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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for NexGard Combo (EMEA/V/C/005094/0000)

INN: esafoxolaner / eprinomectin / praziquantel

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

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Introduction

On 5 November 2020, the CVMP adopted an opinion and CVMP assessment report.

On 06 January 2021, the European Commission adopted a Commission Decision granting the marketing authorisation for NexGard Combo.

On 21 May 2019, an application for a marketing authorisation to the European Medicines Agency (The Agency) for NexGard Combo was submitted, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (optional scope). The initial applicant for the product was Merial; however, due to a company merger, the applicant changed during the procedure to Boehringer Ingelheim Vetmedica GmbH.

The eligibility to the centralised procedure was agreed upon by the CVMP on 20 July 2018 as NexGard Combo contains a combination of active substances (esafoxolaner, eprinomectin and praziquantel), one of which (esafoxolaner) was not authorised as a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004. At the time of submission, the applicant applied for the following indications:

> "For cats with, or at risk from mixed infestations by cestodes, nematodes and ectoparasites. The veterinary medicinal product is exclusively indicated when all three groups are targeted at the same time.
>
> Ectoparasites:
>
> Treatment and prevention of infestations by fleas (*Ctenocephalides felis)*. One treatment prevents further infestations for at least one month.
> Prevention of environmental flea contamination by preventing egg laying for over a month.
> The product can be used as part of a treatment strategy for the control of flea allergy dermatitis (FAD).
> Treatment and prevention of infestations by ticks (*Rhipicephalus sanguineus*, *Ixodes ricinus*, *Ixodes scapularis*, *Amblyomma americanum*). One treatment prevents further infestations for at least one month.
>
> Treatment of ear mange (*Otodectes cynotis*).
>
> Gastro-intestinal cestodes:
>
> Treatment of infestations with tapeworms (*Dipylidium caninum*, *Taenia taeniaeformis*, *Echinococcus multilocularis, Joyeuxiella pasqualei* (adult), and *Joyeuxiella fuhrmanni* (adult)).
>
> Gastro-intestinal nematodes:
>
> Treatment of infestations with gastrointestinal nematodes (L3, L4 larvae and adults of *Toxocara cati*, L4 larvae and adults of *Ancylostoma tubaeforme* and *Ancylostoma ceylanicum*, and adult forms of *Toxascaris leonina* and *Ancylostoma braziliense*).
>
> Cardio-pulmonary nematodes:
>
> Prevention of heartworm disease (*Dirofilaria immitis* larvae) for one month.
>
> Treatment of infestations with feline lungworms (L4 larvae and adults of *Troglostrongylus brevior).*
>
> Vesical nematodes:
> Treatment of infestations with vesical worms (*Capillaria plica*)."

NexGard Combo is a fixed combination of three antiparasitic active ingredients, esafoxolaner, eprinomectin, and praziquantel, which act against ectoparasites, nematodes, and cestodes, respectively. The target species is cats. NexGard Combo is a spot-on solution available in two strengths at a dose volume of 0.3 ml (i.e. 3.6 mg+1.2 mg+24.9 mg) or 0.9 ml (i.e. 10.8 mg+3.6 mg+74.7 mg), and is presented in packs containing 1, 3, 4, 6 (0.9 ml volume only), or 15 applicators.

The rapporteur appointed is Andrea Golombiewski (replacing Gesine Hahn) and the co-rapporteur is Niels Christian Kyvsgaard.

The dossier has been submitted in line with the requirements for submissions under Article 13b of Directive 2001/82/EC – a fixed combination application.

Scientific advice

The applicant received scientific advice from the CVMP on 15/06/2017 (EMA/CVMP/SAWP/178051/2017). The scientific advice pertained to the efficacy/clinical development of the dossier.

Scientific advice EMA/CVMP/SAWP/178051/2017 mainly addresses eprinomectin and praziquantel dose determination, product equivalence as per VICH GL7, and clinical demonstration of the efficacy in endoparasites. The applicant followed CVMP's scientific advice.

MUMS/limited market status

The applicant requested classification for some parasites in this application as MUMS/limited market by the CVMP, and the Committee confirmed that, where appropriate, the data requirements in the relevant CVMP guideline(s) on minor use minor species (MUMS) data requirements would be applied when assessing the application. MUMS/limited market status was granted as the indications for the treatment of infections caused by the tapeworms *Joyeuxiella (J.) pasqualei and J. fuhrmanni*, the lungworm *Troglostrongylus brevior*, the hookworm *Ancylostoma ceylanicum* and the vesical worm *Capillaria plica* in cats are considered a minor use.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant changed during the assessment procedure, as Merial S.A.S. was merged with Boehringer Ingelheim. The new electronic application form reflects that now Boehringer Ingelheim Vetmedica GmbH is the applicant. Accordingly, the latest version of Boehringer's DDPS was submitted, which was already assessed and approved in former procedures as indicated by the applicant. 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 and therefore fulfils the requirements of Directive 2001/82/EC.

Manufacturing authorisations and inspection status

Manufacture of the dosage form takes place in the EU. GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the manufacture of the proposed veterinary dosage form, has been provided.

Batch release takes place at Boehringer Ingelheim Animal Health France SCS, 31000 Toulouse, France which holds a manufacturing authorisation. GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the batch release of such veterinary dosage forms (non-sterile liquids for external use), has been provided.

GMP declarations for the active substance manufacturing sites of the three actives were provided from

the Qualified Person (QP) at the EU batch release site. The declarations were based on audits of the sites within the last three years.

Overall conclusions on administrative particulars

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

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

Part 2 - Quality

Composition

The finished product is presented as non-aqueous spot-on solution containing 12.0 mg/ml esafoxolaner, 4.0 mg/ml eprinomectin and 83.0 mg/ml praziquantel as active substances. Two different single-dose volumes are proposed: 0.3 ml (3.6 mg esafoxolaner, 1.2 mg eprinomectin and 24.9 mg praziquantel) and 0.9 ml (10.8 mg esafoxolaner, 3.6 mg eprinomectin and 74.7 mg praziquantel). Both volumes are filled in the same spot-on applicator system.

The active substances are solubilised in dimethyl isosorbide (DMI), glycerol formal and Butylhydroxytoluene (BHT).

Containers

The primary packaging material, for both presentations, is a 1 ml single-dose applicator. Each spot-on applicator comprises of a clear cyclic olefin copolymer syringe-shaped barrel (siliconised), a polypropylene plunger rod with plunger (siliconised) and a bromobutyl rubber tip cap.

Secondary packaging consists of individual child resistant polyethylene trays with plastic lids. The plastic trays are then packed into cardboard cartons. Pack sizes of 1, 3, 4 or 15 applicator(s) containing 0.3 ml spot-on solution and pack sizes of 1, 3, 4, 6 or 15 applicator(s) containing 0.9 ml spot-on solution are proposed.

The material complies with the relevant European Pharmacopoeia (Ph. Eur.) and EU requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

The pack sizes are consistent with the dosage regimen and duration of use.

Development pharmaceutics

The product is formulated as a spot–on solution in a non-aqueous vehicle consisting of two solvents. Development of NexGard Combo is based on already authorised veterinary medicinal products by the same applicant, the centralised authorisation for Broadline (EMEA/V/C/002700) and national authorisation for Centragard. The same solvent system and packaging material as in Broadline are used in NexGard Combo, except for minor changes in quantity of an excipient and addition of esafoxolaner instead of fipronil and (S)-methoprene. Furthermore, unstabilised glycerol formal is used in NexGard Combo. Development reports of Broadline have been provided that were extended by data on Broadline spiked with esafoxolaner, to simulate the proposed finished product. The choice and amounts of excipients has been adequately justified.

The vehicle is hygroscopic. Therefore, water absorption needs to be restricted to prevent precipitation and/or degradation of the active substances. The stability studies provided demonstrate little water absorption by the formulation when stored at higher temperatures and humidities.

Photolability was observed for eprinomectin and esafoxolaner when exposed to light. The choice of the cardboard carton used for secondary packaging was shown to provide sufficient protection to the product against photodegradation.

All excipients are known pharmaceutical ingredients. The quality of butylhydroxytoluene is compliant with Ph. Eur. standard. The list of excipients is included in section 6.1 of the SPC.

Results of studies designed to determine the residual volume of product after expression of the dose were provided for both volumes. The target fill volume range was determined.

Method of manufacture

The manufacturing process is considered a standard process. Manufacture of the bulk solution and its filling into spot-on applicators is a standard and straightforward process. Process parameters are indicated. The in-process control (IPC) on the bulk solution include tests for fill weight and clarity of solution to be checked before proceeding to the filling process and an integrity test of the primary container performed after filling.

The manufacturing process will be validated after scale-up prior to commercialisation. As the manufacturing process is a standard process, this approach is acceptable. The process validation scheme outlining the formal process validation studies to be conducted on production scale batches has been submitted.

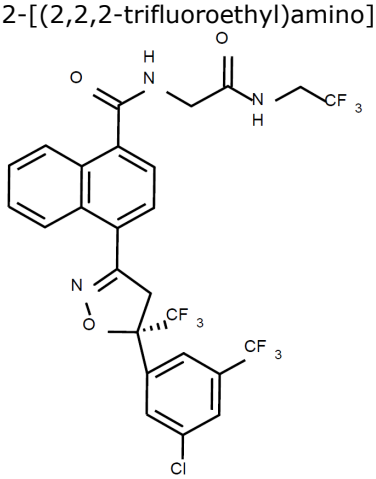
Control of starting materials

Active substance

Esafoxolaner

The active substance esafoxolaner is an ectoparasiticide of the isoxazoline class. The active substance possesses one chiral centre and is the enantiomeric pure S-form of the already authorised racemic afoxolaner. The applicant considered that there are no significant differences in terms of safety and/or efficacy between the already authorised racemate afoxalaner and its S-enantiomer, and esafoxalaner is therefore not classified as a new active substance (in line with the CVMP reflection paper on the chemical structure and properties criteria to be considered for the evaluation of New Active Substance (NAS) status of chemical substances for veterinary medical products, EMA/CVMP/QWP/3629/2016).

The chemical name is 4-[(5S)-5-[3-chloro-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl]-N-{2-oxo-2-[(2,2,2-trifluoroethyl)amino]ethyl}-1-naphthamide. The active substance has the following structure:



The active substance is a white to off-white crystalline powder which is insoluble in water. As a solid, non-solvated substance, esafoxolaner has been observed in amorphous state and in two crystalline forms (Form I and Form II).

The information on the active substance is included in full detail within the dossier.

Esafoxolaner is not subject of a monograph in either the European Pharmacopoeia (Ph. Eur.) or any other pharmacopoeia of the EU. The active substance specification has been established in-house by the applicant and includes tests for: appearance, identity, assay, colour of solution, (R)-afoxolaner content, impurities, residual solvents (GC), water content and sulfated ash.

The specification for the final active substance esafoxolaner controls relevant parameters. Two methods for identification are used. The specification includes limits for single unspecified impurities and total impurities. The limits for the unspecified impurities are in compliance with thresholds in VICH GL10 on impurities in new veterinary drug substances. The absence of tests for particle size distribution and microbiological purity is acceptable considering the intended use.

The analytical methods used have been sufficiently described and non-compendial methods appropriately validated in accordance with the VICH guidelines. These data are satisfactory, and it can be concluded that the methods are suitable for the intended use. Satisfactory information regarding the reference standard used for assay has been presented.

Detailed information on the manufacture of the active substance has been provided. Esafoxolaner is synthesised in three chemical steps and uses three regulatory starting materials. Adequate in-process controls are applied during the synthesis.

The route of synthesis of the starting materials have been provided, including the solvents, reagents, catalysts used. For each starting material a specification including limits for impurities such as specified, unspecified and total impurities, solvents and residue catalysts (as needed) has been provided. The carry-over of potential impurities from the starting materials to the final substance has been discussed. The proposed starting materials are considered acceptable. The names and addresses of the manufacturers of the starting materials are included in the dossier as well.

A specification for each raw material and solvent used in the production is provided. The proposed specification of the catalyst has been adequately justified.

A detailed specification has been provided for the intermediates. Analytical methods have been described and validated. Critical process parameters and in-process controls were identified by the manufacturer.

The chemical structure of the drug substance is controlled during the manufacturing process. Solubility studies are performed in different organic solvents.

Under the manufacturing conditions applied to isolate the final drug substance, both crystalline forms have been obtained and identified

The discussion on the impurity profile of the drug substance is thorough. Possible impurities and their occurrence in starting materials intermediates and the final API have been discussed.

The discussion on residual solvents is considered acceptable. A risk assessment on elemental impurities was performed. No elemental impurities are intentionally introduced in the manufacture of the substance after introduction of the starting materials. Discussion on the presence of mutagenic impurities has been provided.

Batch analysis data (n= 3 and industrial scale) of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

Stability data on 3 batches of active substance from the proposed manufacturer stored in the intended commercial package for 36 months under long term conditions at 30 °C/65% RH and for up to 6 months under accelerated conditions at 40 °C/75% RH according to the VICH guidelines were provided. The commercial packaging of esafoxolaner consists of a primary packaging of double polyethylene bags and

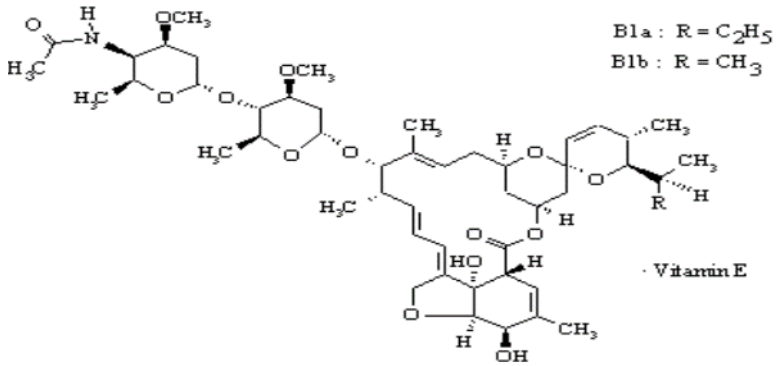
a secondary packaging of high-density polyethylene (HDPE) drums.

All tested parameters were within the specification. Forced degradation studies were performed on esafoxolaner, as part of the development and validation of both HPLC methods for related substances. Esafoxolaner was found to be stable under heat, humidity, light and oxidative conditions but pH sensitive to both acidic and basic conditions.

The stability results indicate that the active substance is sufficiently stable. The stability results justify the proposed retest period of 48 months (based on extrapolation of stability test results) without any storage precaution.

Eprinomectin

The active substance eprinomectin is a mixture of the two components B1a and B1b. The chemical name of component B1a is (4''R)-4"-(acetylamino)-5-O-demethyl-4''-deoxyavermectin A1a, and of component B1b is (4''R)-4"-(acetylamino)-5-O-demethyl-25-de(1-methyl-propyl)-4''-deoxy-25-(1-methylethyl)avermectin A1a. Due to its susceptibility to oxidative degradation, vitamin E (all-rac-alpha-tocopherol as per Ph. Eur. monograph No. 0692) as an antioxidant is added to eprinomectin The active substance has the following structure:



The active substance is a white to off-white crystalline hygroscopic powder with limited solubility in water. Eprinomectin exhibits stereoisomerism due to the presence of 20 (B1a) or 19 (B1b) chiral centres. The mixture is optically active and the specific optical rotation of eprinomectin is routinely controlled. Polymorphism has not been observed for eprinomectin.

The information on the active substance is included in full detail within the dossier.

Eprinomectin is not subject of a monograph in either the European Pharmacopoeia (Ph. Eur.) or any other pharmacopoeia of the EU. The proposed specification is based on the USP monograph for eprinomectin. The active substance specification includes tests for: appearance, identity, assay, impurities, residual solvents, water content, specific optical rotation, heavy metals, and residue on ignition.

The limits for assay are set according to the USP monograph. The proposed limits for specified impurities are based on toxicity studies performed at the time of development of early eprinomectin finished products. The limits applied for oxidative degradation products and Class 2 solvents are consistent with VICH GL18 limits. Total solvents are controlled to a limit consistent with VICH GL18 limits. Limits for microbiological quality of the active substance are included in the specification but the test will be performed if the active substance is to be used in a parenteral product.

The overall proposed specification is considered acceptable and the proposed limits are adequately justified.

The analytical methods used have been sufficiently described and non-compendial methods appropriately validated in accordance with the VICH guidelines. These data are satisfactory, and it can be concluded that the methods are suitable for the intended use. Satisfactory information regarding the reference standard used for assay has been presented.

Detailed information on the manufacture of the active substance has been provided.

The specifications and control methods for the starting material and reagents have been presented. No justification for the choice of the starting material has been presented. However, eprinomectin is a semi-synthetic active substance and it is considered acceptable to define the fermentation product as starting material. The addresses of the manufacturers of the starting material are included in the dossier.

The quality of the water used is in accordance with the CVMP Note for Guidance on quality of water for pharmaceutical use (EMEA/CVMP/115/01-Revision).

The characterisation of the active substance and its impurities are in accordance with the CVMP guideline on chemistry of new active substances. The chemical structure of the drug substance is verified by several methods. Potential and actual impurities were well discussed with regards to their origin and characterised.

Batch analysis data (n= 3 and industrial scale) of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

Stability data on 5 batches of active substance from the proposed manufacturer stored in the intended commercial package for 36 months under long term conditions at 5 °C ± 3 °C and for up to 6 months under accelerated conditions at 25 °C ± 2 °C /60% RH ± 5% according to the VICH guidelines were provided. Results of the stress tests under heat, humidity, light, acid, alkaline and oxidizing conditions were also provided.

The following parameters were tested: appearance, assay, water, related substances, impurities and vitamin E content.

All tested parameters were within the specification.

The stability results indicate that the active substance is sufficiently stable. The stability results justify the proposed retest period of 36 months when stored at less than 8 °C/ambient RH and protected from light in the proposed container.

Praziquantel

Praziquantel is described in the European Pharmacopoeia (Ph. Eur.) and is covered by a certificate of suitability (CEP). A re-test period of 5 years if stored in a PE bag is specified in the CEP. The absence of use of material of human or animal origin in the manufacture of the substance is declared on the CEP. A risk management summary for elemental impurities has been provided.

The manufacturer's specifications are based on the tests included in the Ph. Eur. monograph for praziquantel, and the additional specifications according to the CEP. The active substance specification is considered acceptable.

Excipients

There are no novel excipients used in the finished product formulation. As dimethyl isosorbide is already included in an authorised veterinary medicinal product (Broadline), it is not considered a novel excipient. Dimethyl isosorbide is not described in the Ph. Eur. or any pharmacopoeia of the EU. An acceptable specification for dimethyl isosorbide has been provided controlling identity, physical characteristics, purity and assay.

A suitable specification for glycerol formal has been provided controlling identity, physical characteristics, purity and assay. The used glycerol formal complies with the Ph. Eur. monograph 1671. Butylhydroxytoluene is the subject of a Ph. Eur. monograph.

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

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

None of the starting materials used for the finished product are risk materials as defined in the current version of the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev 3).

Control tests on the finished product

The specifications proposed for use at release and at the end of shelf-life are in general suitable to control the quality of the finished product and relevant physical, chemical and microbiological parameters of the dosage form.

The release specification includes the following tests: appearance, clarity and colour of solution, density, water content, identity and assay of each active substance, specified, unspecified and total impurities respective to each active substance, assay of (R)-afoxolaner, identity and assay of the antioxidant BHT, uniformity of dosage units, delivered dose and microbiological purity.

The methods for density, clarity and colour of solution, uniformity of dosage units and microbiological purity are performed in accordance with the Ph. Eur. The analytical methods used to control the finished product have been adequately described and appropriately validated in accordance with the VICH guidelines.

Information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for 3 pilot scale batches of each presentation manufactured and tested in the USA. Furthermore, batch analysis results on 2 pilot scale batches of the bulk solution and on one production scale bulk batch manufactured at the proposed manufacturing site in the EU are provided. Taking into account the experience of the finished product manufacturer regarding manufacture of spot-on products as well as the provided results for deliverable volume and the calculations to determine the target filling volume, certificates of analysis on bulk batches are accepted. Comparability of the development batches manufactured in the USA with the bulk batches manufactured in the EU is proven. The analysis results are in compliance with the proposed release specification and confirm consistency of the product.

Stability

Stability data of 3 pilot scale batches of each presentation of the finished product stored under long term conditions for 9 months at 30±2 °C/ 65±5% RH and for up to 6 months under accelerated conditions at 40 ºC/75% RH according to the VICH guideline GL3 were provided. Intermediate stability studies are performed at 30±2°C/ 75±5% RH. The batches of finished product were manufactured in the USA. These batches were packed in the proposed primary and secondary packaging material.

Samples were tested for appearance, clarity and colour of solution, water content, assay of each active substance, specified, unspecified and total impurities respective to each active substance, assay of the antioxidant BHT, delivered dose and microbiological purity. The analytical procedures used are stability indicating.

The parameters remained well in their corresponding specified limits except for two out of specification

results at 40±2 °C/ 75±5% RH. No significant changes have been observed. A slight increase in water content was observed at all three storage conditions. A decrease in active substance content of eprinomectin was observed during stability studies under all storage conditions. Total degradation products of eprinomectin decreased in some batches during stability studies under all storage conditions, in other batches no clear trend was observed. A decrease in content of esafoxolaner could be observed under all three storage conditions but no increase of degradation products occurred.

In addition, one batch of each presentation was exposed to light as defined in the VICH GL5 on photostability testing of new veterinary drug substances and medicinal products. As photostability studies demonstrate that the cardboard box is required to protect the product from light, the storage precaution 'Keep the applicator in the outer carton in order to protect from light´ should be used in the product information.

The proposed shelf-life of 3 years when stored in the blister package as stated in the SPC is considered acceptable.

Overall conclusions on quality

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

The quality of the product NexGard Combo is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical aspects relevant to the performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on TSE safety.

Based on the review of the data on quality, the manufacture and control of NexGard Combo are considered acceptable.

The applicant has committed to perform process validation studies on the first 3 commercial batches. In addition, the first 3 batches produced for commercial release will be placed in a stability study for which the protocol has already been approved.

Part 3 – Safety

NexGard Combo is a new fixed combination for cats with the insecticidal/acaricidal substance esafoxolaner and two anthelmintic substances, eprinomectin (nematocide) and praziquantel (cestocide).

Esafoxolaner is considered a novel active compound for use in cats, although afoxolaner (as racemate) is already approved in veterinary medicinal products (NexGard [EU/2/13/159], NexGard Spectra [EU/2/14/177]) for flea, tick and mite control in dogs. Eprinomectin and praziquantel are well-established anthelmintic substances, and already present in authorised veterinary medicinal products for the treatment of mixed infestations by parasites (e.g. Broadline [EU/2/13/157]).

The current application relies on studies previously assessed by the CVMP in the context of the initial marketing authorisation for Broadline (eprinomectin and praziquantel) as well as NexGard and NexGard Spectra (both afoxolaner), supplemented by relevant scientific literature. This approach was agreed by the CVMP in a scientific advice.

Safety documentation

Pharmacodynamics

See part 4.

Pharmacokinetics

The known pharmacokinetic data of (es)afoxolaner, eprinomectin and praziquantel are summarized as follows:

(Es)afoxolaner

After oral administration in dogs, afoxolaner is rapidly absorbed, with an oral bioavailability of approximately 74%. The mean half-life ranged from 7.7 to 17.8 days. Over 99% of afoxolaner is bound to plasma proteins in cats, dogs and rats without having a high potential for saturable protein binding. Clearance of afoxolaner was low after intravenous administration in dogs. Accumulation was not shown in adult dogs receiving three doses of 2.5 mg/kg bodyweight (bw) at monthly intervals by oral gavage. Various metabolites and the parent compound are eliminated mainly via biliary excretion (biliary clearance is about 30%) and to a lesser extent via urine (renal clearance less than 0.01% of the total clearance).

A bridging pharmacokinetic study to compare the pharmacokinetic profile of the S-enantiomer esafoxolaner and afoxolaner (racemate) when administered via oral gavage in rats was provided. The pharmacokinetic profile of esafoxolaner was similar in female rats when the same dose of afoxolaner was administered (the racemate contains 50% esafoxolaner), but the AUC after administration of afoxolaner was increased by 40% compared to the AUC after administration of esafoxolaner in male rats. This might be due to differences in pharmacokinetics, although, considering the variability of data, the difference can be regarded as adequately similar. Nonetheless, even if the differences observed would be due to differences in pharmacokinetics, this would not have an impact on the question on bridging, because the concentrations of esafoxolaner in the plasma samples of rats after administration of afoxolaner were higher than those after administration of esafoxolaner alone. Thus, studies conducted with afoxolaner would rather provide an overestimation of effects of esafoxolaner, if assuming 50% of the racemate consists of this enantiomer and the whole toxicity of the racemate is allocated to esafoxolaner.

Eprinomectin

According to the MRL summary report (EMEA/MRL/114/96-FINAL), eprinomectin is systemically bioavailable after oral ingestion in rats, and only metabolised to a limited extent and excreted predominantly unchanged via faeces. Eprinomectin was mainly distributed to fat, liver, kidneys and muscles. Once absorbed, eprinomectin is highly bound to plasma proteins (> 99%). After dermal exposure, only 29% of the dose was absorbed through the skin in cattle. In cats, dermal bioavailability of 31% has been reported for eprinomectin. Pharmacokinetic behaviour of eprinomectin is similar in different species investigated (cattle, sheep, goats).

Praziquantel

As noted in the MRL summary report (EMEA/MRL/141/96-FINAL), praziquantel is absorbed between 75 and 100% after oral administration in rat, dog and monkey. It is subject to a marked first pass effect in the liver where it is rapidly and extensively metabolised. The highest concentrations of praziquantel were found in the liver and kidneys. None of the other organs and tissues showed specific accumulation. Praziquantel has a moderate tissue distribution, and about 64–84% of praziquantel is bound to plasma proteins. Its elimination from serum and organs is rapid and almost complete within 24 hours, mainly via the urine. The average half-life for praziquantel is 3.08 days.

Toxicological studies

The active substances eprinomectin and praziquantel were previously assessed by the CVMP in the context of the establishment of MRLs and the key findings of the toxicity studies evaluated (see European Public MRL Assessment Reports (EPMAR) EMEA/MRL/114/96-FINAL and EMA/CVMP/607398/2017 as well as EMEA/MRL/141/96-FINAL and EMEA/MRL/523/98-FINAL), as summarised below.

Several new toxicity studies were submitted for esafoxolaner. In addition, toxicity studies or summaries conducted with the racemate afoxolaner that were already reviewed for the marketing authorisation of NexGard (EU/2/13/159) and NexGard Spectra (EU/2/14/177) were provided. Toxicity studies conducted with afoxolaner can be used to describe the toxicological profile of esafoxolaner, as bridging has been successfully proven via a comparability pharmacokinetic and comparability acute and repeat-dose (14 days in rats) toxicity studies.

Single dose toxicity

Esafoxolaner

Afoxolaner (as an equal mixture of (*R*)-afoxolaner and esafoxolaner) was tested in rats after oral and dermal exposure. The oral no observed adverse effect level (NOAEL) is 30 mg/kg bw/day due to significantly decreased bodyweight starting from 100 mg/kg bw. Afoxolaner was of low dermal toxicity with an LD_{50} value higher than 2000 mg/kg bw. Acute oral toxicity of esafoxolaner was investigated in Sprague-Dawley rats and the no observed effect level (NOEL) was 150 mg/kg bw based on bodyweight reduction. In a combined tolerance and pharmacokinetic study (oral administration) in dogs, one dog showed severe neurological symptoms at the highest dose of 100 mg/kg bw/day. Emesis was observed from 45 mg/kg bw/day. The NOAEL of this study is 25 mg/kg bw/day.

Eprinomectin

Acute toxicity studies of eprinomectin were carried out in Sprague-Dawley rats via oral administration as well as in female Crl:CD-1 (ICR) BR mice and female Crl:CD (SD) rats via gavage and after intraperitoneal administration, respectively. Clinical signs on the CNS were observed. The NOEL was 8 mg/kg bw after oral administration, based on a decreased foot splay at 10 mg/kg bw.

Praziquantel

No acute NOEL was provided for praziquantel. The acute oral and parenteral toxicity of praziquantel to mice and rats is low, with oral LD_{50} values being 2249 mg/kg bw in rats and 2454 mg/kg bw in mice.

Repeat dose toxicity

Esafoxolaner

Afoxolaner (as an equal mixture of (*R*)-afoxolaner and esafoxolaner) was tested via the diet in rats (7, 14 and 90 days), rabbits (5 days) and female mice (7 or 12 days). The NOEL for the 14-day study in rats is 10 mg/kg bw, based on decreased defecation (based on decreased food consumption) at higher doses. In the rabbit study, all treated animals had lower bodyweight gains compared to the control group during the treatment period. The LOAEL of this study was therefore the lowest dose tested, i.e. 300 mg/kg bw/day. Toxicity of afoxolaner was tested in female mice after oral administration by gavage at doses of 0, 10, 30, 100 and 550 mg/kg bw/day administered for 7 or 12 consecutive days. The NOEL in mice was 550 mg/kg/day. In the 90 days study in rats, lower food intake, and therefore lesser bodyweight (gain), hypocellularity of the bone marrow, haemoconcentration and slight differences in oestrus cycle stage compared to the control group were observed in all treatment groups (3, 10 and 50 mg/kg bw/day). Mean total urine volume was increased in the 50 mg/kg/day group. A NOAEL of 3 mg/kg bw/day was established.

Esafoxolaner was administered via the diet in rats (14 days and 90 days). Reduction of reticulocytes at 1000 mg/kg bw/day and increases in adrenal gland weights at 250 mg/kg bw/day were observed after a 14-day exposure. A NOAEL for esafoxolaner of 100 mg/kg bw/day was therefore retained for the two-week study. Increased adrenal gland weights starting from 100 mg/kg bw/day was the most sensitive endpoint in the 90-day study. As this was the lowest dose tested, it is considered a LOAEL.

In a 56-day dermal repeated dose study conducted with afoxolaner in rats, a dermal NOAEL of 10 mg/kg bw/day was determined (the lowest dose of 3 mg/kg bw/day was increased to 60 mg/kg bw/day at day 28). Haematological parameters and clinical chemistry were significantly altered starting from 30 mg/kg bw/day. Furthermore, red material that persisted until the end of the study appeared around nose, mouth, dorsal trunk and forelimbs at 60 mg/kg bw/day. Topical effects included slight erythema and desquamation in females and scabbing in males at 30 and 60 mg/kg bw/day. Hyperkeratotic dermatitis occurred at the application site in all animals including controls (acetone was used as vehicle), but the severity was increased starting from 30 mg/kg bw/day.

In a dermal 28-day study conducted with esafoxolaner (100, 300 or 1000 mg/kg bw/day), no dermal NOAEL could be determined, because reduced food consumption and concomitant reduced bodyweights, haematological effects and serum chemistry changes were observed already in the lowest dose group. Furthermore, higher adrenal gland weights, lower kidney, liver, thymic, splenic, pituitary, epididymis, thyroid, testis, prostate/seminal vesicles weights, uterus and lower ovary/oviduct weights, microscopic changes including cortical hypertrophy of *zona glomerulosa* and *zona fasciculata* and vacuolation of the cells of these layers in the adrenal glands, thymic atrophy, decreased lymphocyte cellularity in the spleen and alveolar histiocytosis in the lungs were observed. A benchmark dose (BMD) analysis was conducted based on relative bodyweight reduction from day 0 to day 28. A benchmark dose lower bound (BMDL) of 6.9 mg/kg bw was estimated for male rats and a BMDL of 49.3 mg/kg bw/day was estimated for female rats. However, as this study was not initially designed to derive a BMDL (e.g., few dose groups were used), this value is likely to be over-conservative. Therefore, the NOAEL of 10 mg/kg bw/day derived in the dermal target animal safety and toxicokinetics study with esafoxolaner in cats is employed in the calculations for user risk assessment.

The overall oral short-term toxicological reference value (TRV) for esafoxolaner is 5 mg/kg bw/day. This value results from the oral 14-day study conducted with afoxolaner with a NOAEL of 10 mg/kg bw/day. This NOAEL was divided by two because afoxolaner consists of an equal mixture of esafoxolaner and (*R*)-afoxolaner, and, as a worst-case assumption, the whole toxicity is allocated to esafoxolaner alone.

The overall oral long-term TRV for esafoxolaner is 1.5 mg/kg bw/day. This value results from the NOAEL of 3 mg/kg bw/day derived in the oral 90-day study conducted with afoxolaner.

Eprinomectin

Eprinomectin was tested via the diet in rats (two studies over 4 weeks, and one over 14 weeks) and dogs (6, 14 and 53 weeks). Adverse effects on the central nervous system were consistent between studies. Dogs were the most sensitive species, with a NOEL of 0.8 mg/kg bw/day in the 14-week study and 1 mg/kg bw/day in the 53-week study.

Praziquantel

Praziquantel was tested via the diet in rats and dogs (both 4 weeks) and dogs (90 days). Studies were conducted in the mid-1970s and were therefore not GLP-compliant. The NOAEL for the 4-week study in rats and dogs is 33 mg/kg bw and 60 mg/kg bw, respectively, and 60 mg/kg bw for the subchronic study in dogs. In the latter study, transient signs of vomiting and depressed appetite as well as an increase in liver weight at higher doses were observed.

Tolerance in the target species of animal

The tolerance in the target animal is described under Part 4.

Relevant for user safety assessment is a non-GLP-compliant study on the target animal safety and the toxicokinetics following topical application of esafoxolaner to young healthy cats. The vehicle used was dimethyl isosorbide (DMI), which is also part of the final formulation of NexGard Combo. Esafoxolaner

was administered 3 times in 4-week intervals at the dose levels 3 mg/kg bw, 10 mg/kg bw and 30 mg/kg bw. In addition, esafoxolaner was administered once at 45 mg/kg bw.

Substance-related systemic findings were mainly a decrease in food consumption and bodyweight gain in the group that received 30 mg/kg on three occasions. Local reactions on a histological level (minimal focal cell necrosis) were noted in one animal in the vehicle control group and two animals that received esafoxolaner. One of these animals (30 mg/kg group) showed a focal area of alopecia and hyperplasia following the third treatment, which is possibly treatment related.

The preliminary NOAEL of 10 mg/kg bw/day from this study was considered as dermal short-term and long-term reference value for user risk assessment.

Reproductive toxicity

Study of the effect on reproduction

Esafoxolaner

Two reproductive toxicity studies were conducted with afoxolaner. In a dietary range-finding one-generation study in rats exposed to 0, 3, 10, 30 or 75 mg/kg bw/day for 7 days before mating and continuing throughout mating, gestation and lactation, reduced food intake and bodyweight were observed in the highest dose in F0 animals. At 30 mg/kg bw/day, reduced litter weight and low number of pups per dam were observed and considered to be treatment related. In a one-generation reproductive toxicity study, rats received dietary doses of 0, 1, 5 and 20 mg/kg bw/day afoxolaner per gavage. Effects on food consumption and reduced bodyweight were observed at the highest dose group in F0 animals. This resulted in litter losses in the later period of lactation. The NOEL in this study was 5 mg/kg bw/day.

A GLP-compliant one-generation dose range-finding study (dietary treatment with 50, 100, 500 or 1500 mg/kg bw/day) was conducted with esafoxolaner in rats. Females were treated throughout mating, during pregnancy and lactation. Males were treated for 14 days prior to mating, throughout mating up to euthanasia after 28 doses. No statistically significant effects were observed on the reproductive performance of the F0 generation. However, effects of esafoxolaner on reproductive and other organ weights were observed at doses from 100 mg/kg bw/day on in the F0 generation. A NOAEL of 50 mg/kg bw/day was retained.

In a GLP-compliant one-generation reproductive toxicity study in rats (oral doses of 0, 100, 300 or 1000 mg/kg bw/day), males were exposed 70 days prior to mating. Females received esafoxolaner for 14 days prior to mating, throughout mating, gestation and lactation. There were no statistically significant effects on F0 reproductive performance. However, treatment-related adrenal effects were already observed in the lowest treatment group. No developmental variations or malformations were observed in pups at any dose level. Based on lower mean pup survival at 1000 mg/kg bw/day and lower mean pup bodyweights and bodyweight gains at 300 and 1000 mg/kg bw/day, a dosage level of 100 mg/kg bw/day is considered to be the NOAEL for neonatal and developmental toxicity.

Eprinomectin

A non-GLP-compliant one-generation dose range-finding study (dietary treatment with 0, 7, 36, 181 mg/kg feed before mating, during pregnancy and lactation) and a two-generation reproductive toxicity study (0, 1, 2.8 and 6 mg/kg bw/day before mating, during pregnancy and lactation) were conducted with eprinomectin in rats. In the dose range-finding study, reproductive performance was reduced at the top dose. Body tremors were observed in the litters at dietary concentrations of 36 and 181 mg/kg feed. In the two-generation study, reduced reproductive performance was observed in adults at the highest dose level. Body tremors were observed in the litter at 2.8 and 6 mg/kg bw/day. The latter concentration increased litter mortality, decreased weight and growth. From this study, a NOAEL of

1 mg/kg bw/day was established. A further study showed that the milk-to-plasma ratio of eprinomectin was 3:1, resulting in enhanced neonatal exposure in nursing pups.

Praziquantel

In a reproductive toxicity study conducted in rats (treatment with 0, 30, 100, 300 mg/kg bw/day for 3–10 weeks premating, throughout mating period and up to 7th day of pregnancy), no embryotoxic effects were observed and fertility and reproductive performance were unaffected by treatment with praziquantel.

Study of developmental toxicity

Esafoxolaner

Afoxolaner showed no potential for teratogenicity in rats and rabbits. Since esafoxolaner is a constituent of afoxolaner, it is reasonable to assume that esafoxolaner is not teratogenic.

Eprinomectin

Based on developmental toxicity studies in rats and rabbits, eprinomectin showed no potential for teratogenicity.

Praziquantel

For praziquantel, no foetotoxic or teratogenic effects were observed in teratogenicity studies performed in rats and rabbits at doses between 0–300 mg/kg bw/day.

Genotoxicity

Based on the negative results of *in vitro* (bacterial reverse mutation assay, mouse lymphoma L5178Y/TK+/- mutation assay, chromosome aberration assay, gene mutation test with V-79 Chinese hamster lung cells at the HGRPT locus) and *in vivo* (mouse bone marrow micronucleus assay) tests, eprinomectin, praziquantel and esafoxolaner were not considered to have a genotoxic potential.

Carcinogenicity

No studies were conducted for any of the active substances due to the absence of genotoxicity findings or relevant findings in the repeated dose toxicity studies. Eprinomectin, praziquantel and esafoxolaner are not considered to have a carcinogenic potential.

Studies of other effects

Final formulation studies

The dermal irritation potential of the final formulation was evaluated in New Zealand White rabbits. The formulation was found to be non-irritant. In a local lymph node assay in mice, the final formulation tested at concentrations up to 100% did not show any skin-sensitising potential.

The ocular irritation potential of the final formulation was evaluated in two New Zealand White rabbits. The final formulation caused irreversible eye damage in 1 animal. When rinsed, the formulation was irritating to the eye of 1 animal, although the effects were reversible. A bovine corneal opacity and permeability (BCOP) assay to confirm these effects was submitted; the formulation was moderately irritating. However, the BCOP assay only detects immediate severe effects and is not suited to assess persistent effects.

In order to provide additional data for a weight-of-evidence approach, another BCOP, EpiOcular and Draize test using more animals were performed. In the BCOP assay, a dilution of the final formulation was non-irritant, while no prediction could be made according to GHS classification on other dilutions. However, according to classification by the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) these other dilutions, including the undiluted formulation, can be considered as mild eye

irritants. In the EpiOcular assay, the undiluted formulation was an eye irritant, while all tested dilutions were non-irritant. In the Draize test performed with different volumes of the final formulation and 3 animals per group, slight to moderate but reversible irritation was observed in all animals and—in contrast to the previously performed Draize test using only 2 animals—no serious eye irritation occurred when tested at 100 µl. The lowest volume testedhad no eye-irritating effects.

Other specific studies

No signs of immunotoxicity or neurotoxicity were observed in repeated dose toxicity studies conducted with esafoxolaner. Therefore, no specific studies were performed.

Excipients

The excipients are butylated hydroxytoluene (BHT), unstabilised glycerol formal and dimethyl isosorbide (DMI). These excipients are currently used in veterinary medicine. The toxicological profile of DMI and glycerol formal (unstabilised) was provided. Glycerol formal is included in Table 1 of Regulation (EU) No 37/2010. As part of that assessment, an ADI of 10 µg/kg bw/day was retained from a developmental toxicity study based on a NOEL of 10 mg/kg bw (foetotoxicity and teratogenicity were observed at 75 mg/kg bw and 300 mg/kg bw, respectively) and a safety factor of 1000.

Dimethyl isosorbide (DMI) is a substance used in cosmetic formulations and it has been proposed as an excipient for topically applied pharmaceutical formulations, largely as an absorption enhancer. DMI is not mutagenic. In both dogs and rats, a NOAEL of 100 mg/kg bw/day was determined in 90-day oral toxicity studies. In developmental toxicity studies, major skeletal, external and visceral abnormalities were observed in rabbits and rats starting from 30 and 100 mg/kg bw/day, respectively. However, because the effects occurred also in control animals and/or were not dose-dependent, they were considered not treatment-related.

Eye irritating effects of the excipients DMI and glycerol formal (unstabilised) as well as the placebo formulation (excipients in the same concentration as in the final formulation without active substances) were investigated in several different Draize tests in New Zealand White rabbits. 100% DMI was irreversibly toxic to the eye, whereas glycerol formal (unstabilised) was only slightly eye-irritating and effects resolved after 72 hours. A very high inter- and intra-laboratory variability of ocular score grading of the placebo formulation was observed (Cat 2B, 2A or 1 according to the GHS classification were determined).

No information was provided for the antioxidant BHT (E 321). However, the concentration of BHT in the formulation is very low and it is authorised as a food additive. Hence, it is not expected to pose a risk to humans.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP guideline EMEA/CVMP/543/03-Rev.1 and EMA/CVMP/SWP/721059/2014.

The product is a spot-on solution, with eprinomectin, praziquantel and esafoxolaner as active ingredients. Excipients contained in the product are currently used in veterinary medicine. The toxicological profile of the excipients DMI and glycerol formal was provided. No information was provided for BHT. However, the concentration of BHT in the formulation is very low and it is authorised as a food additive. Hence, it is very unlikely to pose a risk for the user.

The product is presented as single dose applicator (0.3 or 0.9 ml) and indicated for cats with or at risk from multiparasitic infestation/infection. According to the submitted URA, the product may be administered monthly, if considered necessary by the veterinarian. It is intended to be administered by a non-professional user.

The user may be exposed via the dermal, ocular or oral route. Of these routes, dermal contact with skin is most likely. Dermal exposure may occur at any stage before, during or after administration. Oral exposure may occur through accidental ingestion by a child during the pre-application and by an adult during the application phases (rare and infrequent exposure). Furthermore, oral exposure might occur through hand-to-mouth contact (more likely) in the post-application phase. Finally, being a liquid solution, the final product could make contact with the eyes by splashing or hand-to-eye contact.

The product did not show skin-irritating or sensitizing potential in the respective studies. However, irreversible eye irritation has been observed in one test animal (irritation lasting > 21 days is considered irreversible) after exposure to 0.1 ml final formulation. Additional *in vitro* and *in vivo* tests revealed only slight to moderate reversible eye irritation in all animals tested. Overall, taking all results into account, irreversible eye damage seems unlikely—especially in the post-application phase—but cannot be completely excluded concerning the undiluted product in the application phase.

To protect children from accidental exposure during storage, the product is packed in a child resistant packaging and a warning to keep the product out of reach of children is in place. Eye irritation might occur by accidental splashing of the product during the application phase. Irreversible eye damage is unlikely based on the results of several *in vitro* and *in vivo* studies. However, since in the first Draize test eye-irritating effects were only reversible when eyes were washed after 30-second exposure, a user warning to wash eyes after accidental exposure is in place to protect users during application phase. Concerning the post-application phase, exposure of the user to very small volumes of the formulation is unlikely to result in irreversible eye-irritating effects, which is supported by the results of the *in vitro* and *in vivo* assays testing lower volumes and concentrations of the formulation. Nonetheless, appropriate user safety warnings are included in the SPC as precautionary measure to keep the potential exposure as low as possible.

It should be noted that the application site on the skin is often still noticeable even when the site is already dry, and residue product might remain. Therefore, users should avoid contact with the site of application until the treated area site is no longer noticeable. Children should not be allowed to play with treated animals until the application site is no longer visible and it is recommended that recently treated animals do not sleep with owners, especially children. Furthermore, it is recommended to treat animals in the evening.

The applicant performed a wipe test with a formulation nearly identical to NexGard Combo to determine the exposure of the user. The wipe test was not conducted according to the recommended study design. Therefore, default values as lined out by EMA/CVMP/SWP/721059/2014 were used for the exposure assessment.

A quantitative risk is present.

Three types of exposure (pre-application, application and post-application phase) were considered for the active substances and the excipients:

A child might accidentally ingest parts of the product during the pre-application (storage) phase. This scenario results in margins of exposure (MOEs) of below 100 for esafoxolaner, praziquantel, and greater than 100 for eprinomectinrespectively. Consequently, child-resistant packaging and a warning to keep the product out of reach of children are in place.

In the application phase, adult users might accidentally splash product on their skin in a realistic worst-case exposure scenario. The MOEs are far above 100 for all three active substances but below 100 for two excipients, respectively. In both the short-term and the long-term post-application phase, the calculated MOEs are far above 100 for all three active substances after both dermal and hand-to-mouth exposure. Long-term exposure to the excipients is unlikely due to fast evaporation. However, the short-term MOEs after dermal exposure for excipients are below 100. Since the TRV for glycerol formal is based on

foetotoxic and teratogenic effects, risk-mitigating measures for pregnant women are considered necessary. In addition, the risk-mitigating measures already in place to avoid contact with the treated animal until the application site is no longer noticeable would sufficiently reduce the risk for exposure to one excipient.

Environmental risk assessment

A phase I environmental risk assessment (ERA) was provided according to VICH guideline 6. The environmental risk assessment can stop in phase I, question 3, and no phase II assessment is required because the veterinary medicinal product will only be used in non-food animals.

An appropriate risk mitigation measure is included in the SPC and other product literature stating that NexGard Combo or the empty container should not enter water courses, as this may be dangerous for fish and other aquatic organisms. NexGard Combo is not expected to pose a risk for the environment when used according to the SPC.

Residues documentation

Not applicable

Overall conclusions on the safety documentation

Two individual active components of NexGard Combo—eprinomectin and praziquantel—are already used in authorised veterinary medicines, and the toxicological profiles of these individual substances are known. New studies were submitted for esafoxolaner. Esafoxolaner is a single enantiomer of the racemate afoxolaner, which is also used in authorised veterinary medicines and was assessed previously.

In acute toxicity studies, an overall oral short-term toxicological reference value (TRV) of 5 mg/kg bw for esafoxolaner was determined based on an oral 14-day study in rats conducted with afoxolaner (contains an equal mixture of (*R*)-afoxolaner and esafoxolaner). The NOAEL for afoxolaner was 10 mg/kg bw/day based on decreased defaecation.

The most relevant NOEL for eprinomectin was 8 mg/kg bw after oral administration, based on a decreased foot splay at 10 mg/kg bw.

The acute oral and parenteral toxicity of praziquantel to mice and rats is low: Rat $LD_{50\ oral}$ = 2249 mg/kg bw and mouse $LD_{50\ oral}$ = 2454 mg/kg.

In repeat dose toxicity studies, afoxolaner (contains an equal mixture of (*R*)-afoxolaner and esafoxolaner) was tested via the diet in rats (7, 14 and 90 days), rabbits (5 days) and female mice (7 or 12 days), and esafoxolaner was tested via the diet in rats (14 days and 90 days). The overall oral long-term NOAEL for esafoxolaner was determined to be 1.5 mg/kg bw/day. This value results from the NOAEL of 3 mg/kg bw/day derived in the oral 90-day study conducted with afoxolaner.

Dogs were the most sensitive species in repeat dose toxicity conducted with eprinomectin. NOELs of 0.8 mg/kg bw/day in the 14-week study and 1 mg/kg bw/day in the 53-week study were retained. Adverse effects on the central nervous system were consistent between studies.

For praziquantel, a NOAEL of 33 mg/kg bw/day from a 4-week study in rats, a NOAEL of 60 mg/kg bw/day from a 4-week study in dogs and a NOAEL of 60 mg/kg bw/day from a 90-day study in dogs were retained. Transient signs of vomiting and depressed appetite as well as an increase in liver weight were observed at higher doses. A NOAEL of 167 mg/kg bw/day per day was retained from a 21-day dermal toxicity study.

Reproductive toxicity was studied in laboratory animals:

For afoxolaner, effects on food consumption and reduced bodyweights were observed in F0 animals. This resulted in litter losses in the later period of lactation. The NOEL in this study was 5 mg/kg bw/day. Effects of esafoxolaner on reproductive and other organ weights were observed at doses from 100 mg/kg bw/day in the F0 generation. A NOAEL for of 50 mg/kg bw/day was retained for parental toxicity. Based on lower mean pup survival at 1000 mg/kg bw/day and lower mean pup bodyweights and bodyweight gains at 300 and 1000 mg/kg bw/day a dosage level of 100 mg/kg bw/day is considered to be the NOAEL for developmental toxicity.

For eprinomectin, reduced reproductive performance, body tremors in the litter and increased pup mortality, decreased litter weight and pup growth were observed. An enhanced neonatal exposure in nursing pups due to a milk-to-plasma-ratio of 3:1 of eprinomectin was determined.

For praziquantel, fertility and reproductive performance were unaffected in a reproductive toxicity study in rats. No embryotoxic or foetotoxic effects were observed.

Esafoxolaner, eprinomectin and praziquantel did not show a potential for teratogenicity.

Esafoxolaner, eprinomectin and praziquantel are not genotoxic.

Carcinogenicity studies have not been performed and are not requested.

The product was shown not to be a skin irritant nor a skin sensitizer, but a potential irreversible ocular irritant. The data presented are considered adequate to characterise the toxicity profile of the active substances.

A qualitative and quantitative user risk assessment was provided. Risk management and risk communication are sufficient to ensure the safety of the user adequately.

NexGard Combo is not expected to pose a risk for the environment when used according to the SPC. An appropriate risk mitigation measure is included in the SPC and other product literature stating that NexGard Combo or the empty container should not enter water courses as this may be dangerous for fish and other aquatic organisms.

Part 4 – Efficacy

NexGard Combo is a spot-on formulation containing a fixed combination of the insecticidal/acaricidal substance esafoxolaner with two anthelmintic substances, eprinomectin (nematocide) and praziquantel (cestocide), for topical administration to cats suffering from, or at risk of multiple parasitic infections.

Eprinomectin and praziquantel are well-established anthelmintic substances which are already used in authorised veterinary medicinal products such as Broadline (EU/2/13/157). Esafoxolaner is a novel compound for use in cats, although the racemate afoxolaner is used and approved in veterinary medicinal products (NexGard, NexGard Spectra) in dogs. In line with the guideline on Investigation of chiral active substances (3CC29a) and the scientific advice given by the CVMP (EMA/CVMP/SAWP/178051/2017, 15.06.2017), the applicant did not provide new tests on the individual active substances alone but relied on relevant data previously submitted for Broadline (regarding eprinomectin and praziquantel) and NexGard (afoxolaner), supplemented by relevant scientific literature.

Additionally, new studies were submitted to (i) compare the pharmacological profile of esafoxolaner to the racemate, (ii) evaluate the potential for pharmacological interactions between the active substances when administered as fixed combination, and (iii) determine the pharmacokinetic parameters of the product when administered as indicated to the target species. The applicant's approach followed the scientific advice of the CVMP (EMA/CVMP/SAWP/178051/2017, 15.06.2017).

Pharmacodynamics

Esafoxolaner

Afoxolaner is a substance of the isoxazoline group and a racemic mixture, i.e. equimolar mixture of two enantiomers, (R-)*afoxolaner* and es*afoxolaner* (S-*afoxolaner)*. Isoxazolines act at the central nervous system or the neuromuscular junction of the insect, rather than directly on muscles fibres. Isoxazolines are potent inhibitors of the neurotransmitter gamma aminobutyric acid (GABA) receptor function and work by blocking pre- and post-synaptic transfer of chloride ions across cell membranes. This results in uncontrolled activity of the central nervous system and death of insects or acarines.

Additionally, the applicant submitted one *in vitro* and one *in vivo* study to demonstrate that the acaricidal and insecticidal activity of the racemic afoxolaner is mainly related to esafoxolaner:

In an exploratory *in vitro* study, the investigator compared the activity of the racemic substance afoxolaner and its enantiomers (esafoxolaner, (R-)afoxolaner) in 4 *in vitro* assays (flea ingestion (*C. felis*), flea contact (*C. felis*), tick contact (*R. sanguineus, D. variabilis, A. americanum*) and tick ingestion (*R. sanguineus*). Afoxolaner and its enantiomers were not active via contact but only via ingestion against *C. felis* fleas. Esafoxolaner had a more potent pulicidal activity against *C. felis* compared to afoxolaner, the (R)-enantiomer proved to be significantly less potent. Esafoxolaner was shown to be more potent against *R. sanguineus* and *D. variabilis* via contact compared to afoxolaner, while (R)-afoxolaner was significantly less potent. (R)-afoxolaner had little or no activity against *R. sanguineus* via ingestion. Based on these results, it can be assumed that the activity of afoxolaner (as racemate) is mainly based on its (S)-enantiomer, i.e. esafoxolaner.

Similar observations were made in a well conducted experimental randomised, blinded, placebo controlled clinical study conducted to WAAVP guideline (Marchiondo et al., 2013) comparing the efficacy of afoxolaner and esafoxolaner in the treatment of infestations with *D. variabilis* in dogs at an oral dose of 0.5 mg/kg bw and 1.0 mg/kg bw for both substances. Esafoxolaner proved to be substantially more potent than the racemic afoxolaner. The pharmacodynamic effects of afoxolaner in mammals have been extensively evaluated and can be summarised as follows:

A number of pharmacological differences exist between GABA gated chloride channels of insects and vertebrates and selectivity for insect over mammalian GABA receptors has been demonstrated for other isoxazolines. Also, *in vivo* studies (repeat-dose toxicology in laboratory animals, target animal safety, field studies) did not show evidence of neurological or behavioural effects suggestive of GABA-mediated perturbations in mammals. The CVMP therefore concluded that binding to dog, rat or human GABA receptors is expected to be low for afoxolaner.

In vitro studies reported that afoxolaner can bind to dopamine and norepinephrine cellular transport receptor systems and the CB1 receptor. Inhibition of these catecholaminergic systems and certain types of competitive binding at CB1 receptors may mediate pharmacodynamic effects of diuresis, decreased food consumption, and decreased body weight in animals. The oral toxicity profile of afoxolaner consists of a diuretic effect (rats only), effects secondary to a reduction in food consumption (rats and rabbits only) and occasional vomiting and/or diarrhoea (dogs, 120 and 200 mg/kg bw) following high oral doses.

Eprinomectin

Eprinomectin is a well-established substance of the macrocyclic lactone group and consists of a mixture of two homologous compounds with a major component, 4''-epiacetylamino-4''-deoxyavermectin (B_{1A}), and a minor component, differing by a single methylene group (B_{1B}). Eprinomectin binds selectively and with high affinity to glutamate-gated chloride-channels in nerve and muscle cells of invertebrates which leads to an increased permeability of the cell membranes to chloride ions, resulting in paralysis and death of the parasite. Eprinomectin may also interact with other ligand-gated chloride channels such as those

gated by gamma-aminobutyric acid (GABA). Eprinomectin is active against a wide range of gastrointestinal roundworms and extraintestinal worms.

The pharmacological activity of eprinomectin in mammals is low as mammals do not have glutamate-gated chlorid channels, macrocyclic lactones have a lower affinity to other mammalian ligand-gated chloride channels, and these substances typically do not readily cross the blood-brain barrier.

Praziquantel

Praziquantel is a well-established anthelmintic; it is a synthetic isoquinoline-pyrazine derivate with a dose dependent activity against all development stages of intestinal cestodes. Praziquantel is rapidly adsorbed via the surface of the parasites and affects membrane permeability in cestodes, influencing divalent cation fluxes, particularly calcium ion homeostasis, which is thought to contribute to the rapid muscle contraction and vacuolisation. This results in severe damage to the parasite integument, contraction and paralysis, disruption of metabolism and finally leads to the death and expulsion of the parasite. The pharmacological activity of praziquantel in mammals is negligible.

Pharmacodynamic properties in combination / non-interaction assessment

There is no interaction at receptor level to be expected since all three substances have different modes of action and act on different receptors at the parasite site. This assumption based on mode of action has been confirmed in clinical studies in the target species, with the absence of clinical interactions. Nevertheless, macrocyclic lactones such as eprinomectin are known systemically acting antiparasitics with a wide spectrum of activity against endo- and ectoparasites. However, the applicant satisfactorily justified that the acaricidal effect of eprinomectin is expected to be negligible at the dose of 0.5 mg/kg bw.

Development of resistance

Esafoxolaner

Although the binding site of esafoxolaner is located on the GABA and glutamate chloride channel, which is the same target channel as for fipronil, the receptor-binding site for isoxazolines is unique among the chloride channel modulators and absence of cross-resistance with fipronil resistant arthropods has been demonstrated.

Currently, there are no reports on resistance against the enantiomer esafoxolaner in flea or tick species of the cat. However, possible resistance development of esafoxolaner has been considered, and an adequate warning has been added to the product literature.

Eprinomectin

Eprinomectin is on the European market only since 2014, and there is hardly any report of resistance in its targeted parasite species in cats.

Praziquantel

For praziquantel, recent data revealed that in the USA resistant *Dipylidium caninum* cestodes against praziquantel have been identified in dogs aged less than 2 years and more than 5 years, but to date there are no such reports available for Europe. Moreover, recently the existence of a cat-specific genotype (proposed as new feline species: *Dipylidium felineum*) was proven (Beugnet et al., 2018), revealing that emergence of resistance in the dog genotype does not necessarily lead to the development of resistance in the feline genotype as well.

Nevertheless, prudent use of the product is crucial to ensure that the efficacy is maintained and further development of resistance in the future delayed. A general prudent use warning has been added to the SPC that parasite resistance to any particular class of antiparasitic drug may develop following frequent use of a compound of that class. Therefore, epidemiological information about current susceptibility of the

target species should be taken into account in order to limit the possibility of a future selection for resistance**.**

Pharmacokinetics

Comparative pharmacokinetic study ("bridging study") NexGard Combo and Broadline

Following scientific advice of the CVMP, a GLP-compliant comparative pharmacokinetic study was provided to (i) to determine the pharmacokinetic parameters of esafoxolaner when administered topically to cats in the proposed fixed combination and when administered alone topically or intravenously, and (ii) to compare the pharmacokinetics of eprinomectin and praziquantel when administered topically in the new combination product or within another fixed triple combination product containing eprinomectin and praziquantel (Broadline), following single administration at the recommended treatment dose. The study was conducted in clinically healthy cats (8 cats/group) according to the provisions of the EMA/CVMP guideline for the conduct of pharmacokinetic studies in target animal species (EMA/CVMP/133/99-Final), as applicable. The final formulation of NexGard Combo has been used in this study.

Esafoxolaner

For esafoxolaner, a significantly lower C_{max} (125.7 ng/ml vs. 207.2 ng/ml, 90% confidence interval [45.9%-80.1%]) was determined after topical administration in the fixed combination as compared to the administration as mono-substance alone; also, t_{max} was significantly longer (mean 7.13 days vs. 3.75 days). Additionally, it is noted that the AUC_{last} of esafoxolaner is significantly lower when administered in the fixed combination as compared to the administration as mono-substance (AUC 4162 ng/mL * day vs. 6185.6 ng/mL * day, 90% confidence interval for mean ratio [50.7%-89.3%]). It is concluded that not only the absorption of esafoxolaner is significantly prolonged when administered in the fixed combination product, but also exposure (C_{max}, AUC) is significantly lower. In addition, a high inter-individual variability in pk parameters was noted (e.g. t_{max} ranged from 0.3 to 42 days).

Eprinomectin and praziquantel

The pharmacokinetic parameters were evaluated to determine differences but not equivalence. The results indicate a high inter-individual variability of the pk parameters. Nevertheless, based on the absence of statistically significant differences in C_{max}, t_{max} and AUC_{last} there appears to be no relevant differences in the pharmacokinetic behaviour of eprinomectin and praziquantel when administered in NexGard Combo compared to Broadline. Hence, non-interaction between eprinomectin and praziquantel can be assumed. The CVMP, therefore, concluded that extrapolation of efficacy data submitted for Broadline to support the nematocidal/cestocidal efficacy of NexGard Combo can be accepted for this application.

NexGard Combo – Dose linearity and repeated dose studies

Another GLP-compliant study was provided to evaluate pharmacokinetic parameters of esafoxolaner, eprinomectin and praziquantel when administered as fixed combination topically at different dose levels (0.06, 0.12, and 0.24ml/kg bw corresponding to 0.5X, 1X and 2X the recommended treatment dose (RTD)), and after repeated administration of the recommended treatment dose (0.12 ml/kg) at 4-week intervals to clinically healthy cats. Although plasma concentrations were highly variable, mean exposures of esafoxolaner and eprinomectin appeared to be proportional to the dose as stated by the applicant. CVMP's own calculations indicate that for praziquantel the exposure increased statistically significantly with the dose. While no accumulation could be observed after repeated administration for praziquantel, accumulation was observed for esafoxolaner (ratio 3.24 for C_{max} and for AUC 3.09), and for eprinomectin (ratio 1.59 for C_{max} and for AUC 1.87).

Eprinomectin reached steady state by the fifth monthly dose, while praziquantel reached steady state already by the second monthly dose. For esafoxolaner no clear statement on when steady state was

reached is possible on basis of the data provided but based on pharmacokinetic basics steady state was estimated to be reached by the fifth month; the high accumulation ratio indicates that it might be later than after 6 months (see section "Target animal safety", regarding the potential impact of accumulation of esafoxolaner and eprinomectin). Respective information has been provided in section 5.2 of the SPC.

Other studies

The pharmacokinetics of esafoxolaner were evaluated in two exploratory studies in cats in both a single intravenous dose of ~1 mg/kg bw and a single topical dose of 1.5 or 1 mg/kg bw. There was an absolute bioavailability between 54% and 85.5% after topical administration observed. In general, the exposure of the hydroxyl metabolite was much lower than for the parent compound.

Toxicokinetics following 3 topical administrations of different doses of esafoxolaner was also evaluated in a supportive target animal safety study. The study supports the assumption that, due to the long half-life of the substance, esafoxolaner accumulates following monthly administration.

Justification of fixed combination

A satisfactory justification for the combination product, in accordance with the CVMP Guideline on pharmaceutical fixed combination products, was provided. The principal advantage claimed for the combination of esafoxolaner (insecticide/acaricide), eprinomectin (nematocide) and praziquantel (cestocide) is that it broadens the spectrum of activity for simultaneous treatment and/or prevention of several parasitic infections including ectoparasites, gastrointestinal nematodes and cestodes, and systemic nematodes (lungworms and heartworms). The triple combination is considered therapeutically justified based on a literature review provided by the applicant, which indicates that around 12% of cats harbour concurrent ectoparasite and gastrointestinal nematode infections; although the incidence of mixed gastrointestinal nematode/cestode infections is low (3%).

Detailed information is provided in the SPC and other product literature that this triple fixed combination product should only be used in cats with confirmed mixed infestations/infections or significant risk of such mixed infestations with ectoparasites and nematodes (including for heartworm disease prevention) and where concurrent treatment against cestodes is indicated. In the absence of risk of co-infections, the use of a narrow spectrum antiparasitic should be considered as first-line therapy.

Dose justification/ determination / finding studies

The current application relies, in part, on data that have previously been submitted in relation to products containing the individual substances (Broadline and NexGard).

Esafoxolaner

The dose of 1.44 mg/kg bw esafoxolaner was established based on a number of dose determination studies and supported by several dose confirmation studies. *Ixodes ricinus* and *Otodectes cynotis* are the dose limiting parasites.

Eprinomectin and praziquantel

The proposed doses of 0.5 mg/kg bw eprinomectin and 10.0 mg/kg bw praziquantel were established based on data submitted in relation to Broadline.

Dose confirmation studies

Ectoparasites

Esafoxolaner is systemically acting, and fleas and ticks need to attach to the animal and commence feeding to be killed. Consequently, this mode of action only justifies a treatment claim. The proposed claim "Treatment of infestations by fleas (*C. felis*) and ticks (*I. scapularis, I. ricinus*). One treatment

provides immediate and persistent flea killing activity for one month and tick killing activity against *I. scapularis* for one month and against *I. ricinus* for five weeks" is considered acceptable.

Fleas

Ctenocephalides felis (adults)

To confirm the adulticidal efficacy after a single administration of NexGard Combo against *Ctenocephalides felis* on cats the applicant has provided three GCP compliant dose confirmation studies and one dose determination study designed and performed following the provisions of the CVMP guideline on 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.3). All but one studies were performed with the final formulation. The studies were blinded with a negative control (placebo/untreated) group and a treatment group, using a randomized block design based on pre-treatment flea counts. Cats were infested on D-1 and treated once topically on D0 at the minimum RTD of 0.12 ml/kg bw with NexGard Combo. The immediate efficacy was assessed 24h post-treatment and then weekly assessment after infestations was performed for persistent efficacy evaluation. Efficacy calculation based on comparison of arithmetic means of live flea counts in treated and untreated groups.

The results of two of the dose confirmation studies were valid. The studies demonstrated efficacy from 99.7 to 100% up to 42 days and from 98.3 to 100% up to 31 days after administration, respectively. The other studies failed caused by poor infestation rates in control animals or delayed onset of appropriate efficacy (>95%). However, the indication against *Ctenocephalides felis* was adequately confirmed.

Prevention of egg laying

In two of the GCP compliant laboratory dose confirmation studies described above the applicant evaluated the efficacy of the product in preventing environmental flea contamination by preventing egg laying for a month after a single administration of NexGard Combo. Flea eggs fallen off the cats were collected on the first and second day following each artificial infestation and were incubated for 3 days until larval hatching for larval count. The efficacy in preventing egg laying was based on the reduction of egg counts in the treated group compared to the untreated control. The efficacy rates at the first evaluation time point on Day +1 was below 95% (60% and 85.9%), while at all other time points up to Day +31 efficacy was nearly 100%. Larval hatching from incubated eggs and larvae counts was significantly affected in the treated group. Although, both studies were performed outside Europe (South Africa and USA), one study was performed using a flea isolate originates from a European country, which is considered representative for European conditions. Additionally, two different isolates including adequate refreshing cycles of 2-5 years were used which is in line with the relevant Guideline recommendations (EMA/CVMP/EWP/005/2000-Rev3). The claimed "prevention of environmental flea contamination by preventing egg laying" is in principle accepted but it is not a clinical indication as such. Therefore, the following appropriate information was added in section 5.1 of the SPC: `Esafoxolaner kills fleas before egg production and therefore prevents household contamination´.

Use as part of a treatment strategy for control of flea allergy dermatitis (FAD)

In line with EMEA/CVMP/EWP/005/2000-Rev.3 data from a GCP compliant, European multi-centre, positive controlled field study in cats naturally infested with fleas and/or ticks were submitted to support the claim that this product can be used as part of a treatment strategy to control clinical signs of FAD. Treatment was performed once topically on day 0 using the final product formulation at the recommended dose. The presence of FAD was evaluated in all cats enrolled based on clinical signs of FAD prior to commencement of the study and at study termination on Day +28. Extent and intensity of dermatological signs were assessed using a general scoring system. As a result, 95.7% showed a clinical improvement in the overall scoring, compared to 90.5% in the positive control group, and non-inferiority to the comparator product was shown. Hence, the indication was accepted.

Ticks

Five GCP compliant dose confirmation studies have been provided to confirm the efficacy of NexGard Combo for the treatment and prevention of artificial tick infestation on cats after a single topical administration between the shoulders. The studies were conducted and designed in line with the CVMP guidelines on 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.3) and the general CVMP guideline for the "*Demonstration of efficacy of ectoparasiticides*" 7AE17a) and were mainly performed with the final formulation (except two studies). All studies were blinded with a negative control group (untreated/placebo) and a treatment group using a randomised block design based on pre-treatment tick count. Cats were infested on D-2 and treated once topically on D0 at the minimum recommended dose of 0.12 ml/kg of NexGard Combo. The immediate efficacy was assessed 48h post treatment and then weekly assessment after infestations (except for two studies) followed to evaluate persistent efficacy. Calculation of efficacy against ticks was based on arithmetic means. No treatment related adverse events were observed during the studies.

Ixodes ricinus (adults)

Three dose confirmation studies were provided to justify the efficacy of NexGard Combo against *Ixodes (I.) ricinus* in cats. All studies showed efficacy values above the guideline threshold from 91.1% to 100% up to 37 days after treatment. Although one of the studies could only be considered as being supportive, the other two studies were in line with the relevant guidelines (7AE17a, EMEA/CVMP/EWP/005/2000-Rev.3, VICH GL9) and the indication against *Ixodes ricinus was* accepted.

Ixodes scapularis (adults)

Two GCP-compliant dose confirmation studies were performed in the USA either using wild caught ticks collected in Jasper County, South Carolina, USA or isolates of *Ixodes scapularis* maintained and bred in a US laboratory. Ticks originated from replete females collected from cattle in USA. At OSU ticks are maintained and bred on rabbits and sheep, larvae from replete females collected from cattle are introduced into the colony on a yearly basis for genetic enrichment. High persistent efficacy rates against *I. scapularis* of 95.1 to 100% and 98.8 to 98.2% have been shown from D+2 till D+32, respectively. Therefore, the efficacy claim of NexGard Combo against *I. scapularis* was accepted.

Ear mites (*Otodectes cynotis)*

To justify the claim "treatment of infestation by ear mites (*Otodectes cyno*tis)", the applicant provided four laboratory studies (one GCP compliant experimental challenge dose determination and three dose confirmation studies in naturally infested cats, two of which were GCP compliant). The basic study design was largely in accordance with the provisions of the CVMP guideline for the demonstration of efficacy of ectoparasiticides (7AE17a). All studies were blinded with a negative control (untreated/placebo) group and a treatment group, using a randomised block design based on bodyweight (exploratory study) or approximate pre-treatment mite infestation counts. Cats were treated once topically with the final formulation or an identical formulation on D0 at the minimum recommended dose of 0.12 ml/kg bw. Depending on the individual studies, the efficacy was assessed based on ear mite counts after collection of ear contents on either D+27, D+28, D+31 or D+32. Efficacies of the NexGard Combo in comparison to negative control groups were presented on the base of geometric means, supplemented by arithmetic means. In one dose confirmation study, conducted in the USA in cats naturally infested with ear mites, efficacy above the threshold of 90% efficacy was reliably demonstrated at day +32 (99.9%). Another dose confirmation study conducted in the USA, failed due to poor infestation rates of the negative control and is not considered to be conclusive. Two further studies conducted in South Africa and France in cats demonstrated efficacy of more than 90% against infestation with ear mites after 4 weeks, although remaining mites were still present in some treated cats. Although these studies were apparently not

conducted with the final formulation, they can be accepted as representative for the final product formulation intended for marketing.

These studies are supported by a field study, see below. This field study supports the laboratory data with an efficacy of 97.4% or 96.5% based on geometric or arithmetic mean mite counts, respectively. The differences in mite counts were statistically significant between groups ($p < 0.0001$). Although not a predefined objective of another field study for the demonstration of efficacy against ticks and fleas, instruction was given at some study sites to evaluate ear mite infestations. A certain efficacy against ear mites was noted by the applicant. However, since the study was overall not adequately designed to assess efficacy against ear mites, no conclusion can be drawn from this study in regard to the treatment of infestation with ear mites.

Taken together, based on the data presented the claimed "treatment of infestation by ear mites (*Otodectes cynotis*)" in cats can be accepted.

Endoparasites

In line with CVMP's scientific advice the applicant made cross-reference to relevant studies previously submitted in the context of the initial marketing authorisation for Broadline (regarding eprinomectin and praziquantel) to support the claims against endoparasites. The use of this data is supported by the results of a comparative pharmacokinetic study between NexGard Combo and Broadline (see above) which indicate that non-interaction between the active ingredients of NexGard Combo can be assumed. Since the applicant confirmed that the final formulation of NexGard Combo intended for marketing has been used in this study, this approach is accepted by the CVMP.

Nematodes

The applicant considered *Toxocara cati* as the least susceptible nematode based on the dose range studies submitted in support of the Broadline application.

It is acceptable to use *T. cati* as doselimiting species of the nematode group consisting of *A. tubaeforme*, *T. leonina*, *A. braziliense*, *Capillaria plica* and *A. ceylanicum* and *T. brevior, and* the approach of conducting two dose confirmation (DC) studies using *T. cati* as the dose limiting nematode of eprinomectin only, to demonstrate efficacy against all the claimed nematodes is supported. Efficacy of topically applied 0.5 mg/kg bw eprinomectin against *A. tubaeforme* L4 and *T. cati* L3 and L4 is adequately supported by the results of two studies (each) in cats and in an additional exploratory laboratory study in cats showing efficacy of a laboratory formulation against *T. cati* L3 and L4 stages.

Gastrointestinal nematodes

The proposed indications for the treatment of infections with gastrointestinal nematodes include L3, L4 larvae and adults of *Toxocara cati*, L4 larvae and adults of *Ancylostoma tubaeforme* and *Ancylostoma ceylanicum*, and adult forms of *Toxascaris leonina* and *Ancylostoma braziliense*).

Toxocara cati (L3, L4 and adults):
Three GCP-compliant, blinded, randomized, negative controlled dose confirmation studies, conducted in naturally infected cats in Albania and in experimentally infected cats in the USA, were submitted to demonstrate the efficacy of a single topical application of the final formulation of NexGard Combo at the recommended minimum dose of 0.12 ml/kg bw, against *T. cati*. One study investigated adult and L4 stages of *T. cati*, in two other studies adult *T. cati* were investigated. All studies were performed in accordance with VICH GL7, 9 and 20. One study was considered only supportive information, since the level of infestation in control cats did not allow a valid calculation as per VICH GL7 and 20. Based on worm counts at necropsy, efficacy rates above 95% were demonstrated, thus, sufficient dose confirmation data are presented to support a claim against adult *T. cati*. Efficacy against L3 and L4 stages of *Toxocara cati* was established based on Broadline data (containing the same amount of

eprinomectin).

Ancylostoma tubaeforme (L4 and adults):
The claim was bridged from the data previously submitted for Broadline. In summary, five GCP-compliant dose confirmation studies using natural and induced infections, adding to one non-interference study were provided to support the efficacy of Broadline against adult and L4 *A. tubaeforme*. Efficacies were above the 90% threshold in all trials. Consequently, the claim was accepted.

Ancylostoma ceylanicum (L4 and adults):
The claim was bridged from data previously submitted for Broadline. Based on the limited prevalence in Europe, MUMS status was recognised by CVMP for this parasite species. Therefore, the applicant only provided one GCP-compliant, laboratory controlled, randomised, blinded dose confirmation study to confirm the efficacy against L4 and adults of *A. ceylanicum* in artificially infected cats. The study was performed in Germany, using a field isolate originated from Thailand for challenge and was well designed and conducted in line with the relevant guidelines VICH GL7, VICH GL9 and VICH GL20. The study demonstrated 100% efficacy of Broadline against both L4 and adult forms of *A. ceylanicum* compared to untreated control animals on D+31. Consequently, the claim was accepted.

Toxascaris leonina (adults):
The claim was bridged from the data previously submitted for Broadline. In summary, two GCP-compliant, laboratory controlled, randomised, blinded dose confirmation studies in experimentally infected cats were provided which confirmed an efficacy level of more than 90% for Broadline against adult forms of *T. leonina*. Consequently, the claim was accepted.

Ancylostoma braziliense (adults):
The claim was bridged from the data previously submitted for Broadline. In summary, two GCP-compliant, laboratory controlled, randomised, blinded dose confirmation studies confirmed an efficacy level of more than 90% for Broadline against adult forms of *A. braziliense*. Natural infection was used in one study conducted in South Africa, and experimental infection was applied in the other. Consequently, the claim was accepted.

<u>*Cardiopulmonary and vesical nematodes*</u>

The proposed indications for cardio-pulmonary nematodes are: Prevention of heartworm disease (*Dirofilaria immitis*) for one month. Treatment of infections with feline lungworms (L4 larvae and adults of *Troglostrongylus brevior).* The proposed indications for vesical nematodes are: Treatment of infestations with vesical worms (*Capillaria plica*).

Dirofilaria immitis:
Corresponding to CVMP's Scientific Advice (EMA/CVMP/SAWP/157138/2018), due to the public health implications and the complexity of the pathogenesis, the applicant was requested to provide two new dose determination studies in line with VICH GL20 for *Dirofilaria immitis*.

Five dose confirmation studies which were previously provided for Broadline are considered supportive information. Seven new studies GCP-compliant, laboratory controlled, randomised, blinded dose confirmation studies with NexGard Combo were submitted to conclude the efficacy against *D. immitis* in cats.

It is noted that only one study was considered pivotal to justify the efficacy of NexGard Combo against *Dirofilaria immitis* in line with the recommendations of the relevant guidelines, whereas the other six studies failed due to poor infection rates in control animals. However, all studies were well designed, GCP-compliant and in all other aspects in line with the relevant guidelines (VICH GL 7, 9 and 20). Additionally, the applicant provided the results of four pooled studies in a separate document (`justification for heartworm efficacy based on weight of evidence´) to justify an adequate substitute for

a second valid dose confirmation study. Since the VICH GL20 explicitly allows pooling of studies (provided that the study design and the used isolates are identical) in cases if less than 6 control animals were adequately infected and taking into account that the results could be confirmed by the CVMP, the approach is appropriate.
In summary, the results support the efficacy of NexGard Combo against *Dirofilaria immitis* for one month, and the claim was accepted.

Troglostrongylus brevior (adult, L4 larvae):
The claim was bridged from the data previously submitted for Broadline. One GCP-compliant dose confirmation study, conducted in Germany in 2015 in line with VICH GL7 and VICH GL20 was submitted in support of efficacy of a single topical application of Broadline at the recommended dose of 10 mg/kg fipronil, 12 mg/kg (s)-methoprene, 0.5 mg/kg eprinomectin and 10 mg/kg praziquantel, against induced infections with *T. brevior*. It involved 3 groups of 8 animals each; one group was treated 6 days post-infection, one group at 28 days post-infection, and the last group was left untreated. Efficacy based on worm count reduction was 100% for both time points. The reduction in larval shedding in faeces was 100% in animals treated 6 days post-infection but animals of the other group continued to shed larvae for 13 days after treatment. In support of the new claim, cross-reference was made to a field study that was previously submitted and assessed by the committee. Based on the limited prevalence in Europe, MUMS status was recognised by CVMP for this parasite species and, taking into account that an efficacy of 100% is obtained after one single administration in both the laboratory and in the field study, a claim for *T. brevior* was accepted.

Capillaria plica (adults):
The claim was bridged from the data previously submitted for Broadline. Broadline was shown to be 100% effective against the vesical worm *C. plica* in one GCP-compliant, laboratory controlled, randomised, blinded dose confirmation study using groups of 8 naturally infected animals. Given the low prevalence of this parasite species in cats, it can be accepted that efficacy results obtained from this study performed in Albania can be extrapolated to *C. plica* isolates elsewhere in Europe. Additionally, MUMS status was recognised by CVMP for this parasite species. Consequently, the claim can be accepted.

Cestodes

The proposed indications for the treatment of infections with gastro-intestinal cestodes include *Dipylidium caninum*, *Taenia taeniaeformis*, *Echinococcus multilocularis, Joyeuxiella pasqualei* (adult), and *Joyeuxiella fuhrmanni* (adult).

Dose-limiting cestode species:
The applicant makes reference to relevant dose range studies submitted in support of the marketing authorisation of Broadline to identify the dose limiting cestode species for praziquantel. According to the study results praziquantel at a dose of 10 mg praziquantel/kg bw proved to be efficacious against *Dipylidium caninum* in cats (91 to 100%, mean= 95.2%). Lower efficacy rates were calculated at single doses of 6 mg/kg (64%) and 8 mg/kg (78-98%). The calculated efficacy for *Taenia taeniaeformis* (98.5-100%), *Echinococcus multilocularis* (100 %), and *Joyeuxiella spp.* (99.7-100%) were higher revealing that *D. caninum* is the dose-limiting cestode species for praziquantel. VICH GL7 covers such surrogate method. As a consequence, the efficacy data generated with Broadline for *D. caninum* can be extrapolated to all other cestode species of the same class claimed for the new fixed formulation NexGard Combo, except for *Echinococcus* spp. where higher efficacy of always 100% is deemed necessary, considering that echinococcosis is a serious life threatening zoonosis in human beings.

Dipylidium caninum:
Two GCP-compliant randomised, blinded and controlled dose confirmation studies – one with naturally

infected cats and one with artificially infected cats- were conducted with NexGard Combo in accordance with the relevant VICH guidelines 7 and 20. Study results reveal efficacy of >98% after topical application at the minimum recommended dose (1.44 mg/kg bw of esafoxolaner, 0.5 mg/kg bw of eprinomectin and 10 mg/kg bw of praziquantel) in infected cats. Results support the efficacy of NexGard Combo against *D. caninum.*

Taenia (T.) taeniaeformis:
In order to verify the efficacy against *T. taeniaeformis* no specific DC studies have been conducted with the fixed combination product. Two studies previously submitted for Broadline were considered. Both studies involved groups of 10–12 cats and were carried out in naturally infected animals (Albania and countries outside Europe). The calculated efficacy was of 98.5 and 100% after a single topical application of Broadline at the recommended dose of 10 mg/kg fipronil, 12 mg/kg (s)-methoprene, 0.5 mg/kg eprinomectin and 10 mg/kg praziquantel. Results support the efficacy of NexGard Combo against *T. taeniaeformis*.

Joyeuxiella (J.) pasqualei and J. Fuhrmanni:
In order to verify the efficacy against *Joyeuxiella* spp. no specific DC study has been conducted with NexGard Combo. A study previously submitted for Broadline was considered. Based on the limited prevalence in Europe, MUMS status was recognised by CVMP for this parasite species.
The recorded percentage efficacy, based on geometric means, were 99.7% for adult *J. pasqualei*, and 100 % for adult *J. fuhrmanni*, but only 69.8 % for juvenile *Joyeuxiella* spp. after a single topical application of Broadline at the recommended dose of 10 mg/kg fipronil, 12 mg/kg (s)-methoprene, 0.5 mg/kg eprinomectin and 10 mg/kg praziquantel. The juvenile (immature) stage was defined by the impossibility to identify worms up to the species level. The CVMP considers that the data support the efficacy of NexGard Combo against adult *Joyeuxiella* spp., but the user should be warned that the presence of a high number of immature worms may preclude complete eradication. An appropriate warning is included in the SPC.

Echinococcus multilocularis:
Four GCP-compliant randomised, blinded and controlled DC-studies, designed according to VICH GL 7 and 20 have been provided to demonstrate the efficacy of NexGard Combo in the treatment of *E. multilocularis* infections, two studies each were performed in the USA and two in Europe. Both EU studies failed because no infection in control cats were achieved, but the two USA studies, performed with a strain obtained from foxes in the US, indicate 100% efficacy of NexGard Combo in treating *E. multilocularis* infection after artificial infection with 30,000 protoscolices/per cat, although in one USA study adequate worm burdens were only achieved in 5 out of 10 control animals. Nevertheless, a comparative pharmacokinetic study (see A.3) did not show significant differences in the pharmacokinetic parameters of the cestocidal active substance praziquantel when applied either with NexGard Combo or Broadline topically to cats at the same dose, allowing for an extrapolation of the efficacy against cestodes as shown for Broadline to NexGard Combo. Three studies were successfully performed with Broadline showing 100% efficacy using an EU strain of *E. multilocularis* in two studies and 100% efficacy in another dose confirmation study with a strain from the USA. Considering that laboratory data reveal that cats may only play an insignificant role in *E. multilocularis* transmission, as metacestodes most likely did not develop when originated from infected cats, the data sets presented from both Broadline and NexGard Combo are considered sufficient to justify the claim "Treatment of infections with *Echinococcus multilocularis* tapeworms".

Target animal tolerance

NexGard Combo is a new fixed combination antiparasitic topical product for cats, containing the three active substances esafoxolaner, eprinomectin and praziquantel, to be administered at a dose range of 1.44-4.5 mg/kg esafoxolaner, 0.48-1.5 mg/kg eprinomectin and 10-31 mg/kg praziquantel. For all

calculations on target animal safety, the maximum proposed label dose served as a basis. One pivotal GLP-compliant target animal safety study, one exploratory study, and one study on oral toxicity of the product were provided to investigate target animal safety of the final formulation.

All target animal safety studies of the combination were well conducted, using the final formulation. In addition, safety data obtained from the clinical safety and efficacy trial and other efficacy and laboratory studies are available. The target animal safety of esafoxolaner alone following topical administration has also been evaluated. Further information on afoxolaner (racemate) or the eprinomectin/praziquantel combination are available from the public domain or related centralised procedures.

Full study title	Results
A Target Animal Safety and Local Tolerance Study of a Combination of esafoxolaner, praziquanteland eprinomectinwhen Administered Topically at 1, 3 or 5X the Maximum Exposure Dose Six Times at Four-Week Intervals in Kittens	Pivotal study according to GL VICH 43, GLP One serious neurological AE 8hrs after the 3rd 5x dose, including ataxia with inability to stand, disorientation, hypothermia. Washing of administration site, emergency treatment. Cat fully recovered.
Safety and Tolerance in Kittens of NexGard Combo Applied Topically Four Times at Two-Week Intervals at 3x or 5x the Maximum Exposure Dose and of a High Dose of esafoxolaner Applied Two Times at a Monthly Interval	Non-GLP exploratory TAS study Dose-dependent pupil dilation, slight histological tissue damages at application site.
A Study to Determine the Safety in Cats of the Topical Formulation NexGard Combo Adminstered Orally at the Potential Maximum Dose (i.e. 0.375 mL/kg)	GLP, oral administration Profuse transient salivation in all cats. Otherwise well tolerated.
Esafoxolaner: a 12-week topical dosing toxicity study in cats)	Non-GLP, esafoxolaner alone Decrease in food consumption and body weight gain in high dose group. Slight histological damages at application site (also following application of the excipient DMI alone)

Tolerance of the individual active substance(s)

Esafoxolaner

Esafoxolaner is the (S)-enantiomer of afoxolaner and belongs to the isoxazoline group. Neither esafoxolaner nor the racemate are currently authorised for cats, but the racemate is authorised as single active substance or in combination for dogs.

The safety of esafoxolaner alone in cats following topical dosing was evaluated in a first study at 0.7 x , 2.2x and 6.7x of the proposed maximum recommended treatment dose (RTD) of esafoxolaner, administered 3 times at 4 week intervals, and once at 10.2x the proposed maximum dose and in a second study, one treatment group at 23x the proposed maximum dose, repeated after 4 weeks.

In the first study, substance-related systemic findings were limited to a decrease in food consumption and body weight gain in the 6.7 x RTD group. Results indicate a NOAEL of 10 mg/kg bw, but have to be taken with some caution, since the study was not blinded. In the second study, findings following administration of esafoxolaner alone were limited to pupil dilation in 3 out of 6 animals. In addition, some minimal to slight local reactions at the application site were observed in both studies. A significantly lower C_{max} was determined for esafoxolaner after administration in the fixed combination as compared to the mono-substance alone. Thus, the groups in the study would have been exposed to even higher overdoses when considering plasma levels instead of comparing doses in mg/kg bw. Intravenous administration of 1 mg esafoxolaner/kg bw resulted in slight somnolence for max. 2 hours in one pharmacokinetic study.

Eprinomectin and praziquantel

The active substances eprinomectin and praziquantel are active substances in Broadline spot-on for cats, which additionally contains fipronil and (S)-methoprene, and which has the same qualitative excipients in a similar quantity as NexGard Combo (butylhydroxytoluene, glycerol-formal and dimethyl isosorbide).

Eprinomectin is a macrocyclic lactone, thus, overdoses could lead to the typical well-known central nervous signs associated with avermectin intoxications, including ataxia and hypothermia. Praziquantel is also authorised as single active substance in a number of spot-on and tablet veterinary marketing authorisations in the EU. Adverse reactions listed in the product information of authorised spot-ons with praziquantel are local reactions at the administration site and salivation following licking the administration site, and reaction to overdoses is described to be limited to local reactions.

Tolerance of the fixed combination

Systemic toxicity of the fixed combination

Systemic toxicity following overdoses and repeated use was addressed in one exploratory study, with 4 administrations in 2-week intervals at 3x and 5x the proposed maximum recommended treatment dose (RTD), and in one pivotal target animal safety study according to GL VICH 43, with 6 administrations in 4-week intervals at 1x, 3x and 5x the proposed maximum RTD to healthy young cats.

The target organ is the central nervous system. In the pivotal target animal safety study, one cat of the high-dose group experienced a severe neurological adverse reaction including ataxia, inability to stand, slight tremors, pupil dilation and hypothermia. Similar effects are described for overdoses of avermectins. It is therefore likely that these effects are linked to the eprinomectin component in the product. The animal fully recovered and was reintegrated to the study, after a symptomatic and emergency treatment. In addition, the administration site was washed, thus potentially reducing further absorption. The SPC reflects the above overdose symptoms and informs about the symptomatic treatment. Other effects are limited to pupil dilation, which also indicates a neurologic effect. Dose dependent transient pupil dilation of low to medium grade was observed in the exploratory study. Pupil dilation was also noted in a number of animals in the pivotal target animal safety study. Cases were also noted in control animals. Data on pupil dilation following treatment with the label dose are not conclusive. However, pupil dilation is adequately mentioned in section 4.10, overdose, of the SPC, in conjunction with the case of severe neurological adverse events.

No effects on other clincal or on clinical pathology parameters were observed.

Under the conditions of the pivotal target animal safety study, data indicate a NOAEL corresponding to 3x of the recommended maximum dose.

In the field study and in laboratory efficacy studies, cases of hypersalivation, loose faeces/diarrhoea, anorexia, apathy and emesis were observed in cats. The type, severity and frequency of these adverse reactions is adequately reflected in section 4.6 of the SPC.

In another safety study , the final formulation was adminstered orally at the maximum recommended dose (0.375 ml/kg bw) to fasted animals. No apparent systemic adverse reactions were noted except for profuse transient salivation, which was observed in all cats immediately after treatment. In the clinical field study, hypersalivation was also observed in two cases in partner animals licking a treated cat. It is likely that this is a reaction to the taste of the product; praziquantel, e.g. is known be very bitter. Adequate information on this is included in the SPC.

Section 4.5i of the SPC states correctly that the product has not been tested in kittens under 8 weeks of age and that it is not for use in cats younger than that or weighing less the 0.8 kg. This minimum weight is also included in section 4.9 in the dosing information.

Local tolerance of the fixed combination

In the pivotal target animals safety study, with n = 8 cats per group, 3 animals of the high dose group exhibited small dark red subcutaneous areas at the treatment sites, which did not reveal histologic abnormalities. A relation to treatment is at least likely, and this finding is adequately reflected in the SPC.

In a study (see above), in which esafoxolaner alone was administered, a high overdose (6.8xRTD) on three occasions led to dermatitis, focal alopezia and minimal epithelial hyperplasia in 1animal. In addition, focal epidermal cell necrosis was noted in 1animal each in 2 of the 4 groups receiving afoxolaner, and in 1 animal receiving the vehicle dimethyl isosorbide (which is also included in NexGard Combo). Although this finding was minimal and not dose related, it is noteworthy, as it did not occur in the saline control. A similar observation was made in the 5x group exploratory safety study, with repeated application of the final formulation or of esafoxolaner alone (in high overdose, but in an unknown excipient). It therefore appears possible that this local effect is related to dimethyl isosorbide and/or esafoxolaner. While these findings are of minor relevance with regard to skin functioning, it could be of interest in conjunction with ocular toxicity, see below.

In the field study and laboratory efficacy studies, application site reactions and mild pruritus at the application site were observed, which is adequately reflected in section 4.6 of the SPC.

Ocular toxicity, following contact of the product with the eyes

No data in cats on possible effects of ocular contact with product are available. Persistent partial corneal opacity and transient redness and swelling have been observed in 1 animal species and are discussed in Part 3 of this report. The histological findings of the skin of the application site indicate a certain potential of the product for cell damage. None of the findings in the studies performed in cats indicate any localised effect on the eyes of treated cats. However, when interpreting this data, it has to be kept in mind that treatments were administered by professional personnel (or pet owners in one field trial), thus minimising the risk of accidental animal eye contact with the product, e.g. in un-cooperative cats. Risk mitigation warnings addressing accidental contact of the cat's eyes with the product are included in the SPC.

Reproductive safety in the target animal

The applicant did not present studies on reproductive safety in the target animal. The wording in section 4.7 of the SPC is identical to that of the product Broadline spot-on for cats, which includes two of the three active substances of NexGard Combo and has the same excipients. For the third active substance, esafoxolaner, studies in laboratory animals did not reveal teratogenicity for afoxalaner. In consequence, it can be accpeted that studies in laboratory animals have not produced teratogenic, foetotoxic or maternotoxic effects in regard to the active substances.

However, for the excipient glycerol formal, foetotoxic and teratogenic effects are described in its MRL summary report (EMEA/MRL/108/96-FINAL). Foetotoxic effects were noted from 75 mg/kg bw/d, teratogenic effects from 300 mg/kg bw/d. The recommendation "use only according to the benefit-risk

assessment by the prescribing veterinarian" is acceptable in the view of the results of the reproductive safety studies in laboratory animals, and is in line with comparable authorised products.

Repeated use

It has been demonstrated that all active substances accumulate when given at monthly intervals (see section on pharmacokinetics); esafoxolaner and eprinomectin had reached steady state by the fifth monthly dose, while praziquantel had reached steady state already by the second monthly dose. This could become a concern if NexGard Combo would be administered repeatedly at higher frequencies and/over longer than the maximum tested duration in the pivotal target animal safety study, i.e., six months. VICH GL 43 (target animal safety) recommends in cases of concerns about accumulation a longer than 6 months duration of the investigation of target animal safety. However, based on the data provided, a safety margin beyond 6 months could not be established. Taking into account the potential for accumulation of the active substances, and the adverse reactions observed at 5x the maximum recommended treatment dose in a single cat, no more than 6 consecutive treatments are recommended to be given within a 12-month period.

Conclusions

A broad set of data on target animal tolerance following treatment at RTD or at overdoses is provided. When used at the recommended dose and at 3x the maximum recommende dose at monthly intervals, the product was generally well tolerated with mostly uncommon and mild reactions of short duration. However, application of a 5x overdose led to one case of a severe neurological reaction that required treatment.

Clinical field trials

Endoparasites

In line with CVMP's scientific advice the CVMP accepted that if product equivalence between Broadline and NexGard Combo is shown in regard of eprinomectin and praziquantel, the results of the GCP-compliant endoparasite multicentre field study conducted in veterinary practices in different geographic regions in Europe with Broadline (which was previously assessed by CVMP) will also be applicable to NexGard Combo. Therefore, no additional efficacy field trials against endoparasites would be needed.

Summarised Broadline field study against endoparasites:

One GCP EU field trial was carried out for endoparasites, in 8 sites located in 7 European countries. A total of 130 cats were treated with Broadline and 66 with the selected comparator, i.e., emodepside and praziquantel containing product. The recruited cats were 2 months to 13 years old. Both genders and spaying/castration status were adequately represented. Infestations by *T. cati*, hookworms, taeniids, *Dypilidium* spp. and *Capillaria* spp. were diagnosed. In the Broadline treated group, five cats were diagnosed with mixed cestodes/hookworm infections and 18 cats were diagnosed with mixed cestodes/ascarids infections. In the comparator group, five cats were diagnosed with mixed cestodes/hookworm infections and 11 cats were diagnosed with mixed cestodes/ascarids infections.

Efficacy and safety were assessed at Day 14; efficacy was assessed through the faecal egg count and parasite identification, and the per cent efficacy was calculated in comparison with baseline (Day 0) results. The results show near to 100% efficacy for both the candidate product and the comparator against *T. cati*, hookworms, taeniids and *D. caninum*, although the non-inferiority analysis was not powerful enough to be conclusive in the case of *D. caninum*.

It was noted that field data as to *D. immitis* and *E. multilocularis* are not required as per the current guidance (VICH GL 20).

Ectoparasites (ticks and fleas)

The applicant provided one GCP-compliant pivotal European field study, investigating the safety and efficacy of a single dose of NexGard Combo in the treatment of flea and/or tick infestations, and flea allergy dermatitis (FAD) in naturally infested cats (see table below for study design and results).

Effectiveness and Safety of a Topical Treatment with NexGard Combo against Flea and/or Tick Infestations and Flea Allergy Dermatitis on Cats under Field Conditions over a Duration of One Month	
Objectives	To determine the effectiveness and safety of one topical treatment with NexGard Combo in comparison with a positive control product containing a fixed combination of fipronil and methoprene under field conditions, for the treatment and control of flea and tick infestation over a duration of one month, and for the treatment of flea allergy dermatitis (FAD) signs, in cats naturally infested with fleas and/or ticks.
Study sites	European multicentre study
Study design	Positive controlled, blinded, multicentre, efficacy and safety, non -inferiority field study
GL Compliance	GCP
T 1: IVP	NexGard Combo, final formulation: 1.0 to <2.5 kg: 0.3 ml syringe 2.5 to <7.5 kg: 0.9 ml syringe 7.5 to <10 kg: 0.3 + 0.9 ml syringes, once, topically
T 2: control	Authorised in EU for the treatment of flea and tick infestations in cats, containing fipronil/(S)-methoprene
Animals	557 domestic cats (148 f, 149 f spayed, 113 m and 147 m castrated, 8 weeks to 20 yrs) belonging to 309 households of one to three cats each. Mainly European breeds.
Outcomes/ endpoints	Efficacy evaluation: Parasite (tick and flea) counts of sentinel animals Safety evaluations: Sentinel and non-sentinel cats assessed for application site and systemic safety FAD evaluations: The presence, extent and intensity of dermatological signs was scored (absent, mild, moderate, marked).
Results	
Outcomes for endpoints	Tick efficacy:The percent efficacy (GM) evaluated against baseline for the IVP group was above ≥ 98.1% at all timepoints. Because the upper limit of the two-sided 95% CI of the mean tick counts difference of NexGard Combo and control was less than the non-inferiority margin of 1.5 for all four visits, the hypothesis that the IVP group was inferior to the control group was rejected. The IVP was comparable to or better than the control for all four visits. Five species of ticks were identified. Flea efficacy: . The % efficacy (GM) for the IVP group was ≥97.5%at all timepoints. = The IVP group was comparable to or better than the controls for all four visits. Since the upper limit of the two-sided 95% confidence interval of the log-flea counts

	difference of IVP and controls were less than zero for all four visits, IVP group was better than the controls. Clinical signs of FAD: The proportion of cats that improved was numerically higher than the proportion of cats that stayed the same or worsened, 95.7% cats improved in the IVP group. Ear mites (*Otodectes cynotis*): Some sites were instructed to evaluate ear mite infestation before treatment and 1 month after treatment via otoscopic examination. Before treatment, 15 cats were diagnosed with *O. cynotis.* Of these, 7 cats were treated with the IVP and were free of ear mites on D28.
Adverse events	NexGard Combo: six instances of abnormalities reported by the owner on the day of treatment, all likely related to treatment: mild pruritus at the application site, hypersalivation, hypersalivation after application of the product, hypersalivation for 3 hours after application of the product, emesis, 24h apathy, 48h anorexia.

Discussion:

This pivotal field study was designed to demonstrate the efficacy of NexGard Combo against ticks and fleas in cats after one topical treatment and for the treatment of signs of flea allergic dermatitis (FAD). It was a GCP-compliant, blinded and positive-controlled study that has been conducted at several study sites in Europe.

A total of 557 client-owned cats were included and are considered to be representative of the European cat population. The percent efficacy was calculated at all time-points in comparison to baseline values (day 0).

The overall study design is appropriate and is in line with guideline requirements. Based on the primary efficacy parameter the applicant concludes that the test item was non-inferior to the control product. Although there are several concerns on the statistical design that put the conclusions of the applicant into question, the results do not contradict the results of the dose confirmation studies showing sufficient efficacy, and therefore this study is considered supportive.

Ticks:

Although the Guideline EMEA/CVMP/EWP/005/2000-Rev.3 principally allows a non-inferiority design using a positive control, (without stating any further details), the demonstration of non-inferiority as compared to an already authorised comparator product is only meaningful, if assay sensitivity is given. However, in view of the study design, assay sensitivity as requested by the guideline (EMEA/CVMP/EWP/005/2000-Rev.3) was questioned, especially regarding the evaluation of efficacy against ticks. Due to the manual removal of ticks after each visit (which can, however, be generally accepted for ethical reasons and to allow identification of ticks), and in conjunction with the high percentage of cats with only one tick at a single time point, a relevant infestation pressure cannot be extrapolated over the entire study period. Therefore, the placebo cure rate could be quite high. Since no negative-controlled group was included, efficacy in comparison to placebo could not be evaluated. Hence, it is not evident that the control product is more effective than placebo under this study design.

Due to questions on the study design and the statistical analysis, no clear conclusion regarding the efficacy of the NexGard Combo in regard to efficacy against ticks could be drawn from this study. However, given that adequate efficacy of NexGard Combo against the claimed tick species *Ixodes ricinus*

has been shown in the respective dose-confirmation studies (and were not contradicted by the clinical field study), this study can nevertheless be considered as supportive.

Fleas

For fleas, the result for NexGard Combo were comparable to or better than the controls for all four visits, although some issues were raised on the statistical approach.

The majority of fleas identified during this study, corresponds to the species for which a claim is sought for (*Ctenocephalides felis*). Regarding those tick species for which a claim is sought for, field data are only available *Ixodes ricinus* and *Rhipicephalus sanguineus*. Whereas mostly ticks of the species *I. ricinus* were identified, only 10 vials containing at least one *R. sanguineus* were noted, none of them was collected in Central Europe.

Clinical signs of FAD

Clinical signs of FAD were scored altogether based on a general dermatological score. As improvement of clinical signs has been demonstrated in treated cats, the claim that NexGard Combo can be used as part of a treatment strategy against flea allergy dermatitis is considered acceptable provided >95% efficacy rate against *C. felis* is demonstrated.

Target Animal Safety

With regards to target animal safety, the conclusions of the applicant can be followed. The product was overall well tolerated.

Conclusions:
To support efficacy against ticks and fleas, one GCP compliant, positive controlled field study, conducted at multiple sites within the EU has been provided. Whereas non-inferiority to the comparator product has been shown for fleas, efficacy against ticks could not convincingly be shown in this study due to shortcomings in the study design and the statistical evaluation. (However, adequate efficacy of NexGard Combo against the claimed tick species was shown in dose-confirmation studies).

In addition, this study also serves to support the claim that NexGard Combo can be used as part of a treatment strategy against flea allergy dermatitis (FAD).

With regards to target animal safety, the product appeared generally well-tolerated.

Ear mites

The applicant provided one GCP-compliant pivotal European field study investigating the safety and efficacy of a single dose of NexGard Combo in the treatment of naturally acquired *Otodectes cynotis* infestation in cats (see table below for study design and results).
In addition, efficacy of NexGard Combo in the treatment of ear mites was also investigated as part of the European Field study on ticks and/or fleas (see above); however, no conclusion could be drawn from this study with regards to treatment of ear mites.

Effectiveness and Safety of a Topical Treatment with NexGard Combo against Infestations with Otodectes cynotis in Cats under Field Conditions	
Objectives	To determine the effectiveness and safety of one topical treatment with NexGard Combo (IVP) when administered once as a topical solution to cats for the treatment of naturally acquired *Otodectes cynotis* infestation under field conditions.
Study sites	Two sites in Europe
Study design	Negative controlled, assessor-blinded, multicentre, clinical effectiveness and safety field trial.
GL Compliance	GCP, VICH GL 9, section 8.3.1.
T 1: IVP	NexGard Combo, final formulation: 1.0 to <2.5 kg: 0.3 ml syringe 2.5 to <7.5 kg: 0.9 ml syringe 7.5 to <10 kg: 0.3 + 0.9 ml syringes, once, topically
T 2: Negative control	Mineral oil
Animals	115 domestic cats (33 f, 27 f spayed, 29 m and 26 m castrated, 9 weeks to 12 years) belonging to households of one to three cats each. Mainly European breeds.
Eligibility criteria	Households with up to 3 cats. The first cat per household that was infested with live (motile) *Otodectes cynotis* in at least one ear was chosen as sentinel cat. Households with dogs or ferrets and households containing a cat less than 8 weeks old or weighing less than 0.8 kg were excluded from the study. It was not allowed to add other cat(s) to the household during the course of the study. Animals that had been treated with any ectoparasiticide product (either topical or systemic) within 4 weeks of enrolment or within the efficacy duration of a longer acting ectoparasiticide drug were excluded.
Outcomes/ endpoints	**Sentinel cats:** Efficacy evaluation on D 30/31 (±2): Number of live ear mites after ear duct flushing Safety evaluations for application site and systemic safety on D 30/31 (±2) **Non-sentinel cats:** Safety evaluation on D 30/31 (±2)
Results	
Outcomes for endpoints	Only one cat per household (sentinel cats) was included in the effectiveness evaluation. The percent efficacy of a single treatment with NexGard Combo against *O. cynotis* mites was 97.4% or 96.5% based on geometric or arithmetic mean in regard to live mite counts, respectively. The differences in mite counts were statistically significant between groups ($p < 0.0001$). Cats treated with NexGard Combo had significantly better clinical scores in regard to signs of pruritus and quality/quantity of the cerumen compared to those of the

	control group ($p < 0.0001$).
Adverse events	All cats treated with the IVP and all cats treated with the CP were scored good for both, application site and systemic safety. There were no health abnormalities that were considered related or likely related to the IVP treatment.

Discussion:

This pivotal field study was designed to determine the effectiveness and safety of NexGard Combo when administered once as a topical solution to cats for the treatment of naturally acquired *Otodectes cynotis* infestation under field conditions. It was a GCP-compliant, blinded and negative-controlled study that has been conducted at two study sites in Eastern and Southern Europe. The overall study design is appropriate and is in line with guideline requirements.

A total of 115 client-owned cats belonging to 66 households were randomly allocated to either treatment group 1 or negative control group 2 and considered to be representative of the European cat population. In total, 33 sentinel cats were assigned to the NexGard Combo group and the same number of sentinel cats were assigned to the negative control group. Treatment was conducted once topically on D0 according to the proposed dosage or with mineral oil.

The inclusion criteria for the sentinel cat was the presence of viable mites in at least one ear. Since no quantitative assessment of mites was performed on D0, this could also include inclusion of cats with only one mite, which is considered quite low. However, since the households were randomly assigned to either treatment or negative control group and the difference in efficacies on D30 between both groups is statistically significant, this can be accepted. The efficacy was assessed by counting the number of ear mites in the sentinel cats after ear duct flushing on D30 (±2) after treatment.

The results showed a statistically significant reduction of ear mites following the treatment with NexGard Combo; although individual cats in the treatment group still had considerable mite counts on D30 (one cat had up to 106 live mites in one ear). Therefore, an otoscopical re-examination needs to be conducted after 4 weeks and if necessary, an additional treatment with an appropriate mono-substance product should be initiated. This is appropriately reflected in the SPC.

With regards to target animal safety, the conclusions of the applicant can be followed. The product was well tolerated. No local or systemic adverse events have been observed.

Conclusions:
The results of the study confirm an efficacy of more than 90% in the reduction natural infestation with ear mites (*O. cynotis*) in cats at the proposed dose of NexGard Combo. However, since no complete clearance of mites was achieved, an additional warning for ear mites has been introduced to the SPC.

Overall conclusion on efficacy

Pharmacodynamics:

Esafoxolaner is an ectoparasitic substance with killing activity against fleas, mites and ticks. The mode of action has been sufficiently described. The main effect of esafoxolaner is to act as antagonist at 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. This results in uncontrolled activity of the central nervous system and death of insects or acarids.

Activity of eprinomectin is thought to be mediated by its selective, high affinity binding to glutamate-gated chloride channels that are present in invertebrate nerve and muscle cells. Binding leads to an increase in the permeability of the cell membrane to chloride ions causing hyperpolarization of affected

cells and subsequent paralysis and death of nematodes.

Praziquantel is a synthetic isoquinoline-pyrazine derivative with activity against tapeworms. It is rapidly absorbed via the surface of the parasites and affects membrane permeability in cestodes, influencing divalent cation fluxes, particularly calcium ion homeostasis, which is thought to contribute to the rapid muscle contraction and vacuolisation.

Resistance:

The risk of resistance development seems unlikely and not highly critical for a product used in individual companion animals. However, the appropriate use of the product is crucial to ensure that the efficacy is maintained and any development of resistance in the future delayed. The applicant considers this fact by adding a standard warning on the emergence of resistance following frequent use of the fixed combination into the SPC. Recent data revealed that in the USA resistant *Dipylidium caninum* cestodes against praziquantel have been identified in dogs but not in cats. The reported existence of two specific *Dipylidium* spp. genotypes for cats and dogs reduce, however, the risk of the transmission of a resistant dog strain *via* infected fleas to cats.

Pharmacokinetics:

The pharmacodynamic and pharmacokinetic characteristics of NexGard Combo are generally well documented and have been satisfactorily evaluated in cats. Following topical administration, esafoxolaner reaches its T_{max} in plasma between 0.3 and 42 days (due to inter-variability) with an elimination half-life of about 50 days estimated from the observed accumulation following five monthly administrations. Eprinomectin reaches its T_{max} in plasma between 8 and 48 hours, with an elimination half-life of 10 day. Praziquantel reaches it T_{max} in plasma between 2 and 32 hours, with an elimination half-life of 4 to 10 days.

Comparing the fixed combination to esafoxolaner alone, the absorption of esafoxolaner is significantly prolonged when administered in the fixed combination product, and the exposure (C_{max}, AUC) is significantly lower.

Following repeated monthly administration, esafoxolaner and eprinomectin reached steady state by the fifth dose, while praziquantel had reached steady state already by the second dose.

Dose determination:

The single dose of 1.44 mg/kg bw esafoxolaner was established based on two exploratory dose finding studies against *I. scapularis*, *C. felis* and two pivotal dose determination studies against *I. ricinus* and *O. cynotis*, and supported by several dose confirmation studies performed under experimental conditions.

In line with the recommendation of the scientific advice (EMEA/CVMP/SAWP/178051/2017), the effective doses of 0.5 mg/kg bw eprinomectin and 10.0 mg/kg bw praziquantel have been extrapolated from the data on the doses of these substances in the already authorised reference combination product, Broadline.

Tolerance:

Administration of the product in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions include uncommon and transient cases of hypersalivation, diarrhoea, anorexia, lethargy and emesis shortly after administration, and skin reactions at the application site.

Following administration of a 5x overdose, severe neurological reactions and hypothermia were observed in one animal. The animal fully recovered following removal of remaining product from the administration site, and symptomatic/emergency treatment. In some animals, at 5x overdose, dark red subcutaneous areas at the skin treatment sites were observed. These issues are sufficiently addressed

in the SPC.A potential risk for the eyes of the animals following accidental eye contact is sufficiently addressed in the SPC.

Oral administration of the maximum label dose of the product led to profuse salivation.

Efficacy:

Ectoparasites:

The applicant provided a variety of dose determination and dose confirmation laboratory studies, as well as two European field studies, one against ticks and fleas, and a second one regarding ear mites. Although the data from the field studies could only be considered supportive in regard to the tick claims (due to deficiencies in the study design and statistical evaluation), overall, the results of all the pre-clinical and clinical studies together are considered sufficient to support the claimed efficacy against *Ixodes scapularis*, *Ixodes ricinus*, *Ctenocephalides felis* and *Otodectes cynotis*.

Endoparasites, nematodes:

Data are considered sufficient to prove efficacy against adults of *T. cati*, for which three dose confirmation studies were presented.

Efficacy against L3 and L4 stages of *T. cati*, adults of *Toxascaris leonina, Ancylostoma braziliense*, and *Capillaria plica*, as well as L4 and adults of *Ancylostoma tubaeforme* and *Ancylostoma ceylanicum*, and against L4 and adults of *Trogostrongylus brevior* is acceptable, based on bridging from the authorised product Broadline. Data are considered sufficient to prove efficacy against *Dirofilaria immitis*, for which in sum seven dose confirmation studies were provided.

Endoparasites, gastrointestinal cestodes

Efficacy against *Dipylidium caninum, Taenia taeniaeformis, Joyeuxiella pasqualei* (adult) and *Joyeuxiella fuhrmanni* (adult) has been adequately demonstrated.

Part 5 – Benefit-risk assessment

Introduction

NexGard Combo is a fixed combination of three antiparasitic active ingredients, esafoxolaner, eprinomectin, and praziquantel, which act mainly against ectoparasites, nematodes, and cestodes, respectively. NexGard Combo is a spot-on solution available in two strengths at a dose volume of 0.3 ml (i.e. 3.6 mg+1.2 mg+24.9 mg per unit) or 0.9 ml (i.e. 10.8 mg+3.6 mg+74.7 mg per unit), and is presented in packs containing 1, 3, 4, 6, or 15 applicators.

NexGard Combo is intended to be used in cats from 8 weeks of age with, or at risk from mixed infestations by cestodes, nematodes and ectoparasites. The veterinary medicinal product is exclusively indicated when all three groups are targeted at the same time.

Benefit assessment

Direct therapeutic benefit

The efficacy of a single minimum dose of NexGard Combo (1.44 mg esafoxolaner, 0.48 mg eprinomectin and 10 mg praziquantel per kg bodyweight) against defined species of ectoparasites, nematodes and cestodes in cats was demonstrated in a number of laboratory studies. In addition, two European field studies were provided investigating the clinical efficacy against ectoparasites. In regard to endoparasites, in line with CVMP's scientific advice, the CVMP accepted a European field study submitted previously for another centrally authorised product containing the same endoparasitic active ingredients

by the same applicant (Broadline). Overall, the results of all the pre-clinical and clinical studies together are considered sufficient to support the efficacy against ectoparasites (fleas, ticks, ear mites), cestodes and nematodes.

The fixed combination of esafoxolaner, praziquantel and eprinomectin has been justified. The epidemiological and veterinary medicinal relevance of concurrent infection (or risk of) involving the three target parasite groups (ectoparasites, nematodes, and cestodes) was demonstrated through epidemiological data from the literature. The product would benefit a well-defined, but very limited target cat population.

Additional benefits

NexGard Combo is easy to apply by the owner.

The product increases the range of available treatment possibilities for concurrent flea infestations, cestode infections and mixed nematode infections in cats. It can also be used for the prevention of heartworm disease in cats at risk, when these are also infested with ticks or fleas and, concurrently, cestodes.

Risk assessment

Quality:

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

Safety:

Risks for the target animal:

Administration of the product in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions include uncommon and transient cases of hypersalivation, diarrhoea, anorexia, lethargy and emesis shortly after administration, and skin reactions at the application site. Following administration of a 5x overdose, severe neurological reactions and hypothermia were observed in one animal. Adequate risk mitigation measures following contact of the cat's eyes with the product are in place. Oral administration of the maximum label dose of the product led to profuse salivation.

Risk for the user:

Concerns were previously identified in relation to eye irritation and quantitative risk assessment for pregnant women. These concerns are resolved and sufficiently addressed and mitigated by user safety warnings included in the SPC.

Risk for the environment:

Based on the data provided, the ERA can stop at Phase I. NexGard Combo is not expected to pose a risk for the environment when used according to the SPC.

Special risks:

Praziquantel-resistant *Dipylidium caninum* cestodes have recently been identified in dogs in the USA. However, details on the existence of a cat-specific *Dipylidium* genotype has been published, thus the emergence in the dog genotype in the USA does not necessarily ensure the emergence of resistance.

Risk management or mitigation measures

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

To manage the risk of over-treatment, the product should only be used in animals with concurrent infections with parasites or which are at risk of infestation/infection that require treatment with all three active substances.

User safety:

User safety risks have been identified concerning the risks for pregnant women and associated with exposure of the eyes. These concerns are sufficiently addressed and mitigated by user safety warnings included in the SPC.

Environmental safety:

Adequate advice for the disposal of any unused product or waste material is included in the product literature.

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication:

> "For cats with, or at risk from mixed infestations by cestodes, nematodes and ectoparasites. The veterinary medicinal product is exclusively indicated when all three groups are targeted at the same time.
>
> Ectoparasites:
>
> Treatment and prevention of infestations by fleas (*Ctenocephalides felis)*. One treatment prevents further infestations for at least one month.
> Prevention of environmental flea contamination by preventing egg laying for over a month.
> The product can be used as part of a treatment strategy for the control of flea allergy dermatitis (FAD).
> Treatment and prevention of infestations by ticks (*Rhipicephalus sanguineus*, *Ixodes ricinus*, *Ixodes scapularis*, *Amblyomma americanum*). One treatment prevents further infestations for at least one month.
>
> Treatment of ear mange (*Otodectes cynotis*).
>
> Gastro-intestinal cestodes:
>
> Treatment of infestations with tapeworms (*Dipylidium caninum*, *Taenia taeniaeformis*, *Echinococcus multilocularis, Joyeuxiella pasqualei* (adult), and *Joyeuxiella fuhrmanni* (adult)).
>
> Gastro-intestinal nematodes:
>
> Treatment of infestations with gastrointestinal nematodes (L3, L4 larvae and adults of *Toxocara cati*, L4 larvae and adults of *Ancylostoma tubaeforme* and *Ancylostoma ceylanicum*, and adult forms of *Toxascaris leonina* and *Ancylostoma braziliense*).
>
> Cardio-pulmonary nematodes:
>
> Prevention of heartworm disease (*Dirofilaria immitis* larvae) for one month.

Treatment of infestations with feline lungworms (L4 larvae and adults of *Troglostrongylus brevior).*

Vesical nematodes:
Treatment of infestations with vesical worms (*Capillaria plica*)."

The product has been shown to be efficacious for the indications proposed for nematodes and cestodes, and the CVMP accepted the indications as proposed by the applicant. For ectoparasites, the CVMP only accepted indications relating to the treatment (but not the prevention), and the CVMP agreed to the following indications:

"For cats with, or at risk from mixed infections by cestodes, nematodes and ectoparasites. The veterinary medicinal product is exclusively indicated when all three groups are targeted at the same time.

Ectoparasites:
Treatment of infestations by fleas (*Ctenocephalides felis)*. One treatment provides immediate and persistent flea killing activity for one month.
The product can be used as part of a treatment strategy for the control of flea allergy dermatitis (FAD).

Treatment of infestations by ticks. One treatment provides immediate and persistent tick killing activity against *Ixodes scapularis* for one month and against *Ixodes ricinus* for five weeks.
Treatment of infestation by ear mites (*Otodectes cynotis*).

Gastro-intestinal cestodes:
Treatment of infections with tapeworms (*Dipylidium caninum*, *Taenia taeniaeformis*, *Echinococcus multilocularis, Joyeuxiella pasqualei*, and *Joyeuxiella fuhrmanni*).

Nematodes

Gastro-intestinal nematodes:
Treatment of infections with gastrointestinal nematodes (L3, L4 larvae and adults of *Toxocara cati*, L4 larvae and adults of *Ancylostoma tubaeforme* and *Ancylostoma ceylanicum*, and adult forms of *Toxascaris leonina* and *Ancylostoma braziliense*).

Cardio-pulmonary nematodes:
Prevention of heartworm disease (*Dirofilaria immitis*) for one month. Treatment of infections with feline lungworms (L4 larvae and adults of *Troglostrongylus brevior).*

Vesical nematodes:
Treatment of infections with vesical worms (*Capillaria plica*)."

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

Based on the data presented, the overall benefit-risk is considered positive. It is noted that the product would benefit a very limited target cat population.

Conclusion

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP considers that the application for NexGard Combo spot on for cats is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

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