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SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP Assessment Report for NEXGARD SPECTRA (EMEA/V/C/003842/0000)

International non-proprietary name: Afoxolaner / milbemycin oxime

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

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Introduction

On 16 January 2014 the applicant MERIAL submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for NEXGARD SPECTRA, through the centralised procedure falling within the Article 3(2)a of Regulation (EC) No 726/2004 (new active substance).

The eligibility to the centralised procedure was agreed upon by the CVMP on 18 July 2013. NEXGARD SPECTRA is a fixed combination product containing afoxolaner and milbemycin oxime. This combination of active substances was not previously authorised and it is therefore considered a new active substance which is not authorised in the Community. The rapporteur appointed was D. Murphy and the co-rapporteur S. Srčič.

The applicant applied for the following indication: Treatment of flea, tick and nematode infestations and prevention of heartworm disease and angiostrongylosis in dogs.

NEXGARD SPECTRA chewable tablets contain afoxolaner and milbemycin oxime, and are available in five different strengths for dogs of different weights to achieve doses of 2.50–5.36 mg/kg bodyweight (bw) of afoxolaner and 0.50–1.07 mg/kg bw of milbemycin oxime. The tablets are packed in blisters, which are then packed into outer cartons containing either 1, 3 or 6 tablets. The route of administration is oral use.

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

On 6 November 2014 the CVMP adopted an opinion and CVMP assessment report.

On 15 January 2015, the European Commission adopted a Commission Decision granting the marketing authorisation for NEXGARD SPECTRA.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant provided a detailed description of the pharmacovigilance system (dated 31 October 2013) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

NEXGARD SPECTRA chewable tablets are manufactured by MERIAL Saude Animal Ltda, São Paulo, Brazil. Secondary packaging and batch release for the European Union (EU) is carried out by MERIAL Toulouse, France.

All relevant sites have valid manufacturing authorisations or valid good manufacturing practice (GMP) certificates, as appropriate.

For each of the active substances a satisfactory declaration concerning GMP compliance of the active substance manufacture has been issued by the qualified person at the site of batch release of the finished product. The declarations are issued on foot of an audit of the site.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and the GMP certification of the manufacturing sites were considered in line with legal requirements.

Part 2 - Quality

Composition

NEXGARD SPECTRA comprises five strengths of chewable tablets for dogs. The tablets contain two active substances: afoxolaner and milbemycin oxime. The five tablet sizes correspond to 9.375 mg, 18.75 mg, 37.5 mg, 75.0 mg and 150 mg of afoxolaner and 1.875 mg, 3.75 mg, 7.5 mg, 15 mg and 30 mg of milbemycin oxime respectively. The chewable tablets have a mottled red to reddish brown colour and are circular (lowest strength only) or rectangular shaped. The tablets are homothetic, having the same percentage composition, and are compressed from a common blend. No overages are included in the formulation.

The chewable tablets contain the following excipients: macrogol 400, triglycerides medium chain, povidone K-30, macrogol 4000, glycerol, macrogol 15 hydroxystearate, maize starch, soy protein fines, beef braised type flavour, citric acid monohydrate, butylhydroxytoluene. Potassium sorbate is used in initial formulations of the product as an antimicrobial preservative but subsequent preservative efficacy testing demonstrated the final formulation to be self-preserving and potassium sorbate was removed. Butylhydroxytoluene is an antioxidant and as flavouring agent a beef braised type flavour is used. The excipients are considered typical for this type of dosage form and are therefore acceptable.

Container

The primary packaging material is a blister card made of Aclar laminated PVC film with an aluminium foil-paper backing. Blisters are packaged within an outer cardboard carton which contributes to protection of the product from light.

Development pharmaceutics

The aim of developing chewable tablets was to obtain a product appealing to the target species (by making it chewable and adding a flavouring agent) and easy to administer by the owner. The tablet is manufactured in five strengths for different dog weight ranges. The development of the product has been described, the choice of excipients is justified and their functions are explained. The inclusion of a preservative in the formulation was found not to be necessary to ensure acceptable microbiological quality of the product and it was therefore removed from the final commercial formulation. An overview has been provided of the formulations that were used in canine palatability studies and the final formulation used in pivotal clinical studies. Unlike the final commercial formulation where there is no preservative, all clinical studies have been done with a formulation that included the preservative potassium sorbate at a level of 0.3%. Omission of the preservative in the commercial formulation is not expected to have any impact on the quality, safety or efficacy of the tablets.

Method of manufacture

The manufacturing process of the product involves different pre-mixing steps of different combinations of

components introduced sequentially into a mixer before final mixing of the dough. The dough is divided into portions which are then processed into the forming machine to obtain the formed tablets which are subsequently matured in ovens. Validation data are not yet available for the commercial batch size. The manufacturer has considerable experience of the manufacturing process as it is very similar to the process used for several existing products. By virtue of that experience, the process can be considered a standard one for this manufacturer.

A satisfactory validation protocol has been submitted. The information from the process validation studies shall be available for verification post authorisation.

Control of starting materials

Active substances

Afoxolaner

The active substance afoxolaner is a white to off-white solid of the isoxazoline class. The active substance possesses one chiral centre resulting in the formation of a racemate. Full documentation on the active substance has been included in the dossier.

The synthetic process is carried out in 3 chemical steps and uses 3 as starting materials for the manufacture of afoxolaner. The critical steps have been described and are considered acceptable for the synthesis. The discussion on the impurity profile of the active substance is thorough. Possible impurities and their occurrence in starting materials, intermediate and the final active substance have been discussed. The specifications of the intermediate are provided and are acceptable.

The choice of the main starting materials is considered acceptable.

The active substance specification has been established in-house by the applicant. The specification is acceptable in view of the route of synthesis and the various quality guidelines. The limit for impurity Q6S72 is qualified and specified at not more than (NMT) 1.0%. Five related substances, tetrahydrofuran and acetonitrile are specified in active substance specification. The limits for the unspecified impurities are in compliance with thresholds in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) GL10 on impurities in new veterinary drug substances. Limits for total impurities and the assay limit are considered acceptable. Since afoxolaner is in solution within the finished product, particle size distribution is not relevant and it is not included on the specification. Batch analytical data demonstrating compliance with the active substance specification have been provided for a number of commercial scale batches. The data demonstrate consistency in the manufacturing process and compliance with the proposed active substance specification.

The commercial packaging of afoxolaner consists of a primary packaging of double polyethylene bags and a secondary packaging of high-density polyethylene (HDPE) drums.

Appropriate stability data according to VICH conditions is provided to support the proposed re-test period of 36 months, when stored below 30 °C.

Milbemycin oxime

The active substance milbemycin oxime is a white to off-white powder from the group of milbemycins, it is a mixture of milbemycin A3 5-oxime and milbemycin A4 5-oxime at an approximate ratio of 20:80. Data in support of this active substance is provided in an active substance master file (ASMF).

The manufacturing of milbemycin oxime consists of two major stages: the fermentation stage to produce milbemectin from *Streptomyces milbemycinicus*, and the subsequent chemical synthesis stage to manufacture milbemycin oxime (two synthetic steps). Characterisation of the producer micro-organism is described and potential impurities from the fermentation process have been demonstrated to be absent in the final milbemycin oxime. The critical steps in the synthetic step have been described and are considered acceptable for the synthesis.

The active substance specification has been established in-house by the ASMF holder and the applicant. The specification is acceptable in view of the route of synthesis and the various quality guidelines. The discussion on the impurity profile of the active substance is thorough. One identified related substance, milbemycin oxime D, is limited on the specification to 2.0% which is above the VICH qualification limit. However, it is structurally very similar to the milbemycin A3 and A4 oximes which are of low oral toxicity. Taking this into account a limit of 2.0% on the specification is unlikely to pose a safety concern. Other impurity limits are in compliance with thresholds in VICH GL10 on impurities in new veterinary drug substances. Solvents used during the manufacturing process are controlled by the active substance specification or the absence of a limit has been suitably justified. Since milbemycin oxime is in solution within the finished product, particle size distribution is not relevant for this product and is not included on the applicant's specification. Batch analytical data demonstrating compliance with the active substance specification have been provided for a number of commercial scale batches. The data demonstrate consistency in the manufacturing process and compliance with the proposed active substance specifications.

The active substance is filled into a 2-ply polyethylene bag and heat sealed. This bag is placed in a secondary aluminium foil bag, sealed and placed in an aluminium container and sealed.

The stability data provided support the proposed retest period of 24 months in the commercial packaging with no specific storage precautions.

Excipients

The excipients macrogol 400, triglycerides medium-chain, povidone K-30, macrogol 4000, glycerol, macrogol 15 hydroxystearate, maize starch, citric acid monohydrate and butylhydroxytoluene comply with their current European Pharmacopoeia (Ph. Eur.) monographs.

The excipients soy protein fines and beef braised type flavour comply with in-house specifications. The tests and acceptance criteria are considered appropriate to ensure the quality of these excipients.

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

The active substance manufacturer for afoxolaner has provided a transmissible spongiform encephalopathy (TSE) statement to certify that no raw materials used in its synthesis are of animal origin and that the material complies with the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3).

The active substance milbemycin oxime is produced by fermentation followed by chemical synthesis. The active substance manufacturer for milbemycin oxime has provided a TSE statement to certify that the only materials of animal origin used in its manufacture are porcine peptone and skim milk powder. These materials are 'low to no risk' in accordance with EMA/410/01 rev.3.

No materials of animal origin are used in the manufacture of any of the excipients. Relevant bovine spongiform encephalopathy (BSE)/TSE statements are provided.

Control tests during production

The critical steps of the manufacturing process are monitored to ensure that the process is controlled:

- Preparation of the dough: mixing time after the addition of the pre-mix 2 is controlled which is about 8 minutes.
- Forming of the chews: appearance, shape and individual weight of each chew are controlled.
- Maturation of the chews: individual weight of each chew is controlled. The duration of the maturation phase is not less than 7 hours.

The in-process controls established for this pharmaceutical form are considered acceptable.

Control tests on the finished product

The product specification includes tests for appearance, identity, assay, degradation products, uniformity of dosage units, anti-oxidant content, water content, dissolution and microbial quality. The proposed release and shelf life limits are identical except for assay, degradation products and antioxidant limits. The proposed test parameters and limits are appropriate. The analytical methods have been adequately described and suitably validated. Batch analytical data for eleven pilot scale batches have been provided and include at least one batch of each strength. All results comply and are consistent. These batches have been produced at the proposed site of manufacture.

Stability

Stability studies at long-term conditions at 25 °C/60% relative humidity (RH) and 30 °C/65% RH (24 months) and accelerated conditions at 40 °C/75% RH (6 months) support the proposed shelf life of 2 years. The storage precaution 'Keep the container in the outer carton in order to protect from light' is necessary due to the sensitivity of the product to photodegradation.

Differences in the specification at release and end of shelf life have been adequately justified.

Overall conclusions on quality

The dossier provides a suitable description of the formulation and demonstrates that the manufacturing process leads to a stable product with consistent quality.

As numerous development batches have been manufactured and the manufacturer has considerable experience in the manufacture of this particular dosage form, the submission of the protocol for the validation of the manufacturing process with commercial scale batches is considered sufficient.

Neither of the two active substances is described in a pharmacopoeia. Appropriate details of their manufacture and control are provided as are comprehensive specifications for each of them. Most of the excipients are addressed in Ph. Eur. monographs and their specifications are appropriate. There is no monograph in any pharmacopoeia for two of the excipients, but they are not new excipients in veterinary medicinal products. Appropriate and justified specifications are provided for these excipients also.

The finished product is presented in blister packs inside a folding cardboard carton. The outer cardboard carton is functional to improve protection from light as the active substance milbemycin oxime is sensitive to photodegradation.

Finished product specifications limits at release and shelf life are generally appropriate and control appropriate parameters for this dosage form. The analytical methods are well described and validated in accordance with VICH requirements.

Stability data for the active substances and dosage form are sufficient to support the claimed retest periods and shelf life respectively.

Based on the review of the data on quality, the manufacture and control of NEXGARD SPECTRA are considered acceptable.

In addition, the applicant is recommended to perform the manufacturing process validation studies on commercial size batches after authorisation. The data from the validation studies should be available for inspection at the dosage form site.

Part 3 – Safety

Pharmacodynamics

Afoxolaner

Afoxolaner is a member of the isoxazoline family, shown to inhibit insect and acarine ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA). Isoxazolines work by blocking pre- and post-synaptic transfer of chloride ions across cell membranes resulting in its ectoparasitic activity. Prolonged afoxolaner-induced hyperexcitation results in uncontrolled activity of the central nervous system and death of insects and acarines. The binding site is coupled to the avermectin GABA/glutamate chloride channel activator site; however, the binding sites for isoxazolines are distinct and unique among the chloride channel modulators. While the mode of action is demonstrated on insect receptors, both in vitro and in vivo data demonstrate that afoxolaner has no significant interaction with the mammalian GABA receptor.

Milbemycin oxime

The active substance milbemycin oxime is an anthelmintic substance used in dogs alone or in combination (e.g. with praziquantel, lufenuron or spinosad) in several oral veterinary medicinal products authorised throughout the EU. Milbemycin oxime is a member of the macrocyclic lactones family. Milbemycin oxime acts by binding to chloride ion channels in invertebrate nerve and muscle cells. Increased permeability by the cell membrane to chloride ions causes hyperpolarization of affected cells and subsequent paralysis and death of the intended parasites. Milbemycin oxime may also act by disrupting the transmission of invertebrate neurotransmitters, notably GABA.

Potential for interactions

There is no reason to suspect potential interaction in the target animal as afoxolaner and milbemycin oxime bind to distinct and different sites on GABA-gated chloride channels and do not share activity on mammalian receptors.

Data were provided to demonstrate that there is no biologically relevant interaction between the two active ingredients, afoxolaner and milbemycin oxime.

An acute oral toxicity study in rats has been specifically conducted with the combination product. The active substances were dosed alone or in combination in order to define the acute toxicity profile of the combination, as well as to demonstrate a lack of relevant interaction between them. The dose of the combination that was evaluated was 1,070 mg/kg afoxolaner and 203 mg/kg milbemycin oxime which is approximately equal in ratio to the combination product. Based on the results of this study, oral administration of a single dose of afoxolaner either alone or in combination with milbemycin oxime to Crl:CD[SD] rats resulted in the expected afoxolaner-related body weight loss and lower food consumption. Administration of milbemycin oxime alone did not result in any test article-related changes.

Administration of the combination of afoxolaner and milbemycin oxime resulted in effects similar to those observed for afoxolaner alone. That is, the presence of milbemycin oxime in the combination group did not influence the expected effects of afoxolaner.

Milbemycin oxime is a substrate for P-glycoprotein (P-gp) and therefore could interact with other P-gp substrates. The summary of product characteristics (SPC) includes in section 4.8 an adequate statement as regards interactions with substrates for P-glycoprotein (P-gp).

Pharmacokinetics

Pharmacokinetic information on the two active substances, administered alone or in combination was provided in three specific studies. In addition to pharmacokinetic studies using the combination product, proprietary data was provided for afoxolaner alone and published scientific literature for milbemycin oxime alone.

Afoxolaner

Data were provided to establish the pharmacokinetic profile in dogs of afoxolaner when administered alone. Afoxolaner absorption in dogs was fast and bioavailability was high. Absorption was not impacted by the prandial state of the treated dog. Linear kinetics was observed upon multiple monthly afoxolaner dosings, and dose proportionality was established over the range of 1 to 40 mg/kg. Afoxolaner distributed into tissues moderately (volume of distribution (Vd) = 2.68 ± 0.55 l/kg) and was eliminated via metabolism to a hydroxylated metabolite and as parent in the bile. The afoxolaner systemic clearance was low (Cl = 4.95 ± 1.20 ml/h/kg bw).

A good laboratory practice (GLP)-compliant pharmacokinetic study was conducted in 24 dogs in 4 treatment groups to characterise the pharmacokinetic profile of afoxolaner alone and when administered in combination with milbemycin oxime. In this study 2 treatment groups were administered afoxolaner alone. One group received 1 mg afoxolaner/kg bw in the form of a solution injected once intravenously and the other group received 2.5 mg afoxolaner/kg bw orally in the form of chewable tablets once only. A third group was treated with the combination at a dose of 2.5 mg afoxolaner/kg bw and 0.5 mg milbemycin oxime/kg bw in the form of chewable tablets administered orally. A fourth group received milbemycin oxime only as a solution by intravenous route at the dose of 0.3 mg/kg bw. This study demonstrated that the plasma pharmacokinetic profile of afoxolaner is not changed in dogs by the concomitant administration of milbemycin oxime. Consequently, it can be accepted that the absorption, distribution, metabolism and excretion (ADME) profile previously determined for afoxolaner used as a single oral product for dogs is unchanged in the combination product containing afoxolaner and milbemycin oxime. Bioavailability of afoxolaner was 88.3% when administered orally in the combination chewable tablet. The mean maximum concentration (C_{max}) was 1,822 ± 165 ng/ml in plasma found within 2–4 hours (T_{max}).

The relationship of the combination product dose to afoxolaner exposure (dose proportionality) and the pharmacokinetic profile of afoxolaner upon multiple monthly dosing of the combination product were investigated in a study in 8-week old puppies. Between the oral doses of 5 and 25 mg/kg bw afoxolaner exposure increased approximately proportionally. Afoxolaner kinetics were linear following 3 monthly and 3 bimonthly doses with steady state reached by the third monthly dose and minimal accumulation.

Milbemycin oxime

Milbemycin oxime is a mixture of two homologues: approximately 80% 5-didehydromilbemycin (A4 form) and 20% 5-didehydromilbemycin (A3 form). Milbemycin oxime has been used previously in oral products for dogs and the pharmacokinetic profile of milbemycin oxime in dogs is documented in publicly available literature.

A non-GLP pharmacokinetic study with a cross-over design was conducted in 18 dogs to characterise the pharmacokinetic profile of milbemycin alone and when administered in combination with afoxolaner. All treatments were single dose by oral route. In a first period, there were 3 treatment groups. Group 1 received 0.5 mg/kg bw milbemycin oxime in the form of chewable tablets. Group 2 was treated with a combination of 2.5 mg/kg bw afoxolaner and 0.5 mg/kg bw milbemycin oxime as chewable tablets. Group 3 was treated by 0.5 mg/kg bw milbemycin oxime as a marketed reference product. In a second period, group 1 had the treatment previously received by group 3, and group 3 the treatment previously received by group 1. This study demonstrated that the plasma pharmacokinetic profile of milbemycin is broadly similar when the substance is given alone or with the concomitant administration of afoxolaner.

Milbemycin oxime is absorbed quickly from the combination product. The half-lives for the A3 and A4 forms of milbemycin oxime were 1.6 ± 0.4 days and 3.3 ± 1.4 days, respectively. Because of the short half-life relative to the dosing interval of 1 month no accumulation was present following monthly dosing. Published scientific literature showed linear kinetics with over 7 weekly doses or 43 daily doses. Milbemycin oxime exhibits dose proportional pharmacokinetics over the range of 1.0 to 3.0 mg/kg bw and close to proportional kinetics from 1.0 to 5.0 mg/kg bw. Literature reports suggest dose proportionality over a wider range.

Bioavailability of milbemycin oxime administered orally to dogs in the combination chewable tablet was 80.5% and 65.1% for the A3 and A4 forms, respectively. Absorption was quick with a T_{max} of 1–2 hours. The maximum concentrations (C_{max}) were 42 ng/ml ± 11 ng/ml for the A3 form and 246 ng/ml ±71 ng/ml for the A4 form. The volume of distribution is 2.7 l/kg ± 0.4 l/kg and 2.6 l/kg ± 0.6 l/kg for the A4 and A3 forms, respectively. Milbemycin oxime has low systemic clearance of 75 ml/h/kg ± 22 ml/h/kg and 41 ml/h/kg ± 12 ml/h/kg in dogs for A3 and A4 forms respectively. It is metabolised in vivo to form hydroxylated metabolites and it is eliminated in the faeces via the bile.

Toxicological studies

For afoxolaner, a range of toxicology studies have been conducted. In general, these have been conducted in accordance with recognised guidelines of the Organisation for Economic Co-operation and Development (OECD) and in accordance with the principles of GLP.

Milbemycin oxime is an anthelmintic active substance used in veterinary medicinal products. To address most of the toxicity aspects, the applicant refers to published results. No new toxicology studies have been conducted with milbemycin oxime, but new target animal safety studies with the combination (i.e. afoxolaner and milbemycin oxime) have been performed and are described in Part 4.

Single dose toxicity

Afoxolaner

An acute oral GLP compliant toxicity study was conducted in rats. In this study, no severe effects were observed at doses of up to 1,000 mg afoxolaner/kg bw (oral LD_{50} > 1,000 mg/kg bw) and the no-observed adverse effect level (NOAEL) was 300 mg/kg bw.

A single dose acute dermal toxicity study (limit test: dose 2,000 mg/kg bw) using the active substance was performed in rats. The dermal LD_{50} is > 2,000 mg/kg bw. Adverse effects (abnormal gait and stance, decreased body tone, piloerection) were observed at this dose in this study, though these effects were not severe or irreversible.

In conclusion, afoxolaner has low acute oral and dermal toxicity.

Milbemycin oxime

The published results of acute toxicity studies, where milbemycin oxime was administered to mice or to rats, were provided, indicating an oral LD_{50} of 863 mg/kg bw (male) and 532 mg/kg bw (female) in rats, and 946 mg/kg bw (male) and 722 mg/kg bw (female) in mice.

Following intraperitoneal injection, LD_{50} of 454 mg/kg bw (male) and 318 mg/kg bw (female) in rats, and 138 mg/kg bw (male) and 120 mg/kg bw (female) in mice were noted.

Following subcutaneous administration, the LD_{50} in both species and genders was above 3,000 mg/kg bw.

Combination

An acute oral toxicity study was conducted in rats where the active substances were administered alone or in combination. Three different groups of animals received single oral doses of 220 mg/kg bw milbemycin oxime or 917 mg/kg bw afoxolaner or 1,070 mg/kg bw afoxolaner plus 203 mg/kg bw milbemycin oxime. No deaths occurred in any treatment group.

Oral administration of a single dose of afoxolaner either alone or in combination with milbemycin oxime resulted in the expected afoxolaner-related body weight loss and lower food consumption. There were no adverse effects on body weights or food consumption in the milbemycin oxime group.

Administration of the combination did not result in any effects due to the combination, as the magnitude of body weight and food consumption effects in the afoxolaner and the combination groups were proportional to the dose of afoxolaner administered.

In conclusion, the acute toxicities of afoxolaner and milbemycin oxime in rats are not affected by co-administration and there was no indication that co-administration had any synergistic effects.

Repeat dose toxicity

Afoxolaner

Repeat dose toxicity studies were conducted in rats (14-day oral study and 56-day dermal study), mice (12-day oral study), and rabbits (4-day oral study). The repeat-dose toxicity studies were conducted in line with relevant OECD guidelines and in compliance with GLP. Based on the results of these studies, both the oral and dermal NOAEL can be established at 10 mg afoxolaner/kg bw/day for repeated exposure. At higher dose levels, the main signs were a decrease in food intake and consequent outcomes. In rats a diuretic effect was observed as well.

Milbemycin oxime

In a 3-month study, rats were administered oral doses of 0, 3, 15 or 100 mg/kg bw/day milbemycin oxime. At 15 mg/kg bw/day, there were effects on haematological parameters. At 100 mg/kg bw/day, increases in liver weights, fatty change and hepatocyte swellings also occurred. The no-observed-effect level (NOEL) in this study was 3 mg/kg bw/day. Similar findings were reported for a second oral study in rats conducted over 4 weeks (the NOEL in this study was 10 mg/kg bw/day).

Tolerance in the target species of animal

See Part 4.

Reproductive toxicity

Afoxolaner

Developmental toxicity studies were conducted in rats and rabbits. These studies were conducted in accordance with GLP and the relevant OECD guideline. In these studies, there was no evidence of foetal malformation or developmental variations at any dose tested.

Reproductive toxicity studies were conducted in rats. These studies were conducted in accordance with GLP and the relevant OECD guideline. Based on the findings of these studies, afoxolaner has no effect on reproductive performance at doses up to 5 mg/kg bw/day. Doses of 20 mg/kg bw/day were associated with effects on implantation rate, litter size, litter loss during lactation and pup weight.

Milbemycin oxime

Based on information provided from the published literature, milbemycin oxime is not teratogenic in the rat (substance administered between days 7 and 17 of gestation, maximum dose tested 300 mg/kg/day) or the rabbit (substance administered between days 6 and 23 of gestation, maximum dose tested 180 mg/kg/day). In addition, milbemycin oxime had no effects on reproductive performance at doses of 1.5 mg/kg bw/day in male and female dogs.

Combination

No reproductive safety studies using the combination product have been conducted in the target species. Consequently, the following text is included in section 4.7 of the SPC: “Laboratory studies in rats and rabbits have not produced any evidence of teratogenic effects, or any adverse effect on the reproductive capacity in males and females. The safety of the veterinary medicinal product has not been established during pregnancy and lactation or in breeding dogs. Use only accordingly to the benefit-risk assessment by the responsible veterinarian”. This is considered appropriate and is accepted.

Mutagenicity / genotoxicity

Afoxolaner

Afoxolaner was evaluated for genotoxic potential in the bacterial reverse mutation test, the mouse lymphoma mutagenesis assay and the mouse bone marrow micronucleus test. These studies were conducted in accordance with GLP and the relevant OECD guideline. Based on the results of these studies, it is concluded that afoxolaner is not genotoxic.

Milbemycin oxime

Based on information provided from the published literature, milbemycin oxime is not considered to have genotoxic potential (negative in a reverse mutation study, a chromosomal aberration study and an in vivo mouse bone marrow micronucleus test).

Carcinogenicity

Afoxolaner

Carcinogenicity studies have not been conducted with afoxolaner. This is acceptable as afoxolaner has no structural alerts and is not considered to be genotoxic.

Milbemycin oxime

Carcinogenicity studies have not been conducted with milbemycin oxime. This is acceptable as milbemycin oxime has no structural alerts and is not considered to be genotoxic.

Studies of other effects

Afoxolaner

Based on a series of GLP compliant local effect studies conducted in accordance with relevant OECD guidlines, afoxolaner was not irritating to the skin of rabbits, and was not considered to have sensitising properties. However, afoxolaner was considered to be slightly irritating to the eyes.

Milbemycin oxime

Based on information provided from the published literature, it is considered that milbemycin oxime is not likely to be a dermal irritant. In addition, while there are no data on the skin sensitising potential of milbemycin oxime, the related substance, moxidectin, was tested for its ability to induce dermal sensitisation and negative results were obtained. Hence, milbemycin oxime is unlikely to be a skin sensitiser.

Combination

There are no local effect data available for the combination product, afoxolaner and milbemycin oxime. However, due to the presentation, chewable tablets, dermal exposure will be limited to administration of the product to the dog and hence, will be minimal, while eye contact is an unlikely exposure scenario.

User safety

A user safety assessment in line with the CVMP Guideline on user safety for pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-Rev.1) has been presented.

The excipients are generally recognised as safe and/or are already approved for use in the European Union (EU) as pharmaceutical excipients for human and/or veterinary products, or as a human food ingredient or flavouring agent. Therefore, this assessment of human health risk is based only upon the potential active substance hazards in relation to potential user exposures to the formulated product.

The relevant hazards and exposures considered are 1) an oral hazard associated with a child accessing and ingesting a formulated chew (considered a rare, one-time event), and 2) short term dermal exposure following handling (picking up) a chew between the time it is removed from the package and the time it is administered to the dog. Therefore, acute toxicity studies define the most relevant hazard endpoints applicable to these exposure scenarios. For this type of product, ocular or chronic exposure scenarios are not relevant.

For the short term dermal exposure scenario, the applicant argues that the potential for and extent of dermal exposure to the active substances will be very low given the presentation of the product (chewable tablets in blister packs) and the short term contact (minutes at most) with the product. Therefore, this scenario was not considered further. The following precautionary statement is included in the SPC and package leaflet: “Wash hands after use.” This is acceptable.

The applicant suggests that the relevant safety endpoints for margin of exposure (MOE) calculation for acute oral exposure (ingestion by a child) should be taken from the acute toxicity study performed in rats where the active substances were administered alone or in combination. In this study, a NOAEL of 917 mg/kg was established for afoxolaner and a NOEL of 220 mg /kg was established for milbemycin oxime. However, the appropriateness of using the oral NOEL of 220 mg milbemycin oxime/kg from the rat acute toxicity study for purposes of MOE calculation is questionable given that milbemycin related effects have been observed at lower doses in acute studies in puppies. For example, milbemycin oxime administered daily for three days to pups at doses of 7.5 and 12.5 mg/kg bw resulted in adverse effects (trembling/ataxia) (Ide et al., 1993).

A worst-case user exposure to formulated product is estimated to be a 2- to 3-year-old child (15 kg) who obtains, opens and ingests an entire extra-large size chew. The largest chew contains 150 mg afoxolaner and 30 mg milbemycin oxime. Therefore, the estimated total large-dose exposure to the active substances for the formulated product as a whole is:

Estimated afoxolaner exposure = dose available = 150 mg = 10 mg/kg bw

Estimated milbemycin oxime exposure = dose available = 30 mg = 2 mg/kg bw

Comparing the extent of exposure to the relevant NO(A)ELs results in a MOE of 91.7 for afoxolaner and 110 for milbemycin oxime. The applicant advises that while the MOE for afoxolaner is calculated to be slightly less than 100 for this scenario, the defined hazard at the NOAEL was associated with transient reductions in food consumption and body weight. The calculated MOE for milbemycin oxime is above 100 suggesting an acceptable user safety. Overall, the applicant concludes that for this scenario the risk can be considered acceptable. However, as stated above, the appropriateness of using the oral NOEL of 220 mg/kg for milbemycin oxime from the rat acute toxicity study for purposes of MOE calculation is questionable. Use of a lower NOAEL for milbemycin will result in a MOE < 100.

Based on the assessment conducted, the applicant concludes that the combination product is not expected to result in significant user exposures leading to adverse health events. The following precautionary statements are included in the product information: “Keep out of the sight and reach of children” and “Keep tablets in the blister packs until required, and keep the blisters in the outer carton.”

As advised above, the NOEL used in the MOE calculation for the milbemycin component is not accepted. Use of a lower NOEL will result in a MOE less than 100 indicating a potential risk should a young child ingest a whole chew. That said, it can be accepted that the proposed user safety warnings should ensure that the product is stored away from children, in the original packaging and in a closed container or cupboard. Additionally, it is noted that the product is packaged in sealed, blister packs and the secondary container is a tamper-evident cardboard carton. While child resistance has not been confirmed by way of specifically designed studies, it is accepted that the product packaging (blister pack in tamper-evident outer carton) will minimise unintentional and/or child exposure to the product. However, for completeness, the following additional safety statement is in the product information: “In case of accidental ingestion, particularly in the case of children, seek medical advice immediately and show the package leaflet or the label to the physician.”

To conclude, it is accepted that the potential health risk to all users (adults and children) is low and acceptable with respect to typical conditions of use. The proposed user safety statements in the product information are acceptable.

Environmental risk assessment

A Phase I environmental risk assessment (ERA) was provided according to the Guideline on environmental impact assessment (EIAS) for veterinary medicinal products – Phase I (CVMP/VICH/592/98-FINAL).

The veterinary medicinal product will only be used in individual, non-food producing animals, therefore, the ERA can stop at Phase I. NEXGARD SPECTRA is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

Pharmacodynamics

Afoxolaner is a member of the isoxazoline family, shown to inhibit insect and acarine ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA),

thereby blocking pre- and post-synaptic transfer of chloride ions across cell membranes, resulting in its ectoparasitic activity.

Milbemycin oxime is an anthelmintic substance and acts by binding to chloride ion channels in invertebrate nerve and muscle cells. Increased permeability by the cell membrane to chloride ions causes hyperpolarization of affected cells and subsequent paralysis and death of the intended parasites. Milbemycin oxime may also act by disrupting the transmission of invertebrate neurotransmitters, notably GABA.

There is no reason to suspect potential interaction as afoxolaner and milbemycin oxime bind to distinct and different sites on GABA-gated chloride channels and do not share activity on mammalian receptors.

Pharmacokinetics

The data presented are considered adequate to characterise the pharmacokinetics of afoxolaner and milbemycin oxime when administered in combination as the test product. Following administration of the combination product to the target species the pharmacokinetic parameters were as follows:

In dogs the absolute bioavailability of afoxolaner is 88%. The mean maximum concentration (C_{max}) is 1,822 ng/ml ± 165 ng/ml in plasma found 2–4 hours (T_{max}) after a 2.5 mg/kg afoxolaner dose. Afoxolaner distributes into tissues with a volume of distribution of 2.6 l/kg ± 0.6 l/kg and a systemic clearance value of 5.0 ml/h/kg ± 1.2 ml/h/kg. The terminal plasma half-life is approximately 2 weeks in dogs.

In dogs the absolute bioavailability is 81% and 65% for the A3 and A4 forms of milbemycin oxime, respectively. The maximum concentration in plasma (C_{max}) is 42 ng/ml ± 11 ng/ml for the A3 form and 246 ng/ml ±71 ng/ml for the A4 form within the first 1–2 hours (T_{max}) indicating that absorption from the chewable tablet is fast. It distributes into tissues with a volume of distribution of 2.7 l/kg ± 0.4 l/kg and 2.6 l/kg ± 0.6 l/kg for the A3 and A4 forms respectively. Both forms have low systemic clearance in dogs, 75 ml/h/kg ± 22 ml/h/kg for the A3 form and 41 ml/h/kg ± 12 ml/h/kg for A4 form. The terminal half-lives following oral administration are 1.6 days ± 0.4 days for the A3 form and 3.3 days ± 1.4 days the A4 form.

The simultaneous administration of both actives to the target species does not affect the pharmacokinetic profile of the active substances. For both substances, the kinetics are linear following multiple monthly and twice monthly dosing and concentrations increase approximately proportionally to dose over the dose range of from 5 mg/kg to 25 mg/kg of afoxolaner and from 1 mg/kg to 5 mg/kg of milbemycin oxime.

Toxicity

The oral LD_{50} is greater than 1,000 mg afoxolaner/kg bw and oral NOAEL is 300 mg/kg bw in rats. The dermal LD_{50} is greater than 2,000 mg afoxolaner/kg bw in rats. The oral toxicity profile consists of a diuretic effect identified in rats and effects secondary to a reduction in food consumption identified in rats and rabbits. The NOAEL in a rodent 90-day repeated-dose study is 10 mg/kg/day and the NOEL is 3 mg/kg/day. Data in rats and rabbits demonstrate that afoxolaner has no developmental or reproductive toxicity based on available toxicity data. The rat was deemed the most sensitive species and presented adverse effects secondary to the decrease in food consumption such as lower cumulative body weight gains, decreased defecation and transient hemoconcentration, upon oral administration of afoxolaner. These adverse effects were observed only at a higher dose (2,000 mg/kg) in the mouse and were observed in a range-finding but not the definitive developmental toxicity study in rabbits. These effects were not observed in dogs receiving oral doses of afoxolaner. Afoxolaner demonstrated no clinically relevant mutagenic or clastogenic potential in a battery of tests. Afoxolaner is non-irritating to skin, slightly irritating to eyes and does not have sensitization potential.

Milbemycin oxime is currently used in veterinary medicine and has been extensively investigated. The acute oral rat LD_{50} values are reported as 863 mg/kg and 532 mg/kg for males and females, respectively. In 28-day and 90-day repeated-dose rat studies conducted with milbemycin oxime, NOAEL values were 10 mg/kg/day and 3 mg/kg/day, respectively. In a dog study, 8-week-old Beagle puppies dosed with 8.6 mg/kg/day or 14.3 mg/kg/day in the form of milbemycin oxime tablets for 3 consecutive days were noted with transient trembling and/or ataxia. The regimen was repeated each month for a total of 10 months of treatment. No other findings were noted for this study. Findings in a follow-up study evaluating dosing of up to 12.5 mg/kg/day milbemycin oxime for 3 consecutive days in 8, 10 and 12 week old Beagle puppies were limited to transient ataxia and trembling. A reproductive safety study demonstrated no effects on reproduction parameters for both studs and bitches treated daily at 1.5 mg/kg/day throughout mating (males and females) and until one week prior to whelping (females). In developmental toxicity studies in rats and rabbits, milbemycin oxime was not teratogenic. Milbemycin oxime was shown to be non-mutagenic in an Ames test and a chromosomal aberration test.

The data presented are considered adequate to characterise the toxicity profile of both active substances.

User safety

A user safety assessment in line with the CVMP Guideline on user safety for pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-Rev.1) has been presented. Based on that assessment, it is accepted that the potential health risk to all users (adults and children) is low and acceptable with respect to typical conditions of use. The proposed user safety warnings should ensure that the product is stored away from children, in the original packaging and in a closed container or cupboard. It is noted that the product is packaged in sealed, blister packs and the secondary container is a tamper-evident cardboard carton. While child resistance has not been confirmed by way of specifically designed studies, it is accepted that the product packaging (blister pack in tamper-evident outer carton) will minimise unintentional and/or child exposure to the product. The proposed user safety statements are acceptable.

The CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Environmental safety

A Phase I environmental risk assessment (ERA) was provided according to the Guideline on environmental impact assessment (EIAS) for veterinary medicinal products – Phase I (CVMP/VICH/592/98-FINAL) which is considered acceptable.

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

Part 4 – Efficacy

Pharmacodynamics

For information on mode of action and potential for interaction, see Part 3.

Justification for the combination

The product under consideration is an oral endectocide chewable tablet for dogs containing a fixed combination of two active substances: afoxolaner (an ectoparasitic substance) and milbemycin oxime (an anthelmintic substance). The product is intended for use in dogs suffering from or at risk of mixed parasitic infections with ectoparasite, gastrointestinal nematodes and/or heartworm. The combination was justified as follows:

- The parasites targeted by the combination product (fleas, ticks, gastrointestinal nematodes and heartworm) are commonly found in dogs in Europe and can be present simultaneously on the same animal.
- The two active substances included in the combination product have different spectra of activity i.e. afoxolaner with ectoparasitic activity and milbemycin oxime with anthelmintic activity. Each active substance does not interfere with the efficacy of the other one and the combination does not present additional or increased toxicity effects compared to afoxolaner or milbemycin oxime alone. This statement is supported by the pharmacokinetic, safety and efficacy data presented in the dossier. Possible interactions in terms of efficacy have been investigated in GCP clinical studies (see dose determination/justification section below).
- In addition, the product, being a fixed combination, facilitates dog handling by reducing the total number of tablets to be given.

Based on the argumentation presented, it is accepted that the combination has been adequately justified.

The proposed indication as detailed in section 4.2 of the SPC makes it clear that the combination product is only intended for use in situations where both active substances are required.

Development of resistance

Afoxolaner

Afoxolaner is a member of the isoxazoline family. No resistance has been documented for any isoxazoline compound including afoxolaner; however, afoxolaner was only first authorised in 2014 for the use in animals.

Milbemycin oxime

Since the introduction of milbemycin oxime as an anthelmintic there has been no reported evidence of lack of efficacy or resistance in dogs in Europe, neither with respect to gastrointestinal nematodes nor in heartworm. There is, however, evidence of an increase of reports on product prevention failures with respect to *Dirofilaria immitis* ('lack of efficacy' in heartworm prevention) in the USA. It is accepted that macrocyclic lactones continue to be effective in the vast majority of situations and the appropriate use of macrocyclic lactone products as recommended in the SPC is the basis for effective heartworm prevention. Given the concerns regarding resistance development, section 4.4 of the SPC includes warnings relating to the potential for resistance emergence.

Pharmacokinetics

See Part 3.

Dose determination / justification

Afoxolaner

The identification of the minimum recommended dose of 2.5 mg/kg of afoxolaner orally in dogs for a treatment of flea and tick infestations is based on a product from the same company containing afoxolaner only. Given that the pharmacokinetic profile of afoxolaner is unchanged by the concomitant administration of milbemycin oxime (see pharmacokinetics section, Part 3), the minimum recommended dose of afoxolaner of the combination product is the same as for the afoxolaner only containing product, i.e. minimum 2.5 mg/kg body weight. In view of the weight range of animals treated with one tablet, the maximum recommended dose would be approximately 5.4 mg/kg bw.

A non-GCP efficacy study was conducted in the target species to explore efficacy of the combination product at that dosage. In this study, efficacy of the combination milbemycin oxime/afoxoloner (0.5 mg/kg/2.5 mg/kg) against *R. sanguineus* was above 90% up to day 44 and against *C. felis* was

100% up to day 37 following a single dose administration of the test item. These data can be accepted as evidence that presenting afoxolaner in combination with milbemycin does not impact on the expected efficacy of afoxolaner against *R. sanguineus* or *C. felis*.

Milbemycin oxime

The active substance milbemycin oxime is a substance authorised in the EU for use in dogs either alone or in combination (with e.g. praziquantel or lufenuron) at the minimum recommended dose of 0.5 mg/kg of milbemycin oxime. The effectiveness of milbemycin oxime administered orally at a dose of 0.5 mg/kg bw for the treatment and control of infections with adult stages of common gastrointestinal nematodes and for the prevention of heartworm disease in dogs is documented in the published literature. Given that the pharmacokinetic profile of milbemycin oxime is largely unchanged by the concomitant administration of afoxolaner (see pharmacokinetics section, Part 3), the minimum recommended dose of milbemycin oxime for the combination product is the same as the recommended dose for the milbemycin oxime only containing product, i.e. minimum 0.5 mg/kg bw. In view of the weight range of animals treated with one tablet, the maximum recommended dose would be approximately 1.1 mg/kg bw.

Four non-GCP efficacy studies were conducted in the target species to explore efficacy of the combination product at that dosage. These studies showed that a milbemycin oxime dose of 0.5 mg/kg bw, alone or in combination with afoxolaner, yielded adequate efficacy (>90%) against adult *A caninum*, *T. canis* and *T. vulpis* infestations.

Combination

Based on the totality of data provided (pharmacodynamic, pharmacokinetic and preliminary clinical efficacy studies and published literature), it is considered appropriate that minimum doses of 2.5 mg afoxolaner/kg bw and 0.5 mg milbemycin oxime/kg bw were taken forward to clinical efficacy testing.

Target animal tolerance

Afoxolaner

In a GLP compliant target animal safety (TAS) study investigating afoxolaner alone, afoxolaner was well tolerated in 32 Beagle puppies (8 weeks of age at start) at 6.3 mg, 18.9 mg and 31.5 mg afoxolaner/kg bw at three, one-month dose intervals and three, two-week dose intervals. No mortalities occurred, and there was no evidence of test article-related alterations in food consumption; body weight; physical examination variables (heart rate, respiratory rate, body temperature); anatomical or clinical pathology findings; or clinical abnormalities.

Additional tolerance data for the monosubstance product was available from a number of dose determination/confirmation studies along with tolerance data from four field studies. From the data presented it was concluded that afoxolaner is well tolerated in puppies at an age of 8 weeks.

In an exploratory pharmacokinetic/safety study, collies sensitive to ivermectin (MDR1-collies) were treated orally with 25 mg afoxolaner/kg bw. Afoxolaner was generally well tolerated confirming that P-glycoprotein does not play a role in afoxolaner transport. One treated animal had diarrhoea and one animal vomited 6 hours after administration.

Milbemycin oxime

Based on information provided from the published literature, it is evident that, at sufficiently high doses, avermectins and milbemycins (moxidectin) may be toxic to dogs. Signs of toxicity are indicative of neurotoxicity and include lethargy, hypersalivation and ataxia. However, the main concern with these compounds is the increased sensitivity shown by some animals in the collie population and in some other breeds of dog. This sensitivity arises from the MDR1 gene deletion and accompanying defective

P-glycoprotein which permits increased intestinal and brain permeability for some xenobiotics thus resulting in increased gastrointestinal absorption and higher exposure to the brain.

Combination

The safety of milbemycin oxime in 18 sensitive collies was confirmed in a study with milbemycin oxime alone or in combination with afoxolaner. The dogs treated with milbemycin oxime alone were administered 5.0 mg/kg bw once only in a chewable tablet. The dogs treated with the combination product were administered 25 mg/kg bw of afoxolaner and 5 mg/kg bw of milbemycin oxime (5x the maximum recommended treatment dose) once only in a chewable tablet. Signs consistent with avermectin-type toxicity (salivation, incoordination) were observed in some animals administered milbemycin oxime alone at 5.0 mg/kg bw. However, the effects were mild and transient. The toxicity profile of milbemycin oxime in sensitive collies remained similar when given in combination with afoxolaner.

The safety of milbemycin oxime in combination with afoxolaner was also studied in 32 Beagle puppies of 8 weeks of age. This was a GLP-compliant study conducted in accordance with VICH GL43 on target animal safety for veterinary pharmaceutical products. In this study, there were 4 treatment groups with 8 puppies per group. Treatment group 1 was a non-treated control (sham dosed). Treatment groups 2, 3 and 4 were given chewable tablets containing afoxolaner and milbemycin oxime at the following doses respectively: 5 mg afoxolaner per kg/1 mg milbemycin oxime per kg (1x), 15 mg/kg/3 mg/kg (3x) or 25 mg/kg/5 mg/kg (5x). Administration was at three one-month dose intervals and at three two-week dose intervals. In this study, the product was well tolerated at doses up to 5 times the maximum recommended treatment dose, with no evidence of adverse effects on clinical, clinicopathological or pathological parameters. Occasional gastrointestinal disturbance (emesis, diarrhoea) was noted in some pups during the course of the study. While vomiting and diarrhoea were observed sporadically across all groups including the controls, vomiting followed administration of 3x and 5x doses in some treated dogs suggesting a possible relationship with treatment. In all cases, the effects observed were mild, transient and resolved without treatment.

In addition to the specific target animal safety studies, dose determination and dose confirmation studies and 4 field studies were conducted. In these studies, the combination product was administered at the recommended treatment dose. The proposed product was well tolerated. As noted for the target animal safety study, occasional gastrointestinal disturbance (emesis, diarrhoea) was noted in some test animals in some studies. In addition, in the field studies, single instances of anorexia, lethargy and pruritus were recorded. In all cases, the observed effects were mild and transient. While there is no clear association between treatment and the adverse events recorded, a relationship with treatment cannot be excluded. Therefore, information advising of the possibility of such effects following treatment is included in section 4.6 of the SPC. Based on the totality of data provided, it is accepted that administration of the combination product in accordance with the recommendations in the product information is well tolerated in the target species.

Dose confirmation

To demonstrate the efficacy of the combination product, the applicant provided 26 dose confirmation studies. All dose confirmation studies were conducted in accordance with GCP and in accordance with relevant VICH guidance (except where indicated). All studies used the proposed final formulation and the active substances were administered at the minimum recommended treatment dose of 2.5 mg afoxolaner/kg bw and 0.5 mg milbemycin oxime/kg bw. The studies were conducted at various sites worldwide (Europe, USA and South Africa) and involved both natural and induced infestations.

Ectoparasites

Fleas

Two laboratory studies were provided evaluating efficacy against induced infestations of adult stages of *Ctenocephalides felis*.

The first study was undertaken in the USA (2013) in 32 Beagle dogs (18–19 months old), in accordance with World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines. A single administration of the combination product provided 100% efficacy against adult fleas at 24 hours after treatment or weekly re-infestations for at least 1 month and 99.8% efficacy on day 36. The study also supported the effectiveness of a single treatment of the combination product to prevent the production of viable *C. felis* eggs. While it is accepted that the product would appear to be effective at reducing the production of viable eggs, fleas must attach to the host to be exposed to the active substance; therefore a claim for prevention of infestation cannot be accepted.

The second study was undertaken in France (2013) to investigate the efficacy of afoxolaner when administered alone or in combination with milbemycin oxime as a single oral dose for the treatment and control of induced infestations of adult stages of *Ctenocephalides felis* on 24 Beagle dogs (12 to 17 months old) and in compliance with the CVMP Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestations in dogs and cats (EMEA/CVMP/EWP/005/2000-Rev.2). In this study, the combination product provided a high level of efficacy (≥99.9%) against adult fleas at 24 hours after treatment or weekly re-infestations for at least 5 weeks. In addition, the results of this study confirm that the administration of milbemycin oxime in combination with afoxolaner does not interfere with the efficacy of afoxolaner against fleas.

In conclusion, efficacy of the combination product for the treatment of *C. felis* in the proposed dosing regimen was confirmed. The effect persisted for 5 weeks following a single administration of the product. The product NexGard (afoxolaner only) is indicated for the treatment of both *C. felis* and *C. canis*. Given that efficacy against *C. felis* was confirmed for the combination product, and that the pharmacokinetic profile of afoxolaner is unchanged by the concomitant administration of milbemycin oxime, efficacy against *C. canis* can also be accepted for the combination product. In addition, the study data provided indicates an effect of afoxolaner on the production of viable flea eggs (reduction in egg numbers and viability). Given the effect on fleas, it is accepted that this product can be used as part of a treatment strategy for the control of flea allergic dermatitis.

Ticks

For ticks, the assessment of efficacy deviated from the standard approach described in the current CVMP Guideline for the 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) in that the assessment does not take account of dead attached engorged (category 6) ticks. The current CVMP guideline only addresses the evaluation of acaricides that are topically applied, and not those for systemic use. The applicant therefore used an alternative approach for assessment of efficacy to that detailed in the current CVMP guideline, in line with proposals outlined in the published WAAVP guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats (Marchiondo et al., 2013). This approach does not follow the categorisation outlined in the above CVMP guideline, but only focuses on live (failure) and killed (success) ticks (i.e. excluding category 6 ticks). This approach can in principle be accepted for orally administered acaricides provided that the product information clearly states that: the use of the product is for treatment only (not preventive use); ticks and fleas must attach to the host and commence feeding in order to be exposed to the active substance; and parasites need to start feeding on the host to become exposed to afoxolaner (therefore, the risk of the transmission of parasite-borne diseases cannot be excluded).

In the European Public Assessment Report (EPAR) for NexGard it is acknowledged that *Dermacentor* may be the least sensitive tick species for afoxolaner. In view of the above, the applicant has selected *Dermacentor* as the test parasite for the pivotal dose confirmation studies provided in support of the NEXGARD SPECTRA application. Dose confirmation studies showed the efficacy of the dose in the afoxolaner only containing product for various tick species, and as *Dermacentor* was considered the most susceptible species, dose confirmation studies on the combination product were only considered necessary on this tick species. Therefore, confirmation of efficacy against this tick species is to be extrapolated to other claimed tick species, *I. ricinus* and *R. stenocephalus*. This approach was considered acceptable by CVMP.

Two laboratory studies conducted in South Africa (2013) were provided evaluating the efficacy of afoxolaner when administered alone or in combination with milbemycin oxime as a single oral dose for the treatment and control of induced infestations of adult *Dermacentor reticulatus* on dogs. Both studies followed the same protocol, and were conducted in line with VICH GCP. Following initial infestation with ticks, 30 dogs were treated with the test product; re-infestations were performed every week up to day 35 (5 weeks post-treatment). Tick counts were undertaken 48 hours after each infestation.

In the first study, an adequate level of efficacy (>90%) was detected up to day 23 (3 weeks) only. In addition, the results of this study confirm that the administration of milbemycin oxime in combination with afoxolaner does not interfere with the efficacy of afoxolaner against ticks.

The objective and design of the second study was the same as for the first. However, in this study, an adequate level of efficacy (>90%) was detected up to day 37.

Efficacy of the combination product for the treatment of *D. reticulatus* was confirmed in both studies (>90% efficacy by 48 hours after treatment). However, the duration of persistent effect varied between studies, being 3 weeks only in the first and 5 weeks in the second. The applicant suggests that the variability between studies can be explained by delayed and/or insufficient attachment of ticks following transfer to dogs (that is, less vigorous ticks in the first study). This explanation is not accepted, given that there is a clear deterioration in effect with time (that is, failure to achieve adequate efficacy was at the last two sampling time-points), which is unlikely to be related to the vigour of the ticks used.

Based on the outcome of these studies, a five-week duration of persistent effect was initially proposed. Given, however, the variability noted in the studies presented, a four week duration of persistent effect for ticks is accepted considering that persistent effect up to five weeks was confirmed in one study following administration of the combination product; and that persistent effect of 4 weeks against the claimed tick species (*D. reticulatus*, *I. ricinus* and *R. stenocephalus*) has been accepted for NexGard (afoxolaner only); and also, based on available pharmacokinetic data, that afoxolaner plasma pharmacokinetic profile is similar when afoxolaner is administered alone or in combination with milbemycin oxime. In addition, efficacy against ticks for up to four weeks was further confirmed in a large scale European field study.

Gastro-intestinal nematodes

13 dose confirmation studies were provided investigating the efficacy of the combination product against gastro-intestinal nematode infestations (*Toxocara canis, Toxascaris leonina, Ancylostoma caninum, Ancylostoma braziliense* and *Trichuris vulpis)* in dogs, as presented in the overview table below.

Overview of effectiveness against gastrointestinal nematodes (adults):

Parasite	Species	Natural/induced	% efficacy
Hookworm	*Ancylostoma braziliense*	Natural	94.8
		Induced	90.2
	Ancylostoma	Natural	91.7

	caninum	Natural	90.9
		Induced	99.7
Roundworm	*Toxascaris leonina*	Natural	99.4
		Induced	95.8
		Induced	96.0
	Toxocara canis	Natural	97.8
		Induced	Inadequate infection
		Induced	98.0
		Induced	100.0
Whipworm	*Trichuris vulpis*	Natural	100.0
		Natural	98.3

All (valid) dose confirmation studies presented in the dossier show that the combination product was >90% effective against gastrointestinal nematodes. At least two confirmatory efficacy studies are available for each of the claimed gastrointestinal nematode species. These confirmatory studies are supported by European field data confirming efficacy (in terms of reduction in faecal egg output) against the target parasites. Based on these data, the following indication can be accepted:

"Treatment of infestations with adult gastrointestinal nematodes of the following species: roundworms (*Toxocara canis and Toxascaris leonina*), hookworms (*Ancylostoma caninum* and *Ancylostoma braziliense*) and whipworm (*Trichuris vulpis*)."

Heartworm

Seven dose confirmation studies performed in the USA in dogs experimentally infected with third stage larvae of *Dirofilaria immitis* from different US isolates (Duke, Michigan, Pepper, JYD-34) and two dose confirmation studies performed in dogs with European *D. immitis* isolate were provided, as presented in the overview table below.

Isolate	**Treatment Schedule**	**% efficacy**
JYD-34	0	inadequate infections in controls
Duke	0	inadequate infections in controls
JYD-34	0, 30	76.1
	0, 30, 60	72.2
Duke	0, 30, 60, 90, 120, 150	100
Pepper	0, 30, 60, 90, 120, 150	100
Michigan	0, 30, 60, 90, 120, 150	100
JYD-34	0, 30, 60, 90, 120, 150	72
EU (Italian isolate)	0	100 (low infections in controls)
EU (Italian isolate)	0	100

Confirmatory studies to investigate the efficacy of the combination product against heartworm have been provided.

Two studies using USA isolates (Duke and JYD-34) were inconclusive because of inadequate infections in control animals.

In three studies with USA isolates (Duke, Pepper or Michigan) including six consecutive monthly treatments, 100% efficacy was demonstrated.

In a further two studies using the USA isolate JYD-34 efficacy of circa 70% was achieved using two, three or six treatments at monthly intervals. This may indicate reduced susceptibility of this isolate to the effects of milbemycin. There is evidence of an increase of reports on "product prevention failures" with respect to *Dirofilaria immitis* ('lack of efficacy' in heartworm prevention) in the USA and it is now accepted that lack of efficacy related to resistance to macrocyclic lactones in *D. immitis* has developed in the USA.

In one EU study using an Italian isolate and involving one single treatment, 100% efficacy was demonstrated (adequate infection in the control group). In a second EU study, the numbers of adult

D. immitis detected in control animals were low such that the overall validity of the study may be questioned. While the numbers of adults in individual control dogs was low, seven of ten control dogs were harbouring *D. immitis* adults, whereas *D. immitis* adults were not detected in any of the treated dogs (100% efficacy).

Based on the totality of data available, it is accepted that the product is an effective treatment for *D. immitis* prevention. The following indication can be accepted: "Prevention of heartworm disease (*Dirofilaria immitis* larvae) with monthly administration." While the emergence of resistance to macrocyclic lactones in *D. immitis* in the USA is noted, it is accepted that macrocyclic lactones continue to be effective in the vast majority of situations. Further, it is noted that resistance has not yet been documented in Europe. However, given the concerns regarding resistance development, it is considered appropriate that section 4.4 of the SPC include prudent use warnings.

Lungworm

A single study was performed in the EU in dogs experimentally infected with third stage larvae of *Angiostrongylus vasorum* to evaluate the efficacy of two dosage regimens. In this study, the standard efficacy threshold of 90% was not achieved. Therefore, these data are considered inadequate to support the following proposed indication: "In endemic areas administration of the product every four weeks will prevent angiostrongylosis due to *Angiostrongylus vasorum* by reducing immature adult (L5) and adult parasite burden."

The proposed indication for use against *Angiostrongylus vasorum* was therefore withdrawn during the procedure.

Field trials

Four GCP-compliant studies have been submitted in support of the safety and efficacy of the final formulation when administered to dogs under field conditions. Two studies were conducted at multiple sites in Europe to investigate efficacy of the combination product against gastrointestinal nematodes and ectoparasites. Two other studies were conducted to evaluate the efficacy of the combination product for the prevention of *Dirofilaria immitis* infestation under field conditions in the USA and in Japan.

Ectoparasites

The pivotal European ectoparasite study was performed in 2013 in several European countries (Albania, Austria, Bulgaria, France, Germany, Hungary and Italy) involving 324 client-owned dogs of different ages, genders and breeds, naturally infested with fleas and/or ticks. 15 dogs did not complete the study but only 12 were excluded from the analysis. Efficacy of the combination product against ectoparasites was evaluated against an authorised product containing pyriprole as active substance. The study was a positive control, blinded, multicentre, clinical efficacy study conducted in accordance with VICH GL9 on good clinical practices. There were 2 treatment groups. Treatment group 1 (161 dogs) received orally ≥2.5 mg afoxolaner/kg bw and ≥0.5 mg milbemycin oxime/kg bw. Treatment group 2 (163 dogs) received ≥12.5 mg pyriprole/kg bw topically. Following treatment, the dogs were weekly monitored for fleas and ticks (up to day 30). The natural parasite challenge included a variety of flea (C*tenocephalides canis, C. felis, Pulex irritans*) and tick species (*Dermacentor reticulatus, Haemaphysalis concinna, Ixodes hexagonus, I. ricinus, Ixodes* sp*. and Rhipicephalus sanguineus*).

Based on the results of this study, efficacy of the combination product when administered as single oral treatment to dogs infected with ectoparasites was adequate for up to 30 days post treatment (>97.8% reduction in flea count; >95.2% reduction in tick count) under field conditions. The test item was confirmed to be non-inferior to the authorised reference product. The product was well tolerated.

Nematodes

The pivotal European nematode study was performed in 2013 in several European countries (Albania, Austria, Bulgaria, France, Germany, Hungary, Italy, Lithuania and Romania) involving 408 client-owned dogs of different ages, genders and breeds. Data from 404 dogs were evaluated. This was a positive control, blinded, multicentre, clinical efficacy and tolerance study. Efficacy of the combination product against nematodes was evaluated against an authorised product which contains milbemycin oxime and praziquantel as active substances.

Prior to treatment (days from –14 to 0) all dogs were confirmed positive for natural infections of gastrointestinal nematodes using faecal counts; and *Toxocara*, *Toxascaris*, hookworm, *Trichuris* and *Capillaria* infections were demonstrated in 134, 30, 223, 155 or 14 dogs, respectively. On days 9 to 21 a second faecal sample was examined and compared with the pre-treatment counts.

The dogs were divided in 2 treatment groups. Treatment group 1 (207 dogs) received orally ≥ 2.5 mg afoxolaner/kg bw and ≥ 0.5 mg milbemycin oxime/kg bw. Treatment group 2 (201 dogs) received ≥ 0.5 mg milbemycin oxime/kg bw and ≥ 5 mg praziquantel/kg bw orally.

Efficacy in the NEXGARD SPECTRA group was 99.7%, 97.2%, 99.7%, 99.7%, 99.7% for *Capillaria*, hookworm, *Toxascaris*, *Toxocara*, and *Trichuris*, respectively, (as compared to 98.0%, 94.3%, 99.4%, 99.5%, and 99.9% in the positive control). For both treatments over all five genera, there was a significant difference ($p<0.01$) in the faecal egg counts between the two measurement times.

No treatment related adverse events were observed throughout the study.

Based on the results of this study, efficacy of the combination product when administered as single oral treatment to dogs infected with intestinal nematodes was adequate (> 97.2% reduction in faecal egg output) under field conditions and similar to those of the authorised product. For hookworms, *Toxocara*, and *Trichuris*, the test item was non-inferior to the authorised reference product.

Heartworm

The efficacy of the combination product for the treatment of heartworm infection was investigated under field conditions in Japan and the USA, involving 84 (Japan) and 320 (USA) client-owned dogs of different age, gender and breeds. Both studies were positively controlled, blinded, multicentre, clinical efficacy and safety studies.

Dogs were negative for heartworm infection (antigen and microfilariae test) before the first treatment and had no recent history of treatment with a heartworm preventive, and randomly allocated to one of two treatment groups, NEXGARD SPECTRA or a positive control (USA study: chewable tablets containing milbemycin oxime and spinosad, Japan study: tablets containing moxidectin as active substance).

Heartworm antigen and microscopic microfilariae tests were performed prior to treatment and repeated up to six months after the last treatment.

All dogs tested negative on heartworm antigen and microfilaria tests at the day of the last treatment and six months after the last treatment (100% efficacy), and no treatment related adverse events were noted related to the treatment.

Under the conditions of these studies, the test item when administered monthly at the recommended treatment dose was effective at preventing *D. immitis* infection.

Overall conclusion on efficacy

Two dose determination studies and published data were submitted for the individual components of the combination product. Four non-GCP studies were provided to determine the efficacy of the selected dose.

Four dose confirmation studies on ectoparasites and twenty two on nematodes have been submitted to confirm the efficacy of the selected dose of the combination product.

Four GCP-compliant field studies with the final formulation on the target species have been submitted to investigate efficacy of the combination product against gastrointestinal nematodes, ectoparasites and to evaluate the efficacy for the prevention of *Dirofilaria immitis*. A proposed indication against lungworm (*Angiostrongylus vasorum)* was withdrawn during the procedure.

The field studies, in addition to the various dose determination and dose confirmation studies, support the following indications:

"Treatment of flea infestations (*Ctenocephalides felis* and *C. canis*) in dogs for 5 weeks.

Treatment of tick infestations (*Dermacentor reticulatus*, *Ixodes ricinus*, *Rhipicephalus sanguineus*) in dogs for 4 weeks.

Fleas and ticks must attach to the host and commence feeding in order to be exposed to the active substance.

Treatment of infestations with adult gastrointestinal nematodes of the following species: roundworms (*Toxocara canis* and *Toxascaris leonina*), hookworms (*Ancylostoma caninum* and *Ancylostoma braziliense*), and whipworm (*Trichuris vulpis*).

Prevention of heartworm disease (*Dirofilaria immitis* larvae) with monthly administration."

The wording of the indication makes it clear that the combination product is intended for use where both active substances are required. The following text is included at the beginning of section 4.2 of the SPC:

"For the treatment of flea and tick infestations in dogs when the concurrent prevention of heartworm disease and/or treatment of gastrointestinal nematode infections is indicated."

Part 5 – Benefit-risk assessment

Introduction

The product is an oral chewable tablet for dogs containing a fixed combination of two active substances: afoxolaner and milbemycin oxime. The combination is considered a new fixed combination of active substances previously authorised within EU and is therefore considered a new active substance. The product is intended for use in dogs suffering from or at risk of mixed parasitic infections involving ectoparasites, gastrointestinal nematodes and/or heartworm. The product is intended for use as part of a treatment strategy for the control of flea allergy dermatitis.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC.

Benefit assessment

Direct therapeutic benefit

The combination of substances afoxolaner and milbemycin oxime in the proposed product has been justified. Parasites targeted by the combination product (fleas, ticks, gastrointestinal nematodes and heartworm) are commonly found in dogs in Europe and can be present simultaneously on the same animal. In addition, the two active substances have different spectra of activity as afoxolaner is acting as an ectoparasitic effective against infestations of ticks and fleas and milbemycin oxime is an endectocide and active against gastrointestinal nematodes and *Dirofilaria immitis* larvae.

The recommended treatment dose is 2.5 mg afoxolaner/kg bw and 0.5 mg milbemycin oxime/kg bw. The treatment can be repeated at monthly intervals depending on the indication and epidemiological situation.

The benefit of NEXGARD SPECTRA relates to its efficacy in:

- The treatment of flea and tick infestations in dogs when the concurrent prevention of heartworm disease and/or treatment of gastrointestinal nematode infections is indicated;
- The treatment of flea infestations (*Ctenocephalides felis* and *C. canis*) in dogs for 5 weeks;
- The treatment of tick infestations (*Dermacentor reticulatus*, *Ixodes ricinus*, *Rhipicephalus sanguineus*) in dogs for 4 weeks;

  Fleas and ticks must attach to the host and commence feeding in order to be exposed to the active substance;
- The treatment of infestations with adult gastrointestinal nematodes of the following species: roundworms (*Toxocara canis* and *Toxascaris leonina*), hookworms (*Ancylostoma caninum* and *Ancylostoma braziliense*), and whipworm (*Trichuris vulpis*);
- The prevention of heartworm disease (*Dirofilaria immitis* larvae) with monthly administration.

The efficacy of the product in the proposed dose and dosing interval has been confirmed in a large number of well-controlled laboratory and field studies.

Efficacy was not confirmed for the prevention of angiostrongylosis due to *Angiostrongylus vasorum* in endemic areas, based on a single study, and the proposal for this indication was withdrawn during the procedure.

Additional benefits

NEXGARD SPECTRA increases the range of available treatment possibilities for concurrent flea infestations and single/mixed nematode infections in dogs. It can also be used for the prevention of heartworm disease in dogs at risk, when these are also infested with ticks or fleas.

The fixed combination facilitates dog handling by reducing the total number of tablets to be given.

Risk assessment

Main potential risks have been identified as follows:

Quality:

The formulation and manufacture of NEXGARD SPECTRA are well described and specifications set will ensure that product of consistent quality will be produced provided that the validation of the manufacturing process with commercial scale batches is performed, as recommended.

For the target animal:

Administration of the combination product in accordance with SPC recommendations is generally well tolerated. The potential for mild and transient adverse effects such as vomiting, diarrhoea, lethargy, anorexia, and pruritus cannot be excluded.

The safety of milbemycin oxime in avermectin-sensitive collies was confirmed in a study with milbemycin oxime alone or in combination with afoxolaner. Signs consistent with avermectin-type toxicity (salivation,

incoordination) were observed in some animals administered milbemycin at 5.0 mg/kg bw (5x the maximum recommended treatment dose). However, the effects were mild and transient. The toxicity profile of milbemycin oxime in such sensitive collies remained similar when given in combination with afoxolaner.

For the user:

The CVMP concluded that user safety for this product is acceptable when used as recommended and taking into account the safety advice in the SPC.

For the environment:

The product is for individual treatment of companion animals. NEXGARD SPECTRA is not expected to pose a risk for the environment when used according to the SPC.

Special risks:

Lack of efficacy of milbemycin oxime in the prevention of heartworm disease in dogs has been reported outside Europe. Given the concerns regarding resistance development, section 4.4 of the SPC includes warnings relating to the potential for resistance emergence.

Risk management and mitigation measures

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

In addition, the SPC includes warnings relating to the potential for resistance emergence and related prudent use warnings.

Evaluation of the benefit-risk balance

The product has been shown to have a positive benefit-risk balance overall.

The product has been shown to be efficacious for the following indications:

- The treatment of flea and tick infestations in dogs when the concurrent prevention of heartworm disease and/or treatment of gastrointestinal nematode infections is indicated;
- The treatment of flea infestations (*Ctenocephalides felis* and *C. canis*) in dogs for 5 weeks;
- The treatment of tick infestations (*Dermacentor reticulatus*, *Ixodes ricinus*, *Rhipicephalus sanguineus*) in dogs for 4 weeks;

  Fleas and ticks must attach to the host and commence feeding in order to be exposed to the active substance;
- The treatment of infestations with adult gastrointestinal nematodes of the following species: roundworms (*Toxocara canis* and *Toxascaris leonina*), hookworms (*Ancylostoma caninum* and *Ancylostoma braziliense*), and whipworm (*Trichuris vulpis*);
- The prevention of heartworm disease (*Dirofilaria immitis* larvae) with monthly administration.

The formulation and manufacture of NEXGARD SPECTRA are well described and specifications set will ensure that product of consistent quality will be produced.

It is well tolerated by the target animals and presents an acceptable risk for users and the environment when used as recommended and appropriate warnings have been included in the SPC.

Conclusion on the benefit-risk balance

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

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of NEXGARD SPECTRA are considered to be in accordance with the requirements of Directive 2001/82/EC.

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP recommends the granting of the marketing authorisation for NEXGARD SPECTRA.