10 October 2013
EMA/675222/2013
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for Vectra 3D (EMEA/V/C/002555/0000)

International non-proprietary name: Dinotefuran / pyriproxyfen / permethrin

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

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

Introduction

On 28 September 2011, the applicant, Ceva Santé Animale, submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for Vectra 3D, through the centralised procedure falling within Article 3(2)a of Regulation (EC) No. 726/2004 (new active substance).

The eligibility to the centralised procedure was agreed upon by the CVMP at their meeting of 3-5 March 2011 as Vectra 3D contains a new combination of three active substances (dinotefuran, permethrin and pyriproxyfen) which was not authorised in the community on the date of entry into force of the Regulation. In addition, dinotefuran is a new active substance. The rapporteur appointed was C. Ibrahim and co-rapporteur K. Törneke.

Vectra 3D contains three active substances: dinotefuran, permethrin and pyriproxyfen. Vectra 3D is presented in a range of five different pack sizes (volumes) of single dose spot-on applicators for topical spot-on administration to the target species, dogs. The product is intended for the treatment and prevention of flea infestation (*Ctenocephalides felis* and *Ctenocephalides canis*); treatment and prevention of tick infestation (*Rhipicephalus sanguineus*, *Dermacentor reticulatus* and *Ixodes ricinus*); and prevention of bites from sand flies (*Phlebotomus perniciosus*), mosquitoes (*Culex pipiens* and *Aedes aegypti*) and stable flies (*Stomoxys calcitrans*). The product has also persistent insecticidal activity against mosquitoes (*Aedes aegypti*) and stable flies (*Stomoxys calcitrans*).

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

The CVMP adopted an opinion and CVMP assessment report on 10 October 2013.

On 4 December 2013, the European Commission adopted a Commission Decision for this application.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The pharmacovigilance system as described by the applicant fulfils the requirements of Directive 2001/82/EC as amended and provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

A declaration of the compliance of the manufacture of all the active substances with EU GMP requirements for starting materials has been provided from the Qualified Person of the batch release site.

The finished product is manufactured and packaged in the USA and shipped to Europe.

Batch testing and batch release for the EU will be carried out by Ceva Santé Animale, 10 avenue de la Ballastière, 33500 Libourne, France.

Both sites comply with GMP requirements; corresponding GMP certificates have been provided.

Scientific advice

The applicant received scientific advice from the CVMP on 14 January 2010. The scientific advice pertained to clinical aspects of the dossier and has been followed by the applicant.

Overall conclusions on administrative particulars

The pharmacovigilance system as described by the applicant fulfils the requirements.

The GMP certification of the manufacturing sites was considered in line with legal requirements. No inspections were considered necessary.

Part 2 - Quality

Composition

The finished product is a non-aqueous solution for spot-on use and contains the active ingredients dinotefuran, permethrin and pyriproxyfen in a mixture of two non-aqueous solvents, N-methylpyrrolidone and N-octyl-pyrrolidone. The product is presented in single-dose spot-on applicators. There are five different strengths (applicator sizes) for use in dogs of different bodyweights: 0.8 ml applicators for dogs of 1.5 to 4 kg (each containing 44 mg dinotefuran / 3.9 mg pyriproxyfen / 317 mg permethrin); 1.6 ml applicators for dogs of 4 to 10 kg (containing 87 mg dinotefuran / 7.7 mg pyriproxyfen / 635 mg permethrin); 3.6 ml applicators for dogs of 10 to 25 kg (containing 196 mg dinotefuran / 17.4 mg pyriproxyfen / 1,429 mg permethrin), 4.7 ml applicators for dogs of 25 to 40 kg (containing 256 mg dinotefuran / 22.7 mg pyriproxyfen / 1,865 mg permethrin), and 8.0 ml applicators for dogs of > 40 kg bodyweight (containing 436 mg dinotefuran / 38.7 mg pyriproxyfen / 3,175 mg permethrin).

Container

There are 5 different single-dose spot-on applicator sizes which are distinguishable by their differently coloured applicator tips. The applicator bodies are made of a multi-layered complex of aluminium and polyethylene with a neck and shoulder made of high density polyethylene (HDPE). The applicators are top-sealed with an aluminium liner complex (aluminium/polyester/sealable polyethylene layer) and fitted with coloured polypropylene screw applicator tips.

Development pharmaceutics

The aim of the development studies was to formulate a single spot-on solution containing dinotefuran, pyriproxyfen and permethrin. Experience gained by the applicant during the development and manufacture of a product of the same composition, which is manufactured and filled according to the same process at the same manufacturing site and has been on the US market since 2007, was used.

The choice of excipients was adequately justified.

To accommodate a wide range of dog bodyweights, five different strengths (applicator sizes) were required.

The CVMP agreed that the information provided on the development of this product is sufficiently comprehensive and acceptable for this simple solution formulation comprising of the three active substances dissolved in a mixture of two (non-aqueous) solvents.

Method of manufacture

The finished product is a simple solution formulation with no complex processing or packaging operations. Dissolution of the three active substances is the most critical step in the manufacturing process but this is satisfactorily controlled. The solution is then filtered before filling.

All validation parameters are satisfactory and the validation data provided demonstrate that the simple manufacturing process is robust, reproducible and can consistently produce product of the desired quality. Validation data from the manufacturing process at the largest proposed production scale is still outstanding and the CVMP considered it is sufficient to provide a recommendation for such to be conducted following the granting of a marketing authorisation for the product. A satisfactory validation scheme for this was provided.

Control of starting materials

Active substances

Active substance - dinotefuran

Dinotefuran [IUPAC: (RS)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine, CAS: 165252-70-0] is not described in either the European Pharmacopoeia (Ph. Eur.) or any other pharmacopoeia of the EU so is tested according to an in-house monograph.

The data for this active substance are presented in the form of an Active Substance Master File (ASMF) held by the active substance manufacturer. The manufacturing process of dinotefuran is a chemical synthesis. The justification for the designation of the starting materials is considered appropriate. Suitable specifications for the starting materials are provided.

Adequate specifications for the control of the active substance have been provided, and the analytical methods have been sufficiently validated.

Results of several batches were provided and were shown to meet the specifications.

Results of stability studies have been presented which justify the claimed retest period of 24 months.

Active substance - pyriproxyfen

Pyriproxyfen is not described in either the Ph. Eur. or any other pharmacopoeia of the EU so is tested according to an in-house monograph.

The data for this active substance are presented in the form of an ASMF held by the active substance manufacturer. The manufacturing process of pyriproxyfen is a chemical synthesis. The justification for the designation of the starting materials is considered appropriate. Suitable specifications for the starting materials are provided.

Adequate specifications for the control of the active substance have been provided. The potentially genotoxic impurity CRP is specified separately and adequately controlled. Assay and content of impurities are determined by high-performance liquid chromatography (HPLC). The analytical methods have been sufficiently validated. Results of three recent production batches show that they meet the requirements.

A retest period of 36 months has been substantiated by results of stability studies.

Active substance - permethrin

Permethrin is a mixture of two diastereoisomeric forms (cis and trans isomer) each of which is present as a pair of enantiomers.

Permethrin cis:trans/40:60 is not described in either the Ph. Eur. or any other pharmacopoeia of the EU so is tested according to an in-house monograph.

The data for this active substance are presented in the form of an ASMF held by the active substance manufacturer. The manufacturing process of permethrin cis:trans/40:60 is a chemical synthesis. The justification for the designation of the starting materials is considered appropriate. Suitable specifications for the starting materials are provided. The structure and origin of all the related impurities have been presented.

Adequate specifications for the control of the active substance have been provided, and the analytical methods have been adequately validated.

Stability results provided substantiate the claimed retest period of 4 years.

Excipients

N-Methylpyrrolidone, a non-aqueous solvent, is described in the Ph. Eur. and is controlled according to the current monograph.

N-Octylpyrrolidone, a non-aqueous solvent, is not described in the Ph. Eur., but the in-house monograph provided is considered satisfactory to control the quality of this excipient.

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

No material of animal origin is used in the manufacture of any of the ingredients in the finished product. Compliance of the active substance and all the excipients in the product with 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) has been confirmed.

Control tests during production

Not applicable.

Control tests on the finished product

The specification for release testing is appropriate to control the quality of the finished product and is line with VICH guideline GL39 (Test procedures and acceptance criteria for new veterinary drug substances and new medicinal products: chemical substances). Adequate specifications and routine tests have been described to ensure appropriate and constant quality of the finished product. Analytical methods are fully described and validated in accordance with VICH guidelines.

The shelf-life specification is different from the release specification only with regard to lower content limits for the active substances, but the differences are justified.

Results of the analysis of three consecutive batches of finished product (manufactured at the smallest of the proposed production scales of 299.4 litres) were presented which comply with the required specification.

Stability

The stability studies were conducted in line with the relevant stability guidelines.

Results covering a period of 24 months at long term and at intermediate conditions and 6 months at accelerated conditions are available; all results comply with the product's shelf-life specification. The data presented justify a shelf-life of 3 years with no special storage conditions.

A commitment has been provided that samples from the first three full scale production batches will be used to conduct further accelerated (for 6 months) and long term (over the claimed shelf-life) stability studies.

Overall conclusions on quality

The data for each of the three active substances is presented in ASMFs and include comprehensive information on the starting materials, manufacture, characteristics and control of the relevant active substance. None of the three actives are the subject of a monograph of the Ph. Eur. or a pharmacopoeia of any EU member state and are all tested in accordance with in-house monographs.

The two excipients (non-aqueous solvents) used are considered acceptable and the subject of an appropriate specification.

Appropriate information is provided for the packaging materials for these single dose spot-on applicators.

There are no concerns in relation to TSE for any of the ingredients of the product.

The rationale for the choice of the formulation is acceptable.

Manufacturing process validation for production scale batches larger than 300 litres is outstanding but will be finalised before any such sized batches are placed on the market.

The finished product release specifications are considered acceptable. The control methods have been validated and the specification is considered relevant for a product of this type.

Suitable stability studies according to VICH guidelines have been carried out and the data provided support the shelf-life of 3 years. Stability studies are on-going. No special storage precautions are considered necessary.

The quality data and documentation provided are in accordance with the relevant VICH and EU guidelines.

Part 3 – Safety

Safety documentation

Dinotefuran has not been used previously in a veterinary medicinal product in the EU. Full study reports to support the safety profile of this compound have been presented in the application. Dinotefuran has been tested in well executed *in vivo* GLP studies, all of which have been conducted in line with the appropriate guidelines.

Pyriproxyfen has been included in veterinary medicinal products authorised in the EU and indicated for use in non-food producing species. To complete the limited data available in the public domain, full study reports have been presented for pyriproxyfen. The data are relatively recent and the majority of the studies were conducted in accordance with GLP. Satisfactorily reported studies which were not in

conformance with GLP were however performed in accordance with modern protocols. These studies are considered adequate to ascertain the toxicity profile for pyriproxyfen.

Permethrin has been included in EU-authorised veterinary medicinal products indicated for the treatment of dogs, cattle and horses. The studies, mainly published as summaries in a JECFA (1999) report (Toxicological evaluations – Permethrin. Joint meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. Pesticide residues in food), have been evaluated and re-evaluated toxicologically by JMPR (Joint FAO/WHO Meeting on Pesticide Residues) in 1979, 1981, 1982, 1987 and 1999. Furthermore, the CVMP has evaluated these studies during the MRL procedure for permethrin in 1998, 2000 and 2002 (see CVMP Permethrin Summary Report, EMEA/MRL/843/02-FINAL) which resulted in the inclusion of the substance in Table 1 of the Annex to Commission Regulation (EU) No 37/2010.

The excipient N-octyl-2-pyrrolidone (NOP) is already used in EU-authorised veterinary medicinal products, for example, in sheep dip concentrates. It is also used in cosmetic products (e.g., shampoos) as a surfactant (1-2%). It is classified as an inert ingredient by the US Environmental Protection Agency (EPA) when used as a solvent.

The excipient N-methylpyrrolidone (NMP) is used in the manufacture of various compounds including pigments, cosmetics, drugs, insecticides, herbicides and fungicides. In human and veterinary medicine it is used as solubilising agent in parenteral, oral and topical formulations. It has been reviewed by the CVMP during the EU procedure in setting Maximum residue limits (MRLs) in 2008 and it was evaluated by the US EPA in 1998 and the International Program of Chemical Safety (IPCS) in 2001.

Pharmacodynamics

Please see part 4.

Pharmacokinetics

After an oral dose of radiolabelled dinotefuran given to rats, nearly 100% of the radioactivity was rapidly absorbed, with wide distribution throughout the body. Additionally, it is rapidly transferred from maternal blood to milk and widely distributed in foetal tissues. After intravenous and oral administration of radiolabelled dinotefuran to rats, very little metabolism occurs, as over 90% is excreted as unchanged parent compound.

Pharmacokinetic data in neonatal rats indicate that absorption and clearance of radioactivity were slower than in adults and suggest a slower metabolism of ^{14}C-guanidine-dinotefuran in pups, possibly due to incomplete development of their liver function.

Systemic dermal absorption of dinotefuran in the rats was demonstrated to be very low, with only 1.04% of the topically administered dose measured in the systemic circulation.

For pyriproxyfen, absorption and distribution in the body was limited. Pyriproxyfen levels in tissues of rats (with the exception of fat) peaked 2 to 8 hours after oral administration, whereas the peak concentrations in fat were found 12 to 24 hours after dosing. Pyriproxyfen concentrations were found to be highest in the fat, without evidence of accumulation. Pyriproxyfen was highly metabolised and excretion into faeces and urine was demonstrated to be rapid, with the major route of excretion being via the faeces.

Permethrin undergoes slow and partial absorption in rats after oral administration and the oral bioavailability is relatively low (61%). The highest concentrations are found in the brain and sciatic nerve. In mammals, almost all of an oral dose is excreted as metabolites in the urine and faeces within

a few days. Both faecal and urinary excretion has been demonstrated to be the major routes. Permethrin pharmacokinetics studies show some retention of permethrin in fat after repeated oral administration, and the cis-isomer is more readily retained than the trans-isomer.

Pharmacokinetic data on the excipient N-octyl-2-pyrrolidone (NOP) were not provided.

The excipient N-methylpyrrolidone (NMP) is rapidly absorbed after oral administration in humans and rats (70% and 86%, respectively). It is also readily absorbed through skin (rat 60-85%; human 38%). Skin permeability decreases in the following order: mouse, new-born pig, rat, rabbit, man and guinea pig.

Toxicological studies

Single dose toxicity:

As demonstrated in several acute toxicity studies in mice and rats (mostly compliant to GLP), the acute oral and dermal toxicity of dinotefuran and pyriproxyfen are low.

The data summarised in the JECFA report indicate that the dermal toxicity of permethrin to rodents is low, and its oral toxicity is relatively low (LD_{50} of 400 mg/kg body weight (bw) in the rat). It has been shown that the cis-isomer of permethrin is more toxic than the trans-isomer and that toxicity can be influenced by the vehicle.

The acute toxicity studies submitted for the excipients NOP and NMP show that both substances are of low acute oral and dermal toxicity.

The acute toxicity studies in rats performed with the final product demonstrated a low acute oral and dermal toxicity.

The single dose data were considered complete and satisfactory.

Repeat dose toxicity:

Dinotefuran:

Several repeated toxicity studies in different laboratory animals were provided. From these it was not possible to attribute a target organ to dinotefuran toxicity. Clinical adverse signs consisted mainly of reduced food consumption and reduced body weights at higher dose levels. In rats, alterations in adrenal histopathology were evident. The lowest no-observed-effect level (NOEL) for dinotefuran was found to be 34 mg/kg bw/day in male rats, obtained from a 90 day oral repeat dose study.

Dinotefuran did not exert systemic effects after repeated dermal application in rats. Inhalative exposure is not considered a relevant route of exposure in respect to target animal and user safety, taking into account the formulation of the product and its intended topical application and use.

Pyriproxyfen:

Several repeated toxicity studies in rodents (mice and rats) and dogs (the target species, see part 4) were provided. After the repeated oral administration of pyriproxyfen in mice, a dose-dependent mortality was seen at 750 mg/kg bw/day and higher. The main target organ was the kidney. Increases of liver enzymes and liver organ weights were not associated with histopathological findings. A NOEL was established at 38 mg/kg bw/day based on increased cholesterol values in females.

In rats, no mortality was seen up to the highest dose of 784 mg/kg bw/day. The liver was the target tissue and hepatotoxicity of pyriproxyfen was firstly seen at doses of 118 mg/kg bw/day in rats (changes in biochemistry parameters and increases in liver weight). Additionally, changes in red blood

cell parameters were affected and histopathological changes seen in the kidneys. A NOEL was established at 24 mg/kg bw/day in males and 28 mg/kg bw/day in females.

Pyriproxyfen did not exert systemic effects after dermal application in rats. Inhalative exposure is not considered a relevant route of exposure in respect to target animal and user safety, taking into account the formulation of the product and its intended use.

Permethrin:

After repeated oral administration in rats, permethrin causes a high mortality rate at doses ≥ 630 mg/kg bw/day. The main target organs were the liver and the central nervous system. No substance-related effects were seen at ≤ 30 mg/kg bw/day.

In the MRL application for permethrin (see the first MRL Summary Report, EMEA/MRL/112/96-FINAL) studies were mainly performed with cis:trans 40:60 permethrin on mice, rats, dogs and guinea pigs. It was shown that the cis-isomer is more toxic than the trans-isomer. An overall NOEL of 5 mg/kg bw/day was established based on repeated dose studies in rats and dogs.

The excipient NOP showed moderate liver toxicity in repeated toxicity studies. The NOEL was 53 mg/kg bw/day in rats and 30 mg/kg bw/day in dogs (13 weeks study).

The excipient NMP showed moderate systemic toxicity in subchronic toxicity studies in rodents, with alterations in body weight gain and clinical chemical changes. The NOEL was 169 mg/kg bw/day and 217 mg/kg bw/day in male and female rats respectively, and 250 mg/kg bw/day in dogs (13 weeks study).

Tolerance in the target species of animal

Please see part 4.

Reproductive toxicity

The applicant has provided GLP-compliant studies (dinotefuran and pyriproxyfen) and publications (permethrin) on reproductive toxicology, including developmental toxicity, for all three active substances. There were no treatment-related changes regarding reproductive parameters for pyriproxyfen and permethrin.

A two-generation study and a preceding preliminary two-generation study in rats were provided for dinotefuran. The preliminary study showed evidence that dinotefuran at 1,340 mg/kg bw and 1,507 mg/kg bw/day in males and females respectively may cause increased post-implantation losses and decreased litter sizes. Other reproductive parameters were not affected. Possible treatment-related effects in the offspring included changes in organ weights and reduction in body weight. The NOELs for the adults of the parental (P) and F1 generation as well as for pup development were 241 mg/kg bw/day P males, 249 mg/kg bw/day F1 males, 267.9 mg/kg bw/day P females and 292.6 mg/kg bw/day F1 females. The NOELs for reproductive effects and pup behaviour were 822.1 mg/kg bw/day P males, 934.7 mg/kg bw/day F1 males, 907 mg/kg bw/day P females and 1,004.8 mg/kg bw/day F1 females.

In a GLP-conforming two generation study in rats with pyriproxyfen, no treatment-related changes regarding reproductive parameters were observed. However, the study showed a possible treatment-related increase in chronic interstitial nephritis in males of the F1 generation. The parental NOEL was 200 ppm, the maternal and maternal reproductive NOEL was 5,000 ppm and the pup developmental NOEL (F1 and F2 generation) was 1,000 ppm.

Sufficient experimental data from developmental toxicity studies in rats and rabbits (in the case of permethrin also in mice) were provided for the CVMP to conclude that each of the three actives in the product has no teratogenic potential. However dinotefuran increased the rate of abortions in rabbits (at 1,000 mg/kg bw/day, NOEL 300 mg/kg bw/day), pyriproxyfen provoked developmental effects at toxic maternal doses in rats (300 mg/kg bw/day, NOEL 100 mg/kg bw/day), and permethrin increased the number and percentage of early and late resorptions in rabbits at doses of 1,800 mg/kg bw/day (NOEL 1,200 mg/kg bw/day).

The excipient NOP showed no developmental toxicity in rats (NOEL 200 mg/kg bw/day).

Oral administration of the excipient NMP to rats during embryonic and foetal periods caused teratogenic effects at doses below those causing maternal toxicity (maternal NOEL 250 mg/kg bw/day, developmental NOEL 125 mg/kg bw/day). In rabbits, malformations were seen at doses higher than maternal toxicity after oral administration. After dermal application to the rabbit, skeletal variations were observed at doses lower than maternal toxicity (maternal NOEL > 1,000 mg/kg bw/day; developmental NOEL 300 mg/kg bw/day).

Mutagenicity/genotoxicity

A comprehensive set of *in vitro* and *in vivo* genotoxicity tests examining gene mutations and clastogenic endpoints has been conducted for each of the three active substances, dinotefuran, permethrin and pyriproxyfen, as well as for the excipients NOP and NMP, proved to be non-mutagenic.

It was concluded that each of the active substances, dinotefuran, permethrin and pyriproxyfen did not induce gene mutations in bacteria and mammalian cells, chromosomal aberrations, or unscheduled DNA synthesis in mammalian cells *in vitro* and were considered to be non genotoxic *in vivo*. This holds true also for the excipients NOP and NMP.

Since all three active substances were tested adequately and reveal clear negative results it can be assumed that Vectra 3D has no genotoxic potential.

Carcinogenicity

Dinotefuran:

For dinotefuran, a no-observed-adverse-effect level (NOAEL) of 3 mg/kg bw/day in males and 4 mg/kg bw/day in females established by the US EPA in mice, based on decreased spleen weights in males at termination and increased ovarian weights in females at week 53. Since there were no microscopic findings associated with the increases of the spleen weights and the ovarian weights were not clear dose-dependent and not seen at terminal sacrifice (78 weeks), these changes are considered as not treatment-related. The proposed NOAEL of 345 and 441 mg/kg bw/day for males and females respectively can be supported.

In rats, the survival rate of the combined chronic carcinogenicity study (104 weeks) was below 50% in all groups treated with dinotefuran except the highest dose group in males (53%) and did not show a dose-dependence. This is in agreement with published survival rates of this strain (Compilation by Charles River Laboratories). Moreover, pelvic mineralisation and ulceration clearly increased at 991 mg/kg bw/day in males during the carcinogenicity study and may be caused by the treatment.

The presented GLP-compliant carcinogenicity studies show that dinotefuran has no carcinogenic potential in mice and rats.

Pyriproxyfen:

The chronic toxicity and carcinogenicity of pyriproxyfen was studied in GLP-conforming carcinogenicity studies in rats (2 year study) and in mice (78 week study). None of the studies showed any evidence of a carcinogenic potential.

The relevant NOEL in mice was 16 mg/kg bw/day (males) based on a reduced survival rate, increased severity of systemic amyloidosis and histopathological changes in the kidneys.

In rats, the main target organ was the liver. Increases in serum chemistry values (cholesterol, phospholipids) suggest a compound-related change which is likely to be associated with the liver during the first half of the study in the high dose groups. However, these effects were no longer manifest during the latter half of the study. No microscopic hepatic changes due to treatment were identified.

Permethrin:

The CVMP accepted the International Programme on Chemical Safety (IPCS) classification of permethrin as a possible weak rodent carcinogen.

Long term toxicity or carcinogenicity studies were not provided for the excipient NOP.

In the MRL report for the excipient NMP, chronic studies evaluating the potential chronic toxicity and oncogenicity performed in rats and mice were mentioned. There was no evidence of carcinogenicity.

Studies of other effects

These studies address the effects of each of the active ingredients on skin and ocular exposure, as well as the potential for skin sensitisation, neurotoxicity and immunotoxicity.

Dinotefuran has been found to be a mild ocular irritant in one GLP-compliant ocular irritation study in rabbits, leading to a classification of category 2 in accordance with Regulation (EC) No 1272/2008. The results of a second eye irritation study, conducted with a lower concentration, were negative. In a dermal irritation study dinotefuran demonstrated no dermal irritation. In addition, dinotefuran is not a dermal sensitizer. During an acute and sub-chronic neurotoxicity study, decreased motor activity was seen in high dose animals. However, as there were no correlative effects in the functional observational battery (FOB) data evaluations and macroscopic or microscopic examinations, this effect was not considered adverse or a sign of neurotoxicity. Following monoclonal antibody assays, in both mice and rats, no effect on immune response function was seen, demonstrating that dinotefuran does not elicit an immunotoxic effect.

Dermal and ocular irritation studies conducted according to GLP in rabbits show that pyriproxyfen is only minimally irritating to the eyes and is not a skin sensitizer or a skin irritant.

Permethrin is not a skin sensitizer and causes virtually no skin irritation, however, it is minimally irritating to the eyes. There is evidence within the published literature that permethrin gives rise to neurotoxicity at high doses in laboratory animals, however, the CVMP concluded that these effects are reversible.

In three studies in rats, subchronic skin exposure to permethrin resulted in sensorimotor impairment at concentrations of 0.013 mg/kg bw/day. Changes in acetylcholinesterase receptor binding sites and acetylcholinesterase concentration in the cortex, as well as diffuse neuronal cell death and neuronal cytoskeletal abnormalities in the cerebral cortex, the hippocampus formation and the cerebellum were seen at 0.13 mg/kg bw/day.

Studies conducted with the product demonstrated that Vectra 3D is severely irritating to the eyes and moderately irritating to the skin, but is not a skin sensitizer. Topical application of the product resulted in low amounts of permethrin (cis/trans), pyriproxyfen and dinotefuran residues dislodged from treated dogs after petting. Maximum residue levels were dislodged four hours after treatment.

Dinotefuran and pyriproxyfen are not used in human medicinal products and no human data are therefore available for assessment.

Following human poisoning with permethrin, xylene and surfactant, the gastrointestinal tract was the most commonly involved organ. The involvement of the nervous system and lungs was less common, but clinically more significant. Published literature show that the most commonly reported adverse events following topical application of 1% permethrin rinse against lice infestations to humans are pruritus and rash. The rate of reported adverse events was 2.2 per 1,000 permethrin treatments.

A prospective comparative study conducted in pregnant women demonstrated that topical permethrin products indicated for lice infestation are relatively safe during pregnancy. However it was noted that the cited studies reflect the effects of acute permethrin exposure, rather than a chronic or subchronic dermal exposure as might be expected by dog owners coming into contact with a topically applied veterinary medicinal product.

The excipient NOP proved to be non-phototoxic, non-comedogenic and did not induce contact dermal photoallergy in humans.

The excipient NMP is irritating to the skin in humans. Symptoms fade rapidly upon cessation of exposure (within 24 hours). NMP was also shown to be irritating to the skin in guinea pigs, but to have no sensitizing potential and to be moderately irritating to the eyes of rabbits.

User safety

A user risk assessment according to the current CVMP guideline on user safety (EMEA/CVMP/543/2003-Rev.1) has been submitted. Relevant exposure scenarios were considered for the veterinarian and dog owner who apply the product, and also for other people (especially children) who have direct contact with a treated dog.

A quantitative risk assessment of both acute and chronic oral/dermal exposures was performed according to a Standard Operation Procedure (SOP) for user safety risk assessment from the US environmental protection agency (EPA), published in 2012 (SOP EPA 2012) and margins of exposure have been calculated for all potential relevant exposure scenarios:

Acute exposure: a serious risk during the acute exposure of the user and the acute accidental exposure of children towards permethrin was identified. In order to mitigate this risk appropriate warning phrases have been included in the SPC and other product literature.

Chronic exposure: no risk for the user and children from 4 hours onwards after administration of the product was identified.

Therefore the child-resistant packaging and the warning phrases in the SPC and other product literature which are in line with the warnings for other spot-on products containing permethrin in similar amounts are considered adequate to minimise the risk for children and other users:

"Children must not handle treated dogs for at least four hours after administration of the veterinary medicinal product. It is therefore recommended to treat dogs in the evening, or before taking them for a walk. On the day of treatment, treated dogs should not be permitted to sleep with their owners,

especially children. Used applicators should be disposed of immediately and not left within the sight or reach of children."

The two excipients have shown to be of no health concern to the user.

Environmental risk assessment

A Phase I environmental risk assessment (ERA) was provided according to the CVMP Guideline on environmental impact assessment for veterinary medicinal products – Phase I (CVMP/VICH/592/98-FINAL). The environmental risk assessment can stop in Phase I and no Phase II assessment is required because the veterinary medicinal product will only be used for non-food animals.

However, since this product is for topical use and any exposure of the environment is possible via transfer from the dog's coat to the terrestrial and aquatic environments, the following risk mitigation measure is included in the SPC (section 4.5) and other product literature. This is in line with the recommendation of the EMA/CVMP reflection paper on risk mitigation measures related to the environmental risk assessment of veterinary medicinal products (EMA/CVMP/ERAWP/409328/2010) and is considered suitable to protect the environment: "Treated dogs should not be allowed to enter surface water for 48 hours after treatment to avoid adverse effects on aquatic organisms".

Due to the high toxicity of the active ingredients permethrin and pyriproxyfen to aquatic species, the advice included in section 6.6 of the SPC (and other product information) for disposal of any unused product and waste material has been amended to include the following, in addition to the standard disposal warning: "Vectra 3D should not enter water courses as it is dangerous for fish and other aquatic organisms. Do not contaminate ponds, waterways or ditches with the veterinary medicinal product or with used containers".

Overall conclusions on the safety documentation

The acute toxicity of dinotefuran is low regarding dermal and oral exposure. After repeated oral administration in rodents and dogs, no target tissue could be identified. The lowest NOEL was 34 mg/kg bw/day in male rats in a 90-day study. Repeated dermal application of dinotefuran revealed no signs of systemic toxicity up to high doses. Reproductive toxicity studies showed an increased post-implantation loss and decreased litter size at high doses (1,340 mg/kg bw/day and 1,507 mg/kg bw/day in males and females respectively) and reduction in body weights during lactation in the offspring. Dinotefuran is not teratogenic, however it increases the rate of abortions in rabbits (at 1,000 mg/kg bw/day), it is not genotoxic and not carcinogenic. Dinotefuran is minimally irritating to the eye. It is not a skin sensitizer.

The acute toxicity of pyriproxyfen is low following dermal or oral exposure. For pyriproxyfen the main target tissues are the kidney, liver and blood after repeated administration. Hepatotoxicity was noted in rats and dogs and resulted in liver enlargement, chronic inflammation and fibrosis. The lowest NOEL is 24 mg/kg bw/day in male rats. Repeated dermal application of pyriproxyfen revealed no signs of systemic toxicity up to 1,000 mg/kg bw/day. There were no treatment-related changes regarding reproductive parameters for pyriproxyfen, and no teratogenic potential was observed. However, pyriproxyfen provoked developmental effects at toxic maternal doses (300 mg/kg bw/day) in rats. Pyriproxyfen is not genotoxic and not carcinogenic. It is not irritating to eye or skin, and is not a skin sensitizer.

The acute toxicity of permethrin after oral application is moderate. The main target organs after repeated permethrin administration are the liver and central nervous system. An overall NOEL of 5 mg/kg bw/day was established, based on data from repeated oral toxicity studies in dogs and rats.

There were no treatment-related changes regarding reproductive parameters for permethrin, however permethrin increased the number and percentage of early and late resorptions in rabbits at doses of 1,800 mg/kg bw/day. Permethrin is considered non-mutagenic, however permethrin was classified by the CVMP (in line with IPCS) as a possible weak rodent carcinogen.

Permethrin gives rise to neurotoxicity at high doses in laboratory animals, and it has been shown that subchronic skin exposure to permethrin in the rat results in sensorimotor impairment and receptor alterations as well as in diffuse neuronal cell death and neuronal cytoskeletal abnormalities in different regions of the brain. Permethrin is minimally irritating to the eye.

The excipient N-octyl-2-pyrrolidone (NOP) is of low acute oral and dermal toxicity. NOP showed moderate liver toxicity in repeated toxicity studies. There is no evidence of developmental or genotoxic property. Studies on long term toxicity and carcinogenicity were not performed. NOP proved to be non-phototoxic, non-comedogenic and did not induce contact dermal photoallergy in humans.

The excipient N-methylpyrrolidone (NMP) is of low acute oral and dermal toxicity. NMP showed moderate systemic toxicity in subchronic toxicity studies in rodents with alterations in body weight gain and clinical chemical changes. It has been shown to have teratogenic effects in rats and rabbits after oral and dermal application. It is neither genotoxic nor carcinogenic. NMP is irritating to skin in humans and readily absorbed through skin. Skin permeability was 4 times higher in rats than in humans.

Studies conducted with the finished product demonstrated that it is of low acute oral and dermal toxicity. It is severely irritating to the eyes and moderately irritating to the skin, but is not a skin sensitizer.

The user safety assessment showed risks during the acute exposure of the user and the acute accidental exposure of children towards permethrin which make additional warning phrases necessary. The calculation presented by the applicant following a published SOP of US EPA showed no risk for chronic exposure. The user safety warnings, as used on other spot-on products containing comparable amounts of permethrin, are considered sufficient to minimise the risk for children.

Based on the data provided the ERA can stop at Phase I. Vectra 3D is not expected to pose a risk to the environment when used in accordance with the SPC. The product is intended for the individual treatment of non-food animals (dogs). The active ingredients are well known and any exposure of the environment is likely to be via transfer from the animal's coat to the terrestrial and/or aquatic environment. The risk mitigation measure warnings included in the product are considered suitable to protect the environment. Due to the high toxicity of the active ingredients permethrin and pyriproxyfen to aquatic species additional advice for the disposal of any unused product and waste material is also included in the SPC and other product literature.

Part 4 – Efficacy

Pharmacodynamics

Vectra 3D includes three active ingredients, dinotefuran, pyriproxyfen and permethrin.

Pyriproxyfen and permethrin are already widely used as active substances in different ectoparasiticidal veterinary medicines authorised within the European Union. Their modes of action have been well described.

Pyriproxyfen is a photo-stable insect growth regulator that targets the insect endocrine system by mimicking juvenile hormone activity which results in inhibiting embryogenesis, metamorphosis and adult formation. Pyriproxyfen has been proven to act larvicidally and ovicidally.

Permethrin is a third generation synthetic pyrethroid that exerts its effect primarily by modulating gating kinetics of sodium channels in nerves. It interacts preferentially with the open state of the sodium channel. Permethrin shows repellent, insecticidal and acaricidal activity.

Dinotefuran is a new third generation member of the nicotinoid class of insecticides. Its structure is based on the acetylcholine molecule. It disrupts the insect nervous system by mimicking the action of acetylcholine on the postsynaptic nicotinic acetylcholine receptor. Dinotefuran binds to the acetylcholine receptor, however, its precise binding site is still unknown. Dinotefuran has an adulticidal activity against a wide spectrum of insects.

As for the justification of the triple combination, the combination of permethrin and pyriproxyfen is considered justified based on the widened spectrum of activity providing for insecticidal/acaricidal activity plus larvicidal/ovicidal activity.

Regarding the justification of dinotefuran in the combination, synergistic activity was demonstrated in an *in vitro* study, after administration of dinotefuran in conjunction with permethrin, at a fixed combination ratio of 1:7.3 (the same ratio as in Vectra 3D), which resulted in a significantly higher effect on *C. felis* fleas mortality compared to each active alone.

The clinical benefit resulting from the combination of dinotefuran with permethrin was demonstrated in one laboratory dose confirmation study on dogs, which showed a prolongation of the duration of efficacy against *C. canis* fleas (which is a prevalent flea strain in several European member states) for four weeks, compared to a duration of effect of two weeks for permethrin/pyriproxyfen alone. However, this potential advantage was not further explored under field conditions.

Additional benefits resulting from the synergistic activity of dinotefuran and permethrin were shown in a set of GCP-compliant laboratory dose confirmation studies on dogs artificially infested with *C. felis* or *C. canis* fleas. These studies demonstrated a faster onset of insecticidal activity within four to twelve hours after flea infestations, and adequate adulticidal activity against fleas within 12 hours on the first day after application of the product.

In addition, a pronounced killing effect on dislodged or moribund fleas knocked down by permethrin was shown in two GCP-compliant laboratory dose confirmation studies on dogs infested with *C. canis* or *C. felis* and this was attributed to the combination of dinotefuran with permethrin.

The clinical relevance of these additional effects, for example, a better control of flea infestations or improved animal scratching relief and animal welfare has not been shown.

Information on secondary pharmacodynamic effects of all three of the actives substances (permethrin, pyriproxyfen and dinotefuran) has been provided and discussed.

Development of resistance

Literature, and in particular, a recent European field survey of resistance genes on pet fleas in France and the UK (2012) performed by the applicant, indicate a high and increasing prevalence of fleas with reduced permethrin and fipronil sensitivity over the last ten years. The efficacy of Vectra 3D in the treatment and prevention of a flea strain (*C. canis*) with reduced permethrin sensitivity was confirmed in one recent GCP-compliant controlled laboratory study on artificially infested dogs. However, since improved efficacy of Vectra 3D against flea populations with reduced sensitivity or resistance towards permethrin has not been demonstrated under field conditions, no claim or respective information has been included in the SPC and other product literature.
The risk for selecting dual resistance development towards dinotefuran and permethrin associated with the use of this product was discussed by the CVMP. Based on the information provided, the CVMP

concluded that although the risk of selecting dual resistance cannot be properly estimated, there is at present no indication that this would be a major concern for this product.

Pharmacokinetics

The pharmacokinetic behaviour of dinotefuran, pyriproxyfen and permethrin, in the target species (the dog) has been examined *in vitro* and after intravenous, oral and topical administration. The most relevant pharmacokinetic issues have been addressed.

In vitro experiments with skin samples from dogs demonstrated high skin permeation rates for dinotefuran (up to 70% within 24 hours) and low skin permeation rates for pyriproxyfen (maximal penetration rate <0.22%) and permethrin (maximal penetration rate <0.05%). Comparable results were obtained with human skin samples (58.9%, 0.12% and 0.02% respectively).

The pharmacokinetics of dinotefuran, pyriproxyfen and permethrin have been characterised in dogs after separate intravenous injections and revealed rapid elimination from the plasma with mean half-lives of 1.15 hours for dinotefuran, 4.8 hours for pyriproxyfen and 0.96 and 0.33 hours for cis- and trans-permethrin, respectively. A mean of 54% of the injected dinotefuran was eliminated in urine within 24 hours after injection. The levels of pyriproxyfen and permethrin in urine were below the quantification limits over the entire observation period in most dogs.

The pharmacokinetics was probably underestimated because some dogs vomited soon after single oral administration of approximately 63 mg/kg permethrin, approximately 8 mg/kg dinotefuran and approximately 0.7 mg/kg pyriproxyfen. But even with these limitations, all actives had reached the plasma with bioavailability rates of 52%, 30% and 6-8% for dinotefuran, pyriproxyfen and cis- and trans-permethrin, respectively. Elimination from the plasma took on average 3 hours for dinotefuran and 20 hours for pyriproxyfen, but with large inter-individual variations, whilst the half-lives for cis- and trans-permethrin after oral administration were short.

The pharmacokinetics after topical administration was determined at the mean (0.53 ml/kg) of the recommended dose range. When applying the volume of 0.53 ml/kg onto the hair coat of dogs, only dinotefuran entered the systemic circulation. Dinotefuran was 29% bioavailable and was eliminated slowly from the plasma over approximately 25 days. Absorption rates were not determined for permethrin due to levels below the limits of quantification. Pyriproxyfen was detected in plasma within 10 days after treatment only in single dogs, therefore, absorption rate could not be determined for this compound. As Vectra 3D is administered in a wide dose range, the pharmacokinetic results are expected to vary depending on the actual doses administered. Absorption will also be influenced by the type of hair coat of the dogs treated, and also by the amount of product residues licked off from the hair coat.

The metabolism of dinotefuran, pyriproxyfen and permethrin has not been examined in dogs, but was addressed in the applicant's critical summary of the pre-clinical and clinical data on the basis of findings in laboratory animals.

After topical application of the product between the shoulders of Beagle dogs, quantifiable amounts of the 3 actives were detected at different hair sampling sites from the trunk over the claimed 30 day efficacy period. The highest mean concentrations (of 361 µg/g for dinotefuran, 1.48 mg/g for trans-permethrin, 680 µg/g for cis-permethrin and 36 µg/g for pyriproxyfen) were reached on Day 3 after application of the product. Levels of dinotefuran were high, even towards the end of the sampling period, whereas levels of the other substances declined gradually. Concentrations varied notably among the dogs as a result of the many factors that influence distribution of the compounds over dogs' coats.

Although hair coat concentrations of dinotefuran, permethrin and pyriproxyfen only partially reflect the antiparasitic efficacy of these substances, the applicant demonstrated that they correlated well with *in vitro* determined inhibitory concentrations of these substances against fleas and ticks, as well as against flea eggs and the development of adults from flea eggs.

Dose determination/justification

In a dose determination study, point doses of 0.06 ml (0.5X), 0.12 ml (1X), and 0.24 ml (2X) of the final formulation per kg bw were tested against fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus*). Dose of 0.12 ml/kg bw (1X) was kept as the minimum recommended dose as the persistent acaricidal efficacy at 48 hours was > 90% at 3 weeks and 89.6% at Day 30. All dosing groups showed >95% persistent efficacy against fleas for 4 weeks.

Dose confirmation

Twenty-five GCP-compliant dose confirmation studies have been performed with the final product formulation. All studies were conducted according to the European ectoparasiticide guidelines.

1. Insects

1.1 Fleas (anti-feeding, dislodgeability and speed of kill in adult stages, growth inhibition of larval stages)

1.1.1 *Ctenocephalides (C.) felis* (adults)

Several dose confirmation studies have been carried out to examine the speed of killing *C. felis* fleas following application of the final formulation. The first two of the three studies were primarily intended as non-interference studies, but also serve as dose confirmation studies in fleas.

The results of the first non-interference study confirmed both a stable high immediate (99.8% at 24 hours post-treatment) and persistent flea mortality, up to and including Day 30, at 48 hours after each infestation (99.7-100%) at weekly interval based on the arithmetic mean. The "speed of kill" efficacy at 1 hour following infestation on Day 2 and Day 28 was found to be high, reaching values of 92.3% (Day 2) and 65.6% (Day 28), respectively. At 6 hours post-infestation the killing activity was more pronounced with efficacy values of 100% (Day 2) and 98.2% (Day 28), respectively.

A second non-interference study was performed with the minimum recommended dose of 0.12 ml per kg bw of the product. High immediate pulicidal efficacy was demonstrated at 4 hours after administration of the product (87.8%, based on arithmetic means). Thereafter, up to and including Day 28 persistent flea mortality rates at 1 hour after each weekly infestation were in the range of 73.6–93.6%. More than 95% of fleas were killed within 4 hours after each weekly infestation. Similar results were observed in an additional study. The flea mortality rate at 1 hour following infestation up to Day 28 reached values of 94.9% (on Day 14) and 82.3% (on Day 28). At 4 hours post-infestation the killing activity was of 96.4% (on Day 2) and 91.8% (on Day 28).

In the third study the initial speed of killing fleas 2 hours after treatment was low (23%). Thereafter, more than 95% efficacy was already achieved at 2 hours after each flea infestation, up to the end of the study period on Day 28. At 6 hours post-treatment or infestation Vectra 3D achieved more than 95% efficacy.

In a fourth study the anti-feeding activity of the product was investigated in fleas (*C. felis)* collected from artificially infested dogs, based on a quantitative real time polymerase chain reaction (qPCR) targeting a house keeping gene of dogs for the detection of imbibed blood. Starting on Day 8, the feeding of fleas was significantly impaired (p> 0.05) between 5 to 60 minutes post-infestation when

compared to the controls. In a fifth study, starting on Day 2, the feeding of fleas was significantly impaired (p> 0.05) at 5 and 10 minutes post-infestation when compared to the controls.

The impact of both shampooing 2 weeks after treatment and weekly water immersion on the persistent efficacy of the product was investigated on dogs weekly infested with 100 adult fleas (*C. felis*) and 50 adult *R. sanguineus* ticks. Both treatment groups showed > 99-100% persistent adulticidal efficacy against fleas, up to and including Day 30 after treatment. Acaricidal persistent efficacy in ticks was > 90% at 48 hours post-infestation, starting on Day 7 up to and including Day 28 post-treatment.

The advice given in the SPC (section 4.5) that water immersion 48 hours after treatment, repeated weekly for one month as well as shampooing 2 weeks after treatment do not affect efficacy of the product has been adequately demonstrated.

1.1.2 *Ctenocephalides felis* (larval stages)

In order to evaluate the ovicidal and/or the larvicidal efficacy of a single treatment with the product, a simulated home environment laboratory study was carried out in dogs following weekly infestations with mature fed female fleas (which were ready to lay eggs) for a two month period. The adulticidal effect of the product against fleas was additionally assessed at 48 hours after each infestation. It could be demonstrated that Vectra 3D exhibits persistent adulticidal efficacy of > 95% for at least one month after treatment. The rapid speed of kill of the fixed combination in the first 4 weeks after treatment resulted in an egg laying inhibition of > 90% in mature fleas. Although some eggs were harvested during the first month, no hatching of those (unviable) eggs was observed. With the decreasing activity of the product in the fur over time, the laying capacity of fleas increased from Day 36 to Day 64 up to 60%, allowing calculation of the ovicidal efficacy. Inhibition of eggs hatching into larvae was, however, pronounced, constantly reaching 99-100% inhibition *in vitro* and almost no adult fleas emerged from eggs collected from the treatment group (inhibition: 99.8 to 100% based on arithmetic mean). Control group flea emergence was adequate throughout the 2 month study period.

In a second GCP-conforming dose confirmation study, the impact of water immersion and shampooing on the development of flea eggs was determined in treated dogs.

Treated and untreated dogs were shampooed on Day 13 for five minutes and rinsed with water. The control group and a further group of treated dogs were additionally immersed in lukewarm water weekly for one minute. All dogs were infested weekly with 100 fleas each from Day 6 up to and including Day 55. Three days after each flea infestation, up to 50 flea eggs were collected from each of the dogs and hatching was recorded 3 days later by counting the flea larvae. Adult flea counts were then made 35 days following seeding of the larvae in flea growth medium. Both treatment groups showed a consistent > 95% persistent ovicidal efficacy up to and including Day 58 after treatment compared to the controls. The mean numbers of flea larvae hatching from eggs collected from the controls was high (83.3.-96.1%) and confirm the adequacy of the study condition.

The advice that water immersion and shampooing do not affect relevantly the performance of the product also holds for the pre-adult stages of fleas.

1.1.3 *Ctenocephalides canis* (adults)

The insecticidal activity of the product against *C. canis* fleas was evaluated in a dose confirmation study on dogs weekly infested with 100 fleas following exposure to 100 fasted female *Phlebotomus perniciosus* sand flies (see below*)*. The treatment with Vectra 3D demonstrates almost 100% (Day 30: 99.6%) persistent insecticidal efficacy for 4 weeks.

In a further dose confirmation study the efficacy of this fixed combination product was examined against a laboratory flea strain of *C. canis* less sensitive to permethrin. Dogs were treated with the

product at the minimum recommended dose of 0.12 ml/kg bw. In addition to a negative control group, a second group treated with permethrin alone at a dose of 0.11 ml/kg bw was included. Flea infestations were performed on Day –2, Day 2 and then weekly up to and including Day 28. On Day 0 fleas were counted at 8, 12 and 24 hours post-treatment, then weekly up to Day 14 at 2, 3 and 6 hours post-infestation and on Day 21 and Day 28 at 12, 24 and 48 hours post-infestation.

The addition of dinotefuran to permethrin significantly enhanced the immediate speed of kill up to 12 hours post-treatment in dogs when compared to permethrin alone. From Day 14 post-treatment onwards dinotefuran also significantly enhanced the persistent speed of kill activity of the product. Moreover, the persistent efficacy of the product against *C. canis* fleas was extended up to 4 weeks compared to permethrin alone showing 99.1% and 93.9% for dinotefuran plus permethrin compared to 79.3% and 55.2% for permethrin on Day 23 and 30, respectively. The survival rates of live, but moribund, fleas dropping off the dogs treated with the combination were also consistently lower than that recorded for the group treated without dinotefuran.

Based on the study results provided, the claimed indication, treatment and prevention of flea infestation (*C. felis* and *C. canis*) for one month and the prevention of multiplication of fleas for two months after application is justified.

2. Mosquitoes and stable flies (anti-feeding and insecticidal activity)

2.1 *Aedes aegypti*

A dose confirmation study was carried out in France to substantiate the repellent and adulticidal properties of Vectra 3D in dogs experimentally exposed for one hour at weekly intervals to 100 female *Aedes aegypti* mosquitoes, starting on Day 1. The anti-feeding efficacy was > 90% for 3 weeks, and 87% in the fourth week, based on arithmetic means. Live mosquitoes were placed again in separate nets for 24 hours post-exposure to assess the mortality rate after treatment. The calculated efficacy was constantly high, showing a mortality rate of 98.9% (Day 2) to 93.4% (Day 28).

Since *Aedes aegypti* is considered as a minor use nuisance, this single study justifies a prevention claim (anti-feeding) for four weeks.

2.2 *Culex pipiens*

A dose confirmation study was undertaken in France to confirm the repellent and adulticidal properties of Vectra 3D in dogs after their weekly infestation with 100 *Culex pipiens* mosquitoes for one month, starting on Day 1. The anti-feeding efficacy lasted for the entire study period, showing an efficacy of > 98% at each time point tested. In contrast, the mortality rate at 24 hours was constantly below 70%, based on arithmetic mean.

In a second dose confirmation study, dogs were treated with 1.4-1.5X the minimum recommended dose of Vectra 3D. All dogs treated showed constantly high repellent properties of > 95% for 4 weeks, starting on Day 1, based on arithmetic mean. Again the relevant killing activity of the product was not observed.

Based on the results of the studies provided the claimed indication: "It prevents biting from… mosquitoes *(Culex pipiens)*" is justified.

2.3 Stomoxys calcitrans

A dose confirmation study was initiated to determine both the repellent (anti-feeding activity) and killing activity (mortality rate) of Vectra 3D in dogs when exposed weekly for 30 min to 25 *Stomoxys calcitrans* stable flies for one month, starting on Day 1. The results demonstrated > 93% anti-feeding

activity for 3 weeks, but efficacy fell below 80% in the fourth week based on arithmetic means. The fly mortality rate was also high, reaching > 90% for three weeks and 82.4% in the fourth week.

In a second dose confirmation study in dogs both adequate anti-feeding (> 95%) and killing (99–100% for three weeks, starting on Day 1, and 94% in the fourth week) activities for four weeks were demonstrated at the recommended dosage regimen.

Based on the study, the claimed indication: "It prevents biting from stable flies *(Stomoxys calcitrans)* for one month post-application. The treatment also provides persistent insecticidal activity for one month against stable fly (*Stomoxys calcitrans).*" is justified.

2.4 *Phlebotomus perniciosus* (sand flies)

In the dose confirmation study in fleas the repellent and adulticidal efficacy of Vectra 3D on sand flies were examined concurrently. In addition to the flea infestation, all dogs were exposed weekly, starting from Day 1 for four weeks to fasted *Phlebotomus perniciosus* female sand flies for one hour. A sufficient mortality rate (99.8%) at 1 hour after exposure was noted only for one week after treatment, thereafter the mortality rate steadily declined from 73.7% at Day 14 to 39.6% at 4 weeks. The long term anti-feeding efficacy, however, was > 95% until Day 14 after treatment and still remained above 80% for the remaining study period of 4 weeks.

In a further dose confirmation study in dogs, both the anti-feeding and insecticidal efficacy of Vectra 3D at the recommended dosage was investigated. The calculated anti-feeding activity of the product was 88% to 98%, based on arithmetic means, starting from Day 1 and throughout the 4 week study period. There was no insecticidal activity (6-19%).

Results of both studies justify the claimed indication.

3. Ticks (repellent and acaricidal activity)

To confirm both the repellent and acaricidal activities of the final formulation, three dose confirmation studies were conducted. These comprised one short term anti-feeding efficacy study on Day 2 after treatment and at 24 hours after tick infestation, and two studies each over a period of at least 4 weeks, conducted for each of the relevant hard ticks species infesting dogs in Europe: the castor bean tick *Ixodes (I.) ricinus*, the ornate dog tick, *Dermacentor (D.) reticulatus* and the brown dog tick *Rhipicephalus (R.) sanguineus*. With regards to *R. sanguineus* the results are corroborated by the DDS, the non-interference study and the impact of water immersion/shampooing dose confirmation study.

3.1 *Ixodes ricinus* (adults)

A short term dose confirmation study was conducted in dogs to determine repellent (anti-attachment, anti-feeding) or acaricidal (killing) efficacies against *Ixodes ricinus* tick infestations 24 hours after each spot-on application of Vectra 3D or a comparator product (CP) (containing permethrin and imidacloprid in fixed combination). Repellent efficacies of 100% and 99% were found for the investigational medicinal product and the comparator product, respectively.

In a four week dose confirmation study, dogs were infested before treatment (Day –2) and then at weekly intervals up to Day 28 with 50 unfed *Ixodes ricinus* ticks. The three treatment groups received either Vectra 3D or a comparator product containing either fipronil and S-methoprene (CP1) or permethrin and imidacloprid (CP2) in fixed combination. Adequate immediate acaricidal efficacy on Day 2 post-treatment could not be shown for either the investigational medicinal product or for the two comparators. The repellent activity, however, at 24 hours after each weekly infestation lasted for at least three weeks (96-99%) in both the IVP and CP2 groups. In the fourth week (Day 28), the repellent efficacy was marginally below the threshold of 90% in both the IVP and CP2 (85% and 89%) groups. Nevertheless, both the anti-feeding efficacy and the acaricidal efficacy at 48 hours post-

infestation were adequately demonstrated for the entire four week study period (IVP: 94–100%, CP2: 97–100%). CP1, showed satisfactory acaricidal efficacy ≥90% for 2 weeks (Day 9: 97%, Day 16: 90%) but insufficient repellency throughout the study (9-67%).

A 5 week dose confirmation study in dogs was provided to confirm the t efficacy of the final formulation with or without dinotefuran (positive control) at the recommended dose band. Immediate acaricidal efficacy of 91% was observed in the Vectra 3D group compared to 86% in the positive control group. No other differences between the groups were observed from Day 9 to Day 37: Results were > 90% efficacy at each time point until Day 30, and below 80% on Day 37. As regards anti-attachment, anti-feeding, and acaricidal efficacies, there were > 90% efficacies, based on arithmetic means, in both treatment groups, up to and including Day 30.

Results confirm the repellent and acaricidal efficacy of the product in dogs against *Ixodes ricinus* ticks for 4 weeks.

3.2 *Dermacentor reticulatus* (adults)

A short term dose confirmation study was initiated to determine the repellent efficacy of the product against *D. reticulatus* on Day 2 post-treatment. Tick infestations (n=50) were conducted on Day 1. A comparator product (CP) containing permethrin and imidacloprid in fixed combination was run in parallel as a positive control. Repellent (anti-attachment, anti-feeding) efficacies of 100% (Vectra 3D) and 89% (CP) were calculated on Day 2.

In a 4 week dose confirmation study, adult dogs were infested before treatment (Day –2) and then at weekly intervals with unfed *Dermacentor reticulatus*. Two treatment groups were treated with either Vectra 3D or a comparator spot-on product containing permethrin and imidacloprid in fixed combination. A negative control group run in parallel. Adequate acaricidal efficacy at (Day 2) could not be detected for both treatment groups (< 90%) and did not support a treatment claim. Apart from Day 23, where only 85% efficacy was calculated, a persistent acaricidal activity of 90–95% at 48 hours post-infestation was found throughout the four week study period investigated. The comparator product showed sufficient acaricidal efficacy for the entire study period tested (98-100%, arithmetic mean*).* The anti-attachment activity (repellent efficacy) of Vectra 3D at 48 hours post-infestation varied during the study period tested (82-94%).

In a two month dose confirmation study, the persistent acaricidal and repellent efficacy of the product with and without dinotefuran was tested in dogs artificially infested with *Dermacentor reticulatus* once a week*.* Dogs were treated twice on Day 1 and Day 31. A positive control (1–2.5 ml imidacloprid and permethrin) was run in parallel. Following the first treatment, study results of both treatment groups did not demonstrate an immediate acaricidal efficacy at 48 hours post-treatment (60%). Thereafter, the product, as well as the comparator product, reduced the mean tick burden by more than 90% when assessed 48 hours post-infestation on Day 9, Day 16 and Day 23 compared to the untreated control. On Day 30, acaricidal efficacy remained below the threshold (at 83% and 41%, respectively).

Following the second treatment cycle starting on Day 31, the product reduced the mean tick burden by more than 90% when assessed 48 hours post-infestation up to Day 44. On Day 51 and Day 58 the calculated efficacies in the Vectra 3D group were, however, only 78% and 88%, respectively. The comparator product showed > 90% persistent efficacy throughout the second treatment period.

Based on the study results the claimed acaricidal and repellent efficacy against *Dermacentor reticulatus* ticks for up to three weeks is justified.

3.3 *Rhipicephalus sanguineus* (adults)

One short term controlled dose confirmation study was conducted to determine the repellent (anti-attachment) and acaricidal (killing) activity in dogs two days after application of Vectra 3D and one day post-infestation with *Rhipicephalus sanguineus*. A comparator product containing permethrin and imidacloprid in fixed combination was run in parallel. Both, repellent and acaricidal efficacies of > 90%, based on arithmetic means, could be demonstrated for the IVP and the CP. However, it was noted that a few live and attached ticks were found in dogs in both treatment groups.

In a controlled 4 week dose confirmation study, 4 groups of adult dogs were each infested with 50 unfed *Rhipicephalus sanguineus* before treatment (Day –2) and then at weekly interval up to Day 28. Three treatment groups were treated either with Vectra 3D or with comparator products containing either fipronil and S-methoprene (CP2) or permethrin and imidacloprid (CP1) in fixed combination. One control group was run in parallel. Adequate immediate acaricidal efficacy at Day 1 (Vectra 3D: 9.8%, CP1 5.7%, CP2: 9.3%) or Day 2 (Vectra 3D: 53.1%, CP1: 45.5%, CP2: 64.6%) could not be demonstrated for any of the 3 products tested. From Day 7 to Day 28 both the calculated repellent efficacy *in situ* at 24 hours, and the acaricidal efficacy at 48 hours after each re-infestation, always exceeded 90% in the IVP group. The comparator products showed adequate acaricidal efficacy of > 90% for only 3weeks.

The immediate and long term activity of Vectra 3D against *R. sanguineus* can also be drawn from the impact of shampooing or water immersion dose confirmation study already reported for fleas (see above). Dogs were concomitantly infested with *R. sanguineus* ticks on Day –2, 5, 12, 19 and 28. The immediate acaricidal efficacy at 48 hours post-treatment was below 60% in both treatment groups. Both treatment groups showed 95–100% persistent acaricidal or anti-feeding efficacy at 48 hours post-infestation starting from Day 7 (96.7%) up to and including Day 30 (95%). Efficacy results reveal that a single shampooing on Day 14 after treatment, or consecutive weekly immersion with lukewarm water for one month, do not affect the persistent efficacy of the product against *R. sanguineus* ticks.

From the study results provided, the CVMP considered that the claimed acaricidal and repellent efficacy against *Rhipicephalus sanguineus* ticks for one month is justified.

Target animal tolerance

The safety of dinotefuran, pyriproxyfen and permethrin, has been examined in the target animal species dog in several toxicity studies submitted within Part 3 of the documentation. These data are summarised here together with the target animal safety (TAS) data.

A set of oral toxicity tests in young Beagle dogs has been presented with each of the 3 active substances of Vectra 3D administered as single compounds. These data allow for conclusions on the systemic toxicity of each substance under controlled conditions, because oral administration leads to a greater systemic exposition to the substances than the topical (spot-on) route of application for the product.

The topical application of Vectra 3D will result in the administration of lower doses of dinotefuran, pyriproxyfen and permethrin than those which produced specific systemic side effects when administered orally. However, dinotefuran and, in a lesser extent, pyriproxyfen can enter the systemic circulation after topical administration of the product. Systemic availability may be further increased by licking of residues of the applied product from the hair coat.

The safety of the finished product has been further demonstrated when applied topically at the highest recommended treatment dose, as well as at overdoses, and also after repeated administrations. Even

the highest dose (7 applications of 5x the treatment dose in intervals of 14 days) did not produce more than slight adverse effects, which consisted primarily of erythema and transient cosmetic changes at the application sites. This is addressed in section 4.10 of the SPC (and other product literature).

In addition to its skin irritating potential, which was already evident from safety studies in laboratory animals, the product has been classified as an eye irritant on the basis of laboratory animal studies. A related warning is therefore included in section 4.5 of the SPC (and other product literature).

The toxicity of the excipients N-octyl-2-pyrrolidone (NOP) and N-methylpyrrolidone (NMP) has been discussed in the dossier. NMP has shown teratogenic and developmental effects after oral and dermal application in several developmental studies in rats and rabbits. Appropriate information is therefore included in section 4.7 of the SPC (and other product literature). Topical administration of the vehicle at a concentration of 100% had been associated with frequent skin reactions, as well as with general effects like salivation, tremor, reduced activity and loose or soft faeces in the target animal safety study.

The safety of the finished product has been also addressed during a pharmacokinetic study and in a maximum tolerated dose study, by oral administration of the product at the average dose indicated for topical treatment. Accidental oral uptake of Vectra 3D by dogs has therefore been simulated. Vomiting occurred in all treated dogs shortly after (oral) administration, and also salivation and diarrhoea. Information has been given accordingly in section 4.10 of the SPC (and other product literature).

All experiments on toxicity and tolerance have been performed in young Beagle dogs. The target animal safety study used Beagles which were aged 7 to 8 weeks at the start of the study, therefore the safety of the product has been demonstrated in dogs as young as 7 weeks old. The use of Vectra 3D in dogs less than 7 weeks old has therefore been excluded in section 4.5 of the SPC.

In the field studies, the safety of the product has been examined in dogs of a great variety of other breeds and of all ages.

The safety assessment of Vectra 3D has been completed by pharmacovigilance data from the US where the product is already marketed (having been authorised by the EPA). Findings from more than 700 dogs demonstrated that most side effects were of minor severity. Forty-one per cents of the observed side effects associated with the application of Vectra 3D were related to dermal reactions. They consisted of erythema, pruritus, dermatitis and inflammation. In addition, general effects such as emesis in 6% of the cases, and lethargy, anorexia, alopecia, restlessness and hyperactivity, anxiousness/nervousness and diarrhoea in 3–4% of the cases each were reported. The applicant has re-evaluated these data in line with the EU guidelines on pharmacovigilance for medicinal products for veterinary use (Volume 9B of the Rules Governing Medicinal Products in the EU). The CVMP agreed the information on gastrointestinal adverse events, such as vomiting or diarrhoea, for inclusion in the SPC (section 4.6) and other product literature.

Field trials

One GCP multi-centre, randomized, blinded positive-controlled field study with two arms, one for fleas and one for ticks, was conducted in France, Germany, Hungary and Portugal, between April and October 2010. Vectra 3D was used as the test product. Although no negative control group was included, it can be anticipated that flea and tick challenge would have prevailed during that study period. The study was well conducted according to current standards and guidance.

A comparator spot-on product authorised in the EU and containing fipronil and (S)-methoprene (Frontline Combo) was used as a positive control. This positive control product has however insecticidal

and larvicidal/ ovicidal activity, but no repellent (anti-feeding) activity, and is therefore considered of limited value for comparison.

Vectra 3D was administered once at the recommended dose, according to the product literature.

A total of 485 dogs suffering from tick and/or flea infestations were enrolled and these included different breeds, ages and weights, representative of the target population.

Flea species identified at the beginning of the study were both *C. felis* and *C. canis*. The most common tick species present prior to treatment were *I. ricinus, D. reticulatus* and *R. sanguineus.* The distribution pattern of parasite species differed among the countries included. The most frequent tick species in France was *D. reticulatus* (51%) and *I. ricinus* (44%), in Germany was *I. ricinus* (91%), in Hungary *I. ricinus* (90%) and *D. reticulatus* (57%), and in Portugal *R.* spp*.* (87%). The most frequently identified flea species was *C. felis* (52%) and *C. canis* (47%). The most frequently identified flea species in France and Germany was *C. felis* (90% and 87% respectively), in Hungary *C. canis* (83%) and in Portugal *C. felis* (47%) and *C. canis* (33%).

Results for ticks:

In the per protocol (PP) population (125 test animals, 64 control animals), treatment with Vectra 3D resulted in a mean tick reduction of 93.67%, compared to 96.89% in the control group over the entire study period of 4 weeks. The results are largely confirmed in the intention to treat (ITT) population (126 test animals, 64 control animals).

The mean percentage tick reduction by study day 7, 14, 21 and 28 was 93.9%, 93.6%, 95.0%, and 88.7% in the test group, and 94.8%, 94.7%, 97.8% and 96.9% in the control group.

Results for fleas:

In the PP population (111 test animals, 57 control animals), the treatment with Vectra 3D resulted in a mean flea reduction of 79.41%, compared to 56.78% in the control group over the entire study period of 4 weeks. The results are largely confirmed in the ITT population (122 test animals, 64 control animals). When excluding the French study sites where the infestation pressure was distinctly higher as compared to the other study sites, the treatment with Vectra 3D resulted in a mean flea reduction of 84.38% compared to 85.66% for the control group for the entire study period. Again, these results were largely confirmed in the ITT population. The mean percentage reduction of fleas by study Day 14 and Day 28 was 80.8% and 73.1% for the test group, and 39.2% and 79.7% for the control group. The results were also confirmed in the ITT population. When excluding the French study sites, the mean percentage flea reduction was 94.1% and 85.7% on Day 14 and Day 28 for the test group, compared to 84.3% and 98.0% for the control group. As for the ticks, the methods of calculation were not in line with the recommendation of the relevant EU CVMP guideline (Testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats, EMEA/CVMP/EWP/005/2000-Rev.2). According to the CVMP's calculations, the efficacy rates of the test product were always below 95%, with and without the French study sites. The positive control product showed efficacy rates above 95% only on Day 28, and only when French sites are excluded.

Non-inferiority of the test product compared to the positive control product was shown for the treatment and prevention of tick infestations for the entire study period and for flea infestations for 14 days, only, by using a non inferiority margin of – 15%. The lower than expected efficacy against fleas, even when the French study sites were excluded, implies that the favourable results obtained in the laboratory studies was not confirmed during clinical use of the product. The reason for the low efficacy of the test product in France could not be determined.

Given the lower than acceptable of the test product, especially in the case of massive flea infestations, additional advice to clean and treat the home environment with an appropriate insecticide is included in the SPC and other product literature.

The persistent efficacy against ticks was well demonstrated under these field conditions. Since the distribution pattern of tick species initially identified is different between the countries involved, the applicant also calculated the results per tick species separately for each country involved in the clinical field study. However, the number of tick species collected at each time point post-treatment was too small to draw any firm conclusions regarding the effect for each of the species. Nevertheless, based on the results of the laboratory studies, *D. reticulatus* is regarded as the least sensitive species against the product (permethrin) showing adequate acaricidal and/or repellent efficacy at 48 hours post-infestation for only 2–3 weeks as indicated in the SPC. In conclusion, there is insufficient treatment efficacy at 48 hours post-treatment against the tick species *D. reticulatus*.

All dose confirmation studies were conducted on dogs weighing 10–25 kg and using dosages corresponding to 2 times the minimum recommended dose or more. In order to support the efficacy of Vectra 3D in dogs of different weights receiving different quantities of the product and in accordance with the SPC, the applicant also presented the results of the field study separately for the dogs of different body weights as per the SPC, with special attention to dogs at the upper weight range for a certain applicator size, and therefore receiving the minimum recommended dosage. The summary statistics provided did not indicate any reduction in the efficacy against fleas or ticks related to body weight.

Clinical signs of flea allergic dermatitis were reduced in more than 90% of animals treated with either the test product or the reference product by the end of the study. Therefore, the data is acceptable in supporting the use of Vectra 3D as part of a treatment strategy for the control of flea allergic dermatitis.

The incidence of adverse events was low in both the test and control groups. Adverse events were mostly mild to moderate, transient local reactions at the application site, including colour changes of the hair coat and hypersensitivity of the skin. The application site reactions are adequately reflected in the SPC. A second GCP-compliant, well-conducted, multi-centre controlled, randomised, blocked, blinded, clinical field study performed in 2011 was submitted to confirm the efficacy of this fixed combination product against flea infestations. Study sites were located in France, Germany, Hungary and Portugal. The study design is similar to the earlier clinical field study, but treatments were administered three times at monthly intervals, and flea counts were performed at 14 day intervals up to day 84. As in the previous study, Vectra 3D was compared to EU-authorised comparator spot-on product containing fipronil plus (S)-methoprene based on non-inferiority of the average efficacy over the entire study period. As a result the mean flea count reduction in the field over the entire study period was 94.8% for the PP population (n=117) treated with Vectra 3D and 96.7% (n=57) for the comparator product, based on arithmetic means. Non-inferiority of Vectra 3D, based on 15% non-inferiority margin, was confirmed for each post-treatment period and for the total study period as well. Vectra 3D was well tolerated under the conditions of this field study.

The different efficacy outcomes in the two field studies demonstrate that external uncontrolled factors may affect the treatment outcome during clinical use.

Other studies

Studies on the impact of shampooing or water immersion showed that water immersion 48 hours after treatment, weekly repeated for one month, as well as shampooing 2 weeks after treatment do not affect the efficacy of the product.

Overall conclusion on efficacy

Vectra 3D is intended for use on dogs for the treatment and prevention of flea and tick infestations, and the prevention of mosquito, stable fly and sand fly bites with a persistent insecticidal activity against *Aedes* mosquitoes and stable fly. The mode of action and spectrum of activity of each of the active ingredients have been well described.

Based on the entire data package provided, the anticipated clinical benefit of this product, resulting from the addition of dinotefuran was demonstrated in one laboratory study on dogs which showed a prolongation of the duration of efficacy against *C. canis* fleas to four weeks, compared to the duration of efficacy of two weeks for permethrin/pyriproxyfen alone.

A faster onset of activity within four to twelve hours after first administration of the product, and a pronounced persistent adulticidal efficacy of Vectra 3D against fleas, attributable to dinotefuran, has been demonstrated *in vitro* and *in vivo*, but a clinical benefit resulting from this, such as e.g. better control of flea infestations, improved animal scratching relief and animal welfare has not been substantiated by data.

The dosage of Vectra 3D has been well established in a set of confirmatory laboratory studies on dogs with artificially flea and tick infestations, and also to experimental exposure to mosquitoes and stable flies.

The studies confirm the treatment and prevention of flea infestations, with a rapid onset of kill resulting in an adequate adulticidal efficacy within 12 hours after application on the first day of treatment and a persistent pulicidal efficacy within 2 hours and lasting for 4 weeks. The larvicidal/ovicidal effect was shown to persist for 2 months after administration.

A persistent acaricidal and repellent efficacy claim against tick infestations (*Rhipicephalus sanguineus* and *Ixodes ricinus* for 4 weeks, and *Dermacentor reticulatus* for up to three weeks) was adequately demonstrated.

With regard to sand flies, mosquitoes and stable flies a number of negatively controlled dose confirmation studies have been provided showing persistent repellent (anti-feeding) activity in sand flies (*Phlebotomus perniciosus*), mosquitoes (*Culex pipiens, Aedes aegypti*) and stable flies (*Stomoxys calcitrans*) for four weeks post-treatment. Persistent insecticidal activity for four weeks against mosquitoes (*Aedes aegypti*) and stable flies (*Stomoxys calcitrans*) has also been shown. The contribution of dinotefuran to the efficacy of the product against flies and mosquitoes has not been investigated by the applicant. However, when compared to permethrin only products which are already authorised in the EU, the spectrum of activity of this product against flies and mosquitoes is wider, and the duration of efficacy is longer.

A GCP-compliant multi-centre, controlled, randomized, blinded field study with two arms, one for ticks and one for fleas, was provided. The study was designed and conducted according to the relevant guidelines. Non-inferiority was proven for tick infestations for the entire study period. For flea infestations non-inferiority was proven for a period of only 14 days, based on a predefined non inferiority margin of –15% points because of a lower than expected efficacy of the test product, in particular at study sites where the flea infestation rate was very high. Therefore, additional advice to clean and treat the home environment with an appropriate insecticide is included in the SPC.

Clinical signs of flea allergic dermatitis were reduced in more than 90% of dogs treated with either the test product or the reference product by the end of the study. Therefore, the data is considered acceptable to support the use of Vectra 3D as part of a treatment strategy for the control of flea allergic dermatitis.

A second European GCP-compliant, multi-centre controlled clinical field study performed in 2011 confirmed the adequate efficacy of this fixed combination product against flea infestations following repeated administrations at monthly intervals for 3 months. The mean flea count reduction over the entire study period was 94.8% for the study animals treated with Vectra 3D and 96.7% for the study animals treated with an EU authorised comparator product containing fipronil and (S)-methoprene. Non-inferiority of Vectra 3D, based on 15% non-inferiority margin, was proven for each post-treatment period as well as for the whole study period.

The different efficacy outcomes in the two field studies demonstrate that external uncontrolled factors may affect the treatment outcome during clinical use.

Vectra 3D proved to be well tolerated in both field trials as the incidence of adverse events was low. Adverse events were mostly mild to moderate, transient local reactions at the application site, including colour changes of the hair coat and hypersensitivity of the skin. These application site reactions are adequately reflected in the SPC (and other product information). Information on gastrointestinal adverse events, such as vomiting or diarrhoea, which have been reported are included in the SPC (section 4.6) and other product literature.

Part 5 – Benefit-risk assessment

Introduction

Vectra 3D spot-on solution contains a new combination of three active substances (dinotefuran, permethrin and pyriproxyfen). Dinotefuran is a new substance not yet authorised within the European Union. The route of administration is spot-on use. The target species is dogs. The product is presented in single-dose spot-on applicators of five different volumes (strengths) indicated for dogs in five specified body weight ranges. The application is supported by a full dossier.

Benefit assessment

Direct therapeutic benefit

Vectra 3D is intended for the treatment and prevention of flea infestations (*C. felis, C. canis*) and for the prevention of tick infestations (*Rhipicephalus sanguineus, Dermacentor reticulatus, Ixodes ricinus*). The product prevents the multiplication of fleas by ovicidal and larvicidal activity for two months, and can also be used as part of the treatment strategy of flea allergic dermatitis. The product is also effective for the prevention of sand fly (*Phlebotomus pernicious*) bites, mosquito (*Culex pipiens, Aedes aegypti*) bites and the prevention of stable fly (*Stomoxys calcitrans*) bites in dogs. The minimum recommended dose is 46.6 mg/kg bw permethrin, 0.6 mg/kg bw pyriproxyfen, and 6.4 mg/kg bw dinotefuran. Depending on the adult parasite species, the claimed persistent efficacy is for 3 to 4 weeks. The possibility for repeated treatments once a month is provided in the product literature.

The fixed combination includes permethrin (insecticide, acaricide), dinotefuran (insecticide) and pyriproxyfen (insect growth regulator), to provide insecticidal, acaricidal and ovicidal/larvicidal activities in the product, to achieve a broad spectrum of activity. Permethrin and pyriproxyfen are well established substances in veterinary medicine and their combination is justified, that is, broadening of the spectrum of activity. The contribution of dinotefuran to the overall efficacy of the product is based on a synergistic effect on flea mortality which was demonstrated *in vitro* when dinotefuran was administered in conjunction with permethrin. The clinical benefit resulting from the combination of dinotefuran with permethrin was demonstrated in one laboratory dose confirmation study on dogs, which showed a prolongation of the duration of efficacy against *C. canis* fleas for four weeks compared

to the duration of efficacy of two weeks for permethrin/pyriproxyfen. However, this potential advantage was not further explored under field conditions. Additional benefits resulting from the synergistic activity include a faster onset of insecticidal activity within four to twelve hours on the first day after treatment application and a pronounced persistent adulticidal activity on fleas knocked down by permethrin. A potential clinical benefit resulting from these additional benefits, such as better control of flea infestations, improved relief from scratching and animal welfare, has however not been substantiated by data.

Well conducted GCP-compliant controlled clinical studies demonstrated that Vectra 3D is effective in the treatment and prevention of flea and tick infestations in dogs with a persistent efficacy of four weeks and the product proved to be non-inferior to an EU-authorised ectoparasiticide spot-on formulation containing fipronil and (S)-methoprene.

Well conducted, GCP-compliant, negative controlled clinical studies demonstrated that the product has persistent repellent and insecticidal activity against sand flies (*Phlebotomus perniciosus*), mosquitoes (*Culex pipiens and Aedes aegypti*) and stable fly infestations (*Stomoxys calcitrans*) for four weeks. With regard to the spectrum of activity and the duration of efficacy against flies and mosquitoes, this product is superior to products authorised in the EU which contain permethrin only.

Additional benefits

None.

Risk assessment

Quality

As regards quality, the formulation and manufacture of Vectra 3D is well described and controlled, and adequate specifications have been defined.

Target animal safety

The product proved to be well tolerated in dogs.

As the product is an eye irritant probably due to dinotefuran, a warning is indicated in the SPC to avoid contact between the product and the eyes of the dog. After topical application skin reactions (erythema, pruritus, and cosmetic effects at the application site) may develop which are adequately addressed in the SPC. In addition, systemic effects may occur from penetration of the active ingredients through the skin, from licking residues of the product from the hair coat or from accidental oral uptake of the product. Related side effects may consist of salivation, vomiting, soft stools and diarrhoea, representing symptoms of gastric upset. One of the excipients in Vectra 3D, N-methylpyrrolidone, may to a certain extent contribute to the local and systemic side effects.

These potential adverse reactions possible at regular treatment or after overdose or accidental ingestion of the product have been sufficiently addressed in the product literature.

Vectra 3D may be dangerous for cats. Intoxication may arise when cats are treated with the dog product or when cats are kept together with treated dogs. This is adequately addressed in the SPC.

User safety

The user safety risk assessment showed risks during the acute exposure of the user and the acute accidental exposure of children towards permethrin. Adequate precautionary warnings are included in the SPC that children must not handle treated dogs for at least four hours after administration of the product, and that treated dogs should not be permitted to sleep with their owners, especially children.

A health concern regarding the chronic non-dietary hand-to-mouth exposure of children (toddlers) towards permethrin was excluded following a published SOP of the US EPA. These user safety warnings are used on other EU-authorised spot-on products containing comparable amounts of permethrin, and are considered sufficient to minimise the risk for children.

The child resistant nature of the primary packaging was adequately demonstrated.

Environment

Any exposure of the environment associated with the use of this product on dogs is likely to be via transfer from the animal's coat to the terrestrial and/or aquatic environment. As regards the known toxicity of permethrin and pyriproxyfen to aquatic invertebrates, appropriate risk mitigation measure is included in the SPC to minimise the risk to aquatic environment.

Resistance development

There is at present no indication that the use of Vectra 3D would select dual resistance of the ectoparasite species claimed against dinotefuran and permethrin.

Risk management or mitigation measures

Appropriate risk management measures regarding the user, including safety warnings in the SPC and other product literature are in place.

Regarding safety in the target species, adverse effects detected in the target animal safety/field studies and attributed to the product are detailed in the SPC.

Evaluation of the benefit-risk balance

Permethrin, an insecticide and acaricide, and pyriproxyfen, an insect growth inhibitor, are well established in veterinary medicine and their combination is justified based on the broadening of the spectrum of activity.

The anticipated clinical benefit resulting from the synergistic activity of permethrin and dinotefuran as demonstrated *in vitro* is considered limited, however, no outstanding risk could be identified for dinotefuran.

Although the benefit of dinotefuran in the triple combination appears to be limited, the product has been shown to have a positive benefit-risk balance overall.

The product has been shown to be efficacious for the treatment and prevention of flea infestations for four weeks and can be used as part of the treatment strategy of flea allergic dermatitis. The product proved to be efficacious in the treatment and prevention of tick infestations for three to four weeks, depending on the tick species. The product proved to be efficacious in the prevention of bites caused by sand flies, mosquitoes and stable flies and also has persistent insecticidal activity against *Aedes aegypti* and *S. calcitrans* for 4 weeks.

The formulation and manufacture of Vectra 3D is well described and the specifications set will ensure that product of consistent quality will be produced.

Tolerance in the target species has been appropriately investigated and the potential adverse reactions are clearly detailed in the SPC.

Vectra 3D presents an acceptable risk for users and the environment, and appropriate warnings have been included in the SPC.

Conclusion

The overall benefit-risk evaluation for the product is deemed positive with a sufficiently clear and complete SPC and product literature.

Based on the original and complementary data presented, it is concluded that the quality, safety and efficacy of Vectra 3D were considered to be in accordance with the requirements of Directive 2001/82/EC.

Divergent position to the CVMP opinion on Vectra 3D (EMEA/V/C/002555)

Taking into consideration the legal advice given by EMA regarding how to conclude on the benefit risk balance for a combination product, and all documentation provided by the company to support Vectra 3D the members signing the divergent opinion view the benefit risk balance of Vectra 3D as negative. It is considered that the benefit of adding dinotefuran to permethrin has not been substantiated to a sufficient extent, and thus the combination of the two substances is not justified.

The negative conclusion is based on the following circumstances:

- It has been demonstrated in laboratory studies that the adding of dinotefuran to permethrin reduces the time to obtain sufficient effect against fleas (>95% efficacy) by about 12 hours as compared to permethrin-only. Furthermore, it has been demonstrated that survival rate is reduced among dislodged fleas when dinotefuran is added to permethrin.

  The clinical benefit of these effects is regarded only speculative on the following grounds:

  1. The reduced time to reach sufficient efficacy is only noted during the first day of treatment, whereas during the rest of the 28 days-long efficacy period, the effect of the combination is comparable to that of permethrin alone.
  2. The applicant has not provided data to verify that this minor improvement translates into any potential clinical benefit for the dog, with regard to hastened relief of clinical symptoms (e.g. discomfort, itching, scratching, dermatitis signs).
  3. Given that dogs are constantly re-infested with fleas from the environment, it is deemed unlikely that a quicker onset of effect during the first day after treatment would provide any detectable clinical benefit.
  4. The reduced survival rate among dislodged fleas attributed to the adding of dinotefuran has not translated into a clinical benefit with regard to the combating of flea infestation on the dog and in the environment. By contrast, similar advice as for currently authorized products regarding sanitary actions to reduce flea infestation in the immediate surrounding is already recommended for this product.
  5. The clinical data is restricted to the verification that the efficacy of the product is comparable to previously authorized products (non-inferior). In one of the two clinical studies, efficacy against fleas was insufficient for Vectra 3D and this was claimed to be due to a high flea infestation pressure in that region. This puts clinical benefit of the combination into further doubt.
  6. A synergistic effect regarding the treatment of fleas has been demonstrated when permethrin and dinotefuran is combined which would allow for a reduced permethrin dose. However, this synergistic effect is not utilized since the indication also contains ticks and it is mentioned that a higher dose of permethrin is necessary to obtain sufficient effect against ticks.

- According to one laboratory study the efficacy towards *Ct canis* was 4 weeks when dinotefuran was added to permethrin, as compared to 2 weeks with permethrin only. However, it is highly

uncertain that the prolonged effect noted in this study is due to the adding of dinotefuran, given that there are currently permethrin-only products on the market which are authorized for this species with effect duration for up to 4 weeks. Furthermore, there is no information available which indicates that *Ct canis* would inherently be less sensitive to permethrin than *Ct felis*.

- The benefit of adding dinotefuran to permethrin to comb*at flies* and mosquitoes cannot be evaluated due to lack of studies comparing the combination to permethrin-alone. There are currently permethrin-only products on the market containing these indications and where the dose of permethrin is the same as in Vectra 3D. This questions the appropriatenessof adding dinotefuran to permethrin.
- Broadening of the spectrum to include the stable flies *Stomoxys calcitrans*, and the mosquitoes *Culex pipens* and *Aedes aegypti* is not regarded to be a benefit that could be the sole motive for a positive opinion. Furthermore, for these species the benefit of adding dinotefuran to permethrin has not been explored.

The product has been shown to be efficacious for the treatment and prevention of flea and tick infestation, and the infestations of some flies and mosquitoes species. However, it has not been evaluated whether or not dinotefuran is actually needed to obtain the claimed effect for some species included in the indication and furthermore, with regard to fleas where a synergistic effect between dinotefuran and permethrin is confirmed in laboratory studies there is a lack of data to substantiate that this translates into a clinically meaningful effect. Based on the limited effect noted and on the knowledge regarding the epidemiology of flea infestation it is unlikely that a clinical effect would be detectable. Thus sufficient data are not available which justify the adding of dinotefuran to permethrin and pyroproxifen. Due to this it would not be justifiable to expose animals to this new substance and thus the benefit-risk balance is negative.

London, 10 October 2013

Keith Baptiste

Eva Lander Persson

Hans Kristian Østensen

Jean-Claude Rouby