safety. It is considered unlikely that a yearly repeated treatment would increase toxicity in queens to the extent that specific damage to the reproductive system can occur. The applicant has provided a justification, indicating the safety data (for a single application) covers a large part of the life time of a queen in normal apiary practice. Toxicity of repeated yearly application of OA has not been reported in literature. Although the safety of the product over the lifetime of queen bees has not been extensively evaluated, the applicant has sufficiently justified the safety for the queen.

Conclusions on target animal tolerance:

Although the reported publications demonstrating target animal tolerance are of variable quality, and a range of concentrations of oxalic acid and sucrose were used, the application of oxalic acid by trickling resulted generally in a dose-dependent manner in increased bee mortality, a reduction in colony size in winter, compared to untreated control groups, and the colony growth in spring was slightly (but often not significantly) lower in treated groups.

In the field studies conducted by the applicant, bee mortality after treatment (up to 4 weeks after treatment) was higher in the Oxybee group compared to placebo. However, this effect was dose-related, and a statistically significant increase in bee mortality was mostly seen in colonies that received on average a higher total dose per colony (Hungarian trials) or overdose (German trials). No significant difference between the groups was noted in regard to flight activity of the bees, and the colony strength (indicated by colony survival, and colony size) and reproduction (indicated by the size of the brood area, and presence of the queen) in the spring following treatment. The CVMP therefore concluded that Oxybee at the recommended dose is generally well tolerated, when used according to the SPC recommendations.

The CVMP noted that no specific data on reproductive safety (queen and brood) were provided, and that OAD treatment affects the general condition of bee colonies. However, Oxybee treatment is normally applied during November/December, when brood is absent, and the in-hive tasks performance and foraging are reduced. However, based on the (literature) data provided, effects other than direct (caustic) damage to epithelial surfaces of the gut system of bees have not been observed. Moreover, reproductive safety is supported by numerous studies reporting normal development in spring after autumn treatment. It is therefore considered unlikely that a yearly repeated treatment would increase toxicity in queens to the extent that specific damage to the reproductive system can occur.

From the clinical trials it appears that dosing mistakes leading to overdoses are easily made. Since bees may be more or less dispersed on the combs depending on various circumstances, the dosing per occupied seam may not be sufficiently accurate. In order to reduce the risk of overdosing, the applicant provided a maximum target dose per colony as an additional safety precaution.

Clinical studies

The applicant provided two good quality pivotal studies, conducted in Germany and in Hungary in 2013/14, which evaluated the efficacy and safety of Oxybee in bees naturally infested with *Varroa destructor*. These studies were combined dose-confirmation studies, safety studies and field trials, and were performed under GCP as controlled, blinded, randomised critical tests. The recommended dose, as well as an overdose (1.5 x RTD), were tested in the studies.

The study design is outlined in the tables below.

Field study to evaluate the efficacy and safety of the product "Dany's Bienenwohl" containing oxalic acid in bees naturally infested with *Varroa destructor*.	
Objectives	Evaluation of efficacy and safety of the VMP in the field. Dose confirmation.
Study sites	Two sites in Germany (different regions, Site A: Bavaria, site B: Lower Saxony) in apiaries with 100-150 hives.
Hive types	Site A: "German Normal Size 1.5" hives, Site B: Dadant hives.
Study design	Randomised, blinded, placebo and active-controlled, superiority and non-inferiority. Colonies were evenly distributed based on their mite infestation (mite fall counts). Treatments were performed on D0, follow-up treatment (coumaphos) on D11 (site A) or D14 (site B). Dead mite counts were performed daily from D1 to D21.
Compliance with regulatory guidelines	The studies were designed largely in accordance with the CVMP Guideline on veterinary medicinal products controlling Varroa destructor parasitosis in bees (EMA/CVMP/EWP/459883/2008), and VICH GL 9 (Good Clinical Practice).
Interventions: Test product (T1)	Oxybee, 35 g/kg oxalic acid dihydrate Site A (intended dose): 6 ml per occupied seam by trickling, once at Day 0 Site B (unintended <50% overdose): 9 ml per occupied seam by trickling, once at Day 0.
Control product (T2)	Ecoxal, 33 g/kg oxalic acid dihydrate in sugar syrup, 4 ml per occupied seam, by trickling, once at Day 0
Placebo (T3)	NaCl 0.9%, 6 ml per occupied seam, by trickling, once at Day 0
Follow-up treatment	32 mg/ml Coumaphos at D11 or D14,

(all)	50 ml/colony for strong and 25 ml/colony for small colonies, by trickling
Animals	15 colonies per test group. Site A: Apis mellifera carnica, site B: Apis mellifera hybrid (Buckfast). Queens: Site A: ≤1 year, site B: <1 year.
Eligibility criteria	Mite infestation level of 500-13 000 mites per colony. No treatment against Varroa in the 28 days prior to Day -7.
Efficacy parameters	*Primary endpoint:* Mite reduction per colony (in %) *Secondary endpoints*: Mite reduction (in %) in Oxybee group (T1) compared to positive control (T2) Mite reduction (in %) in Oxybee group (T1) compared to placebo group (T3).
Statistical method (efficacy)	*Primary endpoint:* Non inferiority test using a non-inferiority margin (Δ) of 10% and a significance level of 5%. *Secondary endpoint*: - T1 non-inferior to T2: Non inferiority test using a Δ of 15%. - T1 superior to T3: T-test for independent samples.
Safety parameters	- Mean mortality (in %) of dead bees/test group (Day -7, -3/4, 0, 1, 14) - Colony strength (Liebefeld) (Bee numbers: before Day 0, Day 14, next spring, open/closed brood: next spring) - Flight activity (mean number bees in flight/group)(before Day0, day 14, next spring) - Presence of queen (before Day 0, Day 14, next spring).
Statistical method (safety)	Descriptive statistics, groups were compared using Wilcoxon tests or Fishers exact test (queen presence).
Results efficacy	ITT and PP populations were identical. *Primary endpoint*: - Mite reduction for Oxybee (T1) was 97.98% overall (97.2% and 98.86% for A and B sites, respectively). *Secondary endpoints:* - Mite reduction in T2 (positive control) was 91.39% (86.56% and 96.91% for A and B sites, resp.), non-inferiority of T1 to T2 was confirmed; and T1 was even found superior to T2 (p=0.0007). - Mite reduction in placebo controls (T3) was 17.43% (4.29% and 32.45% for A and B sites, resp.), superiority of T1 to T3 was confirmed (p<0.0001).
Results safety	ITT and PP populations were identical (refer to discussion). Bee mortality was similar between groups prior to treatment. Bee mortality after treatment (day 1-14) was higher in the Oxybee group compared to placebo (p=0.019). However, for site A (intended dose) mortality was only numerically higher in the Oxybee group compared to both control groups, while for site B (50% overdose), bee mortality was significantly higher in Oxybee-treated bees compared to placebo, and numerically higher compared to the positive control. Colony strength in the following spring was not significantly different between the groups. Colony survival was 10 (out of 15) in the Oxybee group compared to 11 (out of 15) in the control groups. Colony size at site B (overdose) in the Oxybee group was numerically smaller (approximately 3488 bees/colony) compared to positive (6200) and negative (6680) controls. The size of the brood area in the spring following treatment was similar in all groups. T1

	performed (significantly) better at site A, but numerically less at site B, compared to the placebo colonies. Flight activity after treatment was similar for T1 and T3 (but higher in T1 compared to T2). Queens were present in each surviving colony in spring.
Discussion (efficacy)	The trial was performed in northern and southern Germany, i.e. north-central European regions. The design, conduct and statistical analysis of the study were largely in accordance with the CVMP's Varroa Guideline ((EMA/CVMP/EWP/459883/2008). The use of coumaphos as follow-up treatment for this critical test was justified by the applicant, no resistance was observed in the treated colonies. Site A (intended dose): A significant treatment effect of 97.2% mite reduction was found, which is in accordance with the Varroa guideline requirement of >90% efficacy for non-synthetic substances. Secondary efficacy criteria (non-inferiority to positive control, superiority to placebo) were also met. The conclusion by the applicant that the treatment was effective at this site can therefore be accepted. Site B (overdose): A significant, unintended overdose (>50%) was applied to the T1 colonies, rendering results from this site invalid with respect to efficacy of the proposed dose.
Discussion (safety)	With respect to the parameters colony strengths incl. brood development in the following spring, flight activity and queen mortality, no statistically significant differences were noted between the Oxybee test group and two control groups. At the intended dose (site A), bee mortality was numerically higher in the Oxybee group compared to T2 and T3, but this difference was statistically not significant. Following treatment with a >50%-overdose (site B), bee mortality was significantly increased which is a well-known effect of higher concentrations/doses of OA from literature, and as such the effect is not surprising. Spring development of treated colonies at site B was numerically (but not significantly) reduced. However, winter survival of colonies was not different between treated and placebo groups. Overall, the performance of treated colonies was not significantly different from the placebo colonies, and the results thus support the safety of the product. An appropriate warning against the use of overdoses is included in the SPC.

Study to evaluate the efficacy and safety of Bienenwohl in the control of the natural infestation with *Varroa destructor* as winter treatment in brood-free colonies.	
Objectives	Evaluation of efficacy and safety of the VMP in the field. Dose confirmation.
Study sites	Hungary, two apiaries with 72 and 180 hives, respectively.
Hive types	Large and small Boczonádi hives were used. 25 colonies enrolled at Apiary A1 and 57 at A2.
Study design	Randomised, blinded, placebo, superiority study, winter treatment (December 2015). Colonies were classified based on their mite infestation level (mite fall counts) into low or higher infested colonies, and treated with a flexible dose (batch 1, low and higher infested colonies) or a single (higher) dose (batch 2, higher infested colonies only). Treatments were performed on D0, follow-up treatment on D20 (batch 1) or D14 (batch 2). Mite counts were performed three times weekly until 7 days after follow-up treatment. 51 colonies were enrolled with low mite counts (0.5-1 mite/day), 31 colonies with higher

	mite counts (1-6 mite/day).
Compliance with regulatory guidelines	Same as in the German field study.
Interventions: Test product (T1)	Oxybee, 35 g/kg oxalic acid dihydrate Batch 1 (41 colonies, with low and higher level of infestation); 30-50 ml per colony (dose depending on colony size, 3-10 combs), by trickling, once at Day 0. Batch 2 (41 hives with higher level of infestation, only); 30-42 ml/colony (5-6 ml per occupied seam, by trickling, once at Day 0, i.e. (5-7 combs).
Placebo (T2)	No treatment.
Follow-up treatment (all)	32 mg/ml Coumaphos at D20 or D14 50 ml/colony for strong and 25 ml/colony for small colonies, by trickling.
Animals	41 colonies per test group, A. mellifera carnica, Queens: 1-3 years.
Eligibility criteria	Infestation level 300-500 mites per colony, brood-free at D0, normal colonies, Exclusion: treated against Varroa in last two months prior to D0.
Efficacy parameters	*Primary endpoint*: Mite reduction per colony (in %) *Secondary endpoint*: Mite reduction (in %) in Oxybee group (T1) compared to placebo (T2).
Statistical method (efficacy)	*Primary endpoint*: Non inferiority test using a non-inferiority margin (Δ) of 10%, and a significance level of 5%. *Secondary endpoint*: T1 superior to T2: T-test for independent samples.
Safety parameters	Same as in the German field study (bee mortality, colony strength, flight activity, presence of queen).
Statistical method (safety)	Same as in the German field study.
Results efficacy	Efficacy was calculated based on PP populations (1 colony (from T1, A1) was excluded from the ITT population). *Primary endpoint*: - Mite reduction in T1 (Oxybee) was 94.9% (for ITT and PP) and 9.1% for the placebo group (T2). For the first batch enrolled (low mite count) this was 94.4% (94.3% for PP) in T1 and 10.7% in T2. For the second batch (higher mite count) mite reduction was 95.8% in T1 and 6.6% in T2. *Secondary endpoint*: - Superiority of mite reduction in the Oxybee treatment group over placebo was supported (P<0.0001), for both enrolment batches, and for PP and ITT populations.
Results safety	Bee mortality in the Oxybee group (T1) was statistically significantly higher on D4/5, D16 and D2/3 after follow-up treatment. This effect was mostly due to the colonies in the first enrolment batch, which received on average a higher total dose/colony. Here the relative number of dead bees over the period between treatment and follow up treatment was significantly higher (3.1 vs. 1.9 dead bees/1000 bees; p=0.002). For the second batch, absolute numbers of dead bees were not statistically different between T1 and T2

	(placebo). In spring, no differences were noted between T1 (both enrolment batches) and T2 in regard to the average colony size (T1: 2290, T2: 2280, p=0.982) or brood area (p=0.963 for open brood, p=0.192 for closed brood), also colony loss was not significantly different between groups (5 colonies out of 41 in T1, and 6 out of 41 in T2) and similar to the average EU wide colony losses in the winter of 2014/2015 (COLOSS).
Discussion (efficacy)	The test was performed in Hungary near lake Balaton, which can be considered representative for a Mediterranean climate. The design, conduct and statistical analysis of the study were largely in accordance with the CVMP's Varroa guideline (EMA/CVMP/EWP/459883/2008). It is however a deficiency of this study that no positive control treatment was included. The use of coumaphos as follow-up treatment for this critical test was justified by the applicant; no resistance was observed in the treated colonies. Pre-treatment mite counts were comparable for both treatment groups, albeit somewhat low in the first batch (mite fall corresponded to an infestation of 125-250 mites/colony, which is actually below the inclusion criteria). Mite counts were only performed three times weekly, this is not fully in accordance with recommendations; however, this was appropriately justified by the applicant and is not expected to have affected the outcome. A significant treatment effect of 94.3% mite reduction was found in the first batch, and 95.8% in the second batch, which are both in accordance with the CVMP's Varroa guideline requirement of >90% efficacy for non-synthetic substances. The secondary efficacy criterion (superiority of mite reduction in Oxybee versus placebo) was also met. It can therefore be concluded that the treatment was effective at reducing V. destructor infestation in brood-free colonies in winter.
Discussion (safety)	The number of dead bees after Oxybee treatment was significantly higher compared to the placebo group. However, the development of colonies in spring was not significantly (or numerically) different in treated and placebo groups. Other safety parameters were not significantly different between treated and placebo groups. The safety profile of Oxybee is therefore similar to what can be expected for treatment with OA by trickling.

Both studies were designed, performed and evaluated largely in accordance with the CVMP Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA/CVMP/EWP/459883/2008) and in accordance with GCP requirements.

The field study performed in Germany, is considered the pivotal study, as it included a positive control group, whereas the study in Hungary did not. Also, in the Hungarian study, mite counts were not performed at the frequency suggested in the guideline, this is however not considered to have affected the study outcome.

Both studies investigated the use of the product as recommended in the SPC with respect to colony phase (brood-free), outside temperature (>3 °C) and applied dose. Based on the locations in both field trials, it is considered that European climates are generally covered by the field studies.

Conclusions:

Efficacy: The efficacy levels in regard to mite mortality achieved in both studies the required threshold defined in the CVMP's Varroa guideline (more than 90% efficacy for non-synthetic substances, primary endpoint), and also showed superiority to placebo (secondary endpoint). Therefore, it can be concluded that a single dose of 5-6 ml Oxybee per seam occupied by bees,

applied once is effective as treatment for varroosis in brood-free colonies.

Tolerance: The product has an acceptable safety profile that is comparable to other trickling treatments based on OAD as the active substance.

Bee mortality after treatment (up to 4 weeks after treatment) was higher in the Oxybee group compared to placebo. However, this effect was dose-related, and a statistically significant increase in bee mortality was mostly seen in colonies that received on average a higher total dose per colony (Hungarian trials) or an (unintended) 50% overdose (German trials). This clearly demonstrates that doses must be carefully calculated and distributed over the hive. An appropriate warning against the use of higher than recommended doses is included in the SPC.

However, although the effects resulted in a temporary increase in bee mortality, this did not significantly affect the development of the hives in the long term, as shown by the development of the colonies in spring. No significant difference between the groups was noted in regard to flight activity of the bees, and the colony strength (indicated by colony survival, and colony size) and reproduction (indicated by the size of the brood area, and presence of the queen) in the spring following treatment.

Overall conclusion on the efficacy

Pharmacodynamics:

Oxalic acid dihydrate is a substance with killing activity against *Varroa destructor* mites; its mechanism of action is not completely known, but direct contact between oxalic acid and the mite is required. The pharmacodynamic action of OA is mainly attributed to its low pH (1.3).

Resistance:

So far, resistance of Varroa mites to OA has not been reported in the literature. Oxybee is therefore unlikely to pose a risk in regard to the development of resistance to *Varroa destructor.*

Pharmacokinetics:

Pharmacokinetics studies have shown that, in bees, OA was absorbed, distributed and metabolised after oral and topical administration. OA penetrates bee keratin and reaches internal structures, which can explain the toxic effect on honey bees. After topical administration, peak levels of OA in internal organs of bees are generally within 4-6 hours.

Dose determination:

Dose justification was based on published clinical data on trickling treatments with oxalic acid solutions. The dose of Oxybee was supported by two dose confirmation studies performed under clinical field conditions.

Tolerance:

Oxybee was in general well-tolerated in two clinical field studies at the recommended single dose of 5-6 ml per seam occupied by bees, applied once during winter time in the broodless period. Bee mortality after treatment (up to 4 weeks after treatment) was higher in the Oxybee group compared to placebo. However, this effect is dose-related, and did not significantly affect the development of the hives in long term, as shown by the development of the colonies in spring. An appropriate warning is included in the SPC and product literature.

Efficacy:
The results from two clinical field trials conducted in Hungary and Germany showed that the product is effective as autumn/winter treatment for varroosis in brood-free colonies at the proposed single

dose of 5–6 ml per seam occupied by honey bees (*Apis mellifera*).

Part 5 – Benefit-risk assessment

Introduction

Oxybee bee-hive dispersion contains oxalic acid dihydrate as the active substance, and is available in two packs sizes, a bottle of 500 ml containing 375 g OAD plus 1 sachet, and a bottle of 1000 ml containing 750 g OAD plus 2 sachets. The proposed withdrawal period for honey is zero days.

The applicant applied for the following indication: For the treatment of varroosis (*Varroa destructor)* of honey bees (*Apis mellifera)* in brood-free colonies. The final/ready-to-use dispersion of Oxybee is prepared by the bee keeper by mixing 1–2 sachet(s) of sucrose powder into a bottle (500 or 1000 ml) containing the active substance (17.5 g OAD in 375 g of solution, and 35 g OAD in 750 g of solution, respectively); the final bee-hive dispersion is for in-hive use. The proposed dose of 5–6 ml of the mixed/ready-to-use bee-hive dispersion is to be trickled onto the bees using a suitable dosing device (e.g. disposable syringe).

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

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

Benefit assessment

Direct therapeutic benefit

The benefit of Oxybee is its efficacy in the treatment of varroosis due to *Varroa destructor* in honey bee colonies.

Efficacy of the product in the treatment of varroosis due to *Varroa destructor* was investigated in two well-conducted controlled clinical trials, which demonstrated that the product is efficacious in the treatment of varroosis at the proposed dose (single dose of 5–6 ml Oxybee per occupied seam). The clinical trials were conducted in two different geographical locations during the winter period, i.e. at a time when there is no brood or honey expected to be in the hive, and efficacy results above 90% were obtained.

Additional benefits

Oxybee increases the range of available treatment possibilities for varroosis in honey bees, which is an indication classified as MUMS/limited market.

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:

Administration of Oxybee in accordance with SPC recommendations is generally well tolerated. The use of the product is associated with dose-dependent reduced tolerance in bees in terms of bee mortality and colony development, and warnings have been included to avoid overdosing. Adverse effects of oxalic acid on individual worker bees cannot be excluded.

Risk for the user:

User safety risks have been identified in relation to exposure to the product. The main risks for the user are associated with the irritating properties of oxalic acid. 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. Standard advice on waste disposal and a warning regarding aquatic organisms are included in the SPC.

Risk for the consumer:

The product is not intended to be used during honey flow. The withdrawal period established 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, user, environment and consumer, and to provide advice on how to prevent or reduce these risks.

Conditions or restrictions regarding supply and use:

The CVMP considered the request and agreed that provided that the assessment of the application would confirm that the product complies with all the criteria prescribed in Article 2 of Commission Directive 2006/130/EC, the application for exemption from the requirement for the veterinary medicinal product to be dispensed only against veterinary prescription could be acceptable.

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 or to the person administering the product. The veterinary medicinal product should not enter water courses as this may be dangerous for fish and other aquatic organisms;

- The summary of product characteristics 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 subject to the following storage conditions:
  "Keep the bottle and the sachet in the outer carton in order to protect from light.
  After mixing the bee-hive dispersion: Store in a refrigerator (2 °C – 8 °C).
  Store away from food.";
- 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 CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned medicinal product.

The product has been shown to be efficacious for the treatment of varroosis in honey bee colonies in the brood-free period, and the CVMP agreed with the indication as proposed by the applicant: "For the treatment of varroosis (*Varroa destructor)* of honey bees (*Apis mellifera)* in brood-free colonies".

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 on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) considers that the application for Oxybee 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).