8 December 2016
EMA/849714/2016
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Stronghold Plus (EMEA/V/C/004194/0000)

International non-proprietary name: selamectin / sarolaner

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

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Introduction

The applicant Zoetis Belgium SA submitted on 28 January 2016 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Stronghold Plus spot-on solution for cats, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 8–10 April 2015 as Stronghold Plus contains a new combination of two existing active substances, selamectin and sarolaner, which were not authorised in combination as a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004.

CVMP appointed Rory Breathnach as rapporteur and Peter Hekman as co-rapporteur for the assessment of the application.

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

Stronghold Plus is a spot-on solution for topical use on cats which contains selamectin and sarolaner. The product is available in three different strength unit dose polypropylene pipettes containing either 0.25 ml (delivering 15 mg selamectin and 2.5 mg sarolaner), 0.50 ml (delivering 30 mg selamectin and 5 mg sarolaner), and 1 ml (delivering 60 mg selamectin and 10 mg sarolaner). The outer packs contain 3 or 6 pipettes. The full recommended indication is as follows:

For cats with, or at risk from, mixed parasitic infestations by ticks and fleas, lice, mites, gastrointestinal nematodes or heartworm. The veterinary medicinal product is exclusively indicated when use against ticks and one or more of the other target parasites is indicated at the same time.

Ectoparasites:

- For the treatment and prevention of flea infestations (*Ctenocephalides* spp.). The veterinary medicinal product has immediate and persistent flea killing activity against new infestations for 5 weeks. The product kills adult fleas before they lay eggs for 5 weeks. Through its ovicidal and larvicidal action, the veterinary medicinal product may aid in the control of existing environmental flea infestations in areas to which the animal has access.
- The product can be used as part of a treatment strategy for flea allergy dermatitis (FAD).
- Treatment of tick infestations. The veterinary medicinal product has immediate and persistent acaricidal effect for 5 weeks against *Ixodes ricinus* and *Ixodes hexagonus*, and 4 weeks against *Dermacentor reticulatus* and *Rhipicephalus sanguineus*.
- Treatment of ear mites (*Otodectes cynotis*).
- Treatment of biting lice infestations (*Felicola subrostratus*).

Ticks must attach to the host and commence feeding in order to be exposed to sarolaner.

Nematodes:

- Treatment of adult roundworms (*Toxocara cati*) and adult intestinal hookworms (*Ancylostoma tubaeforme).*
- Prevention of heartworm disease caused by *Dirofilaria immitis* with monthly administration.

On 8 December 2016 the CVMP adopted an opinion and CVMP assessment report.

On 9 February 2017, the European Commission adopted a Commission Decision granting the marketing authorisation for Stronghold Plus.

Scientific advice

The applicant received scientific advice from the CVMP on 8 May 2014 and follow-up scientific advice on 15 January 2015. The scientific advice pertained to clinical development of the dossier.

The CVMP considered that the applicant, in general, followed the advice of the CVMP. Specific comment is provided in the relevant sections of the assessment report comparing the study data provided and the scientific advice given.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

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

It may be concluded that the pharmacovigilance system is adequate and will permit the applicant to discharge their pharmacovigilance responsibilities in accordance with regulatory requirements.

Manufacturing authorisations and inspection status

Manufacture of the dosage form takes place outside the EEA. GMP certification has been provided which confirms the date of the last inspection and shows that the site is authorised for the manufacture of such veterinary dosage forms.

Batch release takes place within the EU at Zoetis Belgium SA which holds a manufacturing authorisation issued by Federal Agency for Medicines and Health Products, Belgium. GMP certification has been provided which confirms the date of the last inspection and shows that the site is authorised for the manufacture of such veterinary dosage forms.

Manufacture of the active substances selamectin and sarolaner takes place outside the EEA. GMP declarations for each of the manufacturing sites are provided from the Qualified Person (QP) at the EU batch release site. The declarations are issued on the basis of on-site audits which have taken place within the last 3 years.

Overall conclusions on administrative particulars

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

The GMP statuses of all the active substance and finished product manufacturing sites have been

satisfactorily established and are in line with legal requirements.

Part 2 - Quality

Composition

Stronghold Plus is presented as a spot-on solution in three different strength single dose translucent polypropylene pipettes containing 0.25 ml (delivering 15 mg selamectin and 2.5 mg sarolaner), 0.50 ml (delivering 30 mg selamectin and 5 mg sarolaner), and 1 ml (delivering 60 mg selamectin and 10 mg sarolaner).

Other ingredients are dipropyleneglycol monomethylether (DPGMME) (cosolvent), isopropyl alcohol (IPA) (cosolvent) and butylated hydroxytoluene (antioxidant).

Containers

The primary packaging is translucent polypropylene unit dose pipettes with polystyrene caps. Intermediate packaging in the form of strips of cold form aluminium and aluminium/PVC blisters is used to protect the product from light, and also to reduce both evaporation of the solvent from the filled pipettes and their absorption of moisture. The blisters are supplied in cardboard boxes (outer packaging). The caps of the pipettes are colour coded as follows:

- Pipettes with yellow caps contain 0.25 ml of product and deliver 15 mg selamectin and 2.5 mg sarolaner.
- Pipettes with orange caps contain 0.50 ml of product and deliver 30 mg selamectin and 5 mg sarolaner.
- Pipettes with green caps contain 1 ml of product and deliver 60 mg selamectin and 10 mg sarolaner.

The material of construction of the pipettes complies with the relevant Ph. Eur. and EU requirements (including Regulation (EU) No 10/2011 on plastic materials intended to come into contact with foods). The choice of the container closure system is supported by stability data and is adequate for the intended use of the product.

Each strength of the product is packaged individually or in strips of 3 pipettes with perforations between each blister. In addition to these presentations, a 6 pipette presentation is also available (2 x strips of 3 pipettes) for each strength.

Development pharmaceutics

Selamectin as a single substance is already authorised for use in cats (Stronghold, EU/2/99/014/xxx) also as a spot-on solution, at a concentration of 60 mg/ml. The excipients in this existing product are the same but with different quantities, dipropyleneglycol monomethyl ether, isopropyl alcohol and butylated hydroxytoluene.

Pharmaceutical development of this new combination product focused on the already authorised Stronghold formulation, the solubility of both of the active substances, excipient compatibility and selection of an antioxidant.

The need to include an antioxidant in the formulation was demonstrated and several antioxidants were investigated, with butylated hydroxytoluene evaluated as the most effective antioxidant. A further

series of experiments, including stability evaluations, established the appropriate level of it to be 0.2 mg/ml.

Method of manufacture

The manufacturing process involves sequential mixing of the actives and other excipients in the formulation with recirculation through an in-line homogeniser to achieve dissolution of the actives. Filtering of the solution through a polypropylene filter is necessary to remove the insoluble sarolaner isomer which may be present as an impurity of the sarolaner active substance.

Pipettes are then filled and sealed in a single operation.

Small filling overages, based on data generated during the manufacture of full scale batches, are used to ensure the deliverable volume from the pipettes. The filling overages are centred on the declared volume and ensure compliance of the delivered dose with the release specification in terms of concentration of active substance per pipette.

During manufacturing process development, 3 pilot scale batches were manufactured and used for VICH stability testing. Following the manufacture of these VICH stability batches, changes were made to the manufacturing process for commercial scale. Three full scale batches were manufactured using the commercial process and it has been demonstrated to consistently produce product of the required quality. The description of the process and appropriate in-process controls have been established based on the manufacture of these three full scale batches. The manufacturing process is very similar to the registered process for the existing Stronghold product which is manufactured at the same manufacturing site and can be considered to be a standard one. Therefore in accordance with the CVMP Guideline on Process validation for finished products (EMA/CHMP/CVMP/QWP/BWP/70278/2012-Rev.1) process validation data may be generated post-authorisation and will be available at the manufacturing site during future inspections. A process validation protocol has been provided and is considered acceptable.

Control of starting materials

Active substances

Selamectin

Selamectin is a semi-synthetic avermectin, derived by chemical modification of doramectin which is produced from fermentation of *Streptomyces avermitilis*. Selamectin is manufactured from doramectin in a three/four step synthetic process. Full documentation is provided in the dossier.

Doramectin is the starting material for the manufacture of selamectin and is also a long established and well characterised active substance. A summary of the doramectin manufacturing process has been provided detailing the preparation of the media, inoculation, fermentation, recovery, crystallisation and drying steps, together with process flow charts and details of the in-process controls applied, along with a list of all solvents, reagents and auxiliary materials used in the manufacture of the starting material. Information on the characterisation of the producer microorganism used in the manufacture of the starting material (doramectin) has been provided. Further documentation regarding the comparability of the mutagenised strain with the originator strain was however accepted to be provided post authorisation as a recommendation. In addition, information has been provided on the purification processes used along with supporting data to demonstrate the absence of any host DNA in the starting material.

Two different processes are described for the manufacture of the active substance selamectin. Sufficient detail on the manufacturing process has been included in the dossier.

Selamectin is monographed in the European Pharmacopoeia and the limits in the specification provided comply with those of the Ph. Eur. monograph. The absence of mutagenic potential for one process impurity has been demonstrated using the in silico tool, DEREK and statistical based methodology confirming that the impurity is not a mutagenic concern was accepted to be provided post authorisation as a recommendation. Test methods are either pharmacopoeial or in-house methods which have been appropriately validated in accordance with VICH guideline GL2 on Validation of analytical procedures: Methodology. Batch analysis data is provided for the active substance which includes batches manufactured at each of the two sites of manufacture following both manufacturing processes. All results comply with both the specification and the Ph. Eur. monograph.

Stability studies were initiated on 3 pilot-scale batches of the active substance, manufactured in the site approved for the manufacture of selamectin in the original Stronghold application. They were stored up to 18 months at long-term (25 °C/60% RH) and intermediate (30 °C/60% RH) conditions, and up to 6 months at accelerated (40 °C/75% RH) conditions. The samples on stability were packaged in a container-closure system that simulates that used for the bulk active substance. All results were within specification, with only increasing trends in water content, seen for all batches at all conditions. Updated stability data have been provided for 3 full scale batches of the active substance manufactured at one of the proposed sites following one of the manufacturing processes. They were stored at 25 °C/60% RH, with data for the initial and 12 month time-points for the first batch; initial, 3 month and 6 month time-points for the second batch and the initial time-point only for the last batch. All results comply with the specifications, and no trending was apparent. Stability data were also provided for the first 3 validation batches manufactured at the other site, which were manufactured by the second process. Data was provided for the initial and 3 month time-points at 25 °C/60% RH, and for the initial, 1 month and 3 month time-points at 40 °C/75% RH. Results were provided for appearance, assay, specified, unspecified and total impurities and water content, according to the proposed specification. All results were within specification and only an increasing trend in water content was noted for all the batches on accelerated conditions. No trending was possible for the long-term conditions with only 2 time-points available. All results for specified, unspecified and total impurities were well within specification. The data supports the proposed re-test period of 2 years.

It is considered that the proposed re-test period of two years with no specific storage precautions is supported by the stability data provided.

Sarolaner

The active substance sarolaner is a member of the isoxazoline class of parasiticides and is in a veterinary medicinal product (Simparica) already authorised in the EU. Full documentation is provided in the dossier.

Sarolaner is manufactured in a four step synthetic process using three starting materials. One of the starting materials is considered to be quite complex, however, acceptable justification has been provided for its choice as a starting material, coupled with a detailed characterisation of the starting material and its potential impurities, along with a specification that is deemed to be suitable for its control. As such, the starting material is considered to be acceptable and batch data has been provided for active substance manufactured from the starting material from the different proposed suppliers. The level of detail provided for the active substance manufacturing process is sufficient.

Sarolaner is not monographed in a pharmacopoeia. The proposed in-house specification is acceptable Test methods are well described and are validated in accordance with VICH guideline GL2 on Validation of analytical procedures: Methodology. Batch analysis data is provided of the active substance manufactured throughout the development of the synthetic process, and for batches of the active substance manufactured using the proposed manufacturing process.

Stability studies were initiated on three batches of the active substance manufactured at another manufacturing site, at approximately one-third of the production scale. 18 months data at 25 °C/60% RH and 30 °C/75% RH and 6 months data at 40 °C/75% RH are currently available. In addition, two batches manufactured at the proposed site were also placed on stability, one pilot-scale batch and one production-scale batch. Data up to 12 months is available for one of the batches stored at 25 °C/60% RH and 30 °C/75% RH and 6 months data at 40 °C/75% RH. Up to 6 months data is available for the other batch, for all three storage conditions. Finally, 24 months supporting stability data at 25 °C/60% RH has been provided on a pilot-scale batch of the active substance but which was not manufactured at the proposed manufacturing site. The samples on stability were packaged in a container-closure system that simulates that used for the bulk active substance. All results were within the proposed specifications with only minor decreasing trends or overall decreases in assay noted for some of the batches. The proposed re-test period of two years with no specific storage precautions is supported by the stability data provided.

Excipients

Isopropyl alcohol and butylated hydroxytoluene meet the specifications of their respective current Ph. Eur. monographs. Representative certificates of analysis are included in the dossier.

Dipropyleneglycol monomethylether is not monographed and an appropriate in-house specification is provided. The specification includes suitable tests and limits for identification (IR), assay, appearance, acidity, colour and residual solvents. Analytical methods and, where relevant, their validation are provided.

There are no novel excipients used in the finished product formulation.

The list of excipients is included in section 6.1 of the SPC.

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. The product is therefore out of scope of the relevant Ph. Eur. monograph and 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 during production

In-process controls are appropriately defined within the dossier.

Control tests on the finished product

The finished product specifications proposed for use at release and at the end of shelf life include the parameters appropriate for the dosage form. In accordance with the CVMP Guideline on the quality aspects of single-dose veterinary spot-on products (EMEA/CVMP/QWP/54461/2007), the assay is

expressed in terms of the quantity by mass of the active substance in a container of average delivered mass or volume.

The analytical methods used have been adequately described and appropriately validated in accordance with the VICH guideline GL2 on Validation of analytical procedures: Methodology.

Satisfactory information regarding the reference standards for each active substance is provided.

Batch analyses results are provided for three full scale batches filled in each of the three different pipette sizes.

Stability

Stability studies are presented for three pilot scale batches which were 10% of the proposed commercial scale. Each bulk solution was filled into the three fill volumes in 1 ml polypropylene pipettes stored in protective aluminium and aluminium/PVC blisters as proposed for marketing. Samples were placed on stability under real time (25 °C/60% RH), intermediate (30 °C/65% RH) and accelerated conditions (40 °C/75% RH). Data is presented up to 24 months storage at 25 °C/60% RH and 30 °C/65% RH and 12 months storage at 40 °C/75% RH.

The analytical procedures used are stability indicating and have been appropriately validated.

The product is very stable with little inter-batch variation observed in stability studies. Some decrease in antioxidant is observed over time and some decrease in pipette weights. A small number of the results for sarolaner are outside the proposed stability specification in the 0.25 ml pipette but the overfill volumes have been amended since manufacture of these stability batches which will rectify this and future batches can be expected to be within specification. A storage precaution of "Store below 30 °C." is included to mitigate against evaporation of solvent in the formulation.

The existing marketed selamectin spot-on solution (Stronghold) is sensitive to light with unacceptable levels of degradation reported in the dossier for that product. Therefore photostability studies were not performed as part of the VICH stability program for this combination product. Furthermore, the aluminium and aluminium/PVC blisters in which the product is stored are completely impermeable to light and the outer carton contains the warning "Do not remove the pipette from the blister until ready to use." The absence of a photostability study is therefore considered acceptable and the product packaging and storage precautions provide adequate protection of the product from light.

The stability data presented is considered adequate to support the proposed shelf life of 30 months when stored according to the recommended storage conditions: "Store below 30 °C", and "Do not remove the pipette from the blister until ready to use".

Overall conclusions on quality

Stronghold Plus is presented as a spot-on solution in three different strength single dose translucent polypropylene pipettes. Pharmaceutical development of this new product focused on the currently authorised Stronghold formulation, the solubility of both of the active substances, excipient compatibility and antioxidant selection.

The manufacturing process involves sequential mixing of the actives and antioxidant in the formulation with recirculation through an in-line homogeniser to achieve dissolution of the actives. Filtering of the solution through a polypropylene filter is necessary to remove the insoluble sarolaner isomer which may be present as an impurity of the sarolaner active substance.

Process validation studies should be performed on the first 3 commercial batches. As the manufacturing method is a relatively simple standard process and is very similar to the registered process for the existing Stronghold product, manufactured at the same site, it was accepted that full scale process validation would be performed post-authorisation, in accordance with the CVMP Guideline on Process validation for finished products (EMA/CHMP/CVMP/QWP/BWP/70278/2012-Rev.1). The applicant had provided both a protocol for the process validation study and a commitment to submit the data post-authorisation.

Full documentation is provided for the active substance selamectin, a semi-synthetic avermectin, derived by chemical modification of doramectin, which is produced by fermentation by *Streptomyces avermitilis*. Information on the characterisation of the producer microorganism used in the manufacture of the starting material (doramectin) has been provided. Further documentation regarding the comparability of the mutagenised strain with the originator strain will be provided post authorisation. Selamectin is manufactured from doramectin in a three/four step synthetic process. Selection of the starting material is appropriate. Selamectin is monographed in the European Pharmacopoeia and its specification complies with the Ph. Eur. monograph although there is an outstanding issue regarding the threshold for a potential impurity. The absence of mutagenic potential for one process impurity has been demonstrated using the in silico tool, DEREK and statistical based methodology confirming that the impurity is not a mutagenic concern is to be provided post authorisation. Stability studies are provided which support the proposed re-test period of two years with no specific storage precautions.

Full documentation is provided for the active substance sarolaner, a member of the isoxazoline class of parasiticides. Sarolaner is manufactured in a four step synthetic process using three starting materials. One of the starting materials is considered to be quite complex, however, acceptable justification has been provided for its choice as a starting material, coupled with a detailed characterisation of the starting material and its potential impurities, along with a specification that is deemed to be suitable for its control. As such, the starting material is considered to be acceptable. The level of detail provided for the active substance manufacturing process is sufficient. The specification for this active substance is acceptable. Stability studies are provided which support the proposed re-test period of two years with no specific storage precautions.

The finished product specifications proposed for use at release and at the end of shelf-life include the parameters appropriate for the dosage form. In accordance with the CVMP Guideline on the quality aspects of single-dose veterinary spot-on products (EMEA/CVMP/QWP/54461/2007) active substance assay limits are expressed in terms of the quantity by mass of the active substance in a container of average delivered mass or volume and the proposed limits have been justified. Satisfactory batch data for three full scale batches manufactured at the proposed site is provided.

The product is very stable with little inter-batch variation observed in stability studies. The stability data presented is considered adequate to support the proposed shelf life of 30 months when stored according to the recommended storage conditions: "Store below 30 °C.", and "Do not remove the pipette from the blister until ready to use."

Based on the review of the data on quality, the manufacture and control of Stronghold Plus are considered acceptable.

In addition, the applicant is recommended to provide the following information post-authorisation and prior to marketing:

Results of a statistical based methodology to confirm that one of the process impurities of selamectin is of no mutagenic concern.

Comparative analyses (morphological, culture characteristics) between the originator strain and the mutagenised producer strain for the production of selamectin's starting material.

Commitments to submit data to address the two above points post-authorisation (and prior to marketing) have been received from the applicant and the Committee considered these acceptable.

Part 3 – Safety

The active substances in Stronghold Plus, selamectin and sarolaner, are a new combination of established active substances not authorised in combination for a veterinary medicinal product in the EU before. A full safety file in accordance with Article 12(3)(j) of Directive 2001/82/EC has been provided. The application makes reference to the dossier and previous assessment of the CVMP in the context of the initial marketing authorisation applications for the products Stronghold spot-on solution for dogs and cats (selamectin) and Simparica tablets for dogs (sarolaner).

Pharmacodynamics

See Part 4.

Pharmacokinetics

See Part 4.

Toxicological studies

The toxicology profiles for the selamectin-sarolaner combination consists of studies conducted with the individual active substances, sarolaner and selamectin, and studies conducted with the combination product. All pivotal studies were conducted in accordance with GLP and the relevant OECD guidelines.

Single dose toxicity

The acute oral LD_{50} in of sarolaner in rats was estimated to be 783 mg/kg (95% confidence interval of 550–2000 mg/kg bw). The most sensitive acute study with sarolaner was the acute oral tolerance study in dogs where no significant adverse reactions were observed at single oral doses up to 25 mg/kg bw. However, serious adverse effects were observed at 62.5 mg/kg bw (vomiting, stiff jerky movements, convulsions). Convulsions observed in this study are consistent with the GABA-antagonist activity of sarolaner. In a cat dermal tolerance study with sarolaner, adverse neurological reactions were observed in one cat administered a dose of 50 mg/kg bw.

The minimal oral lethal dose of selamectin in mice and rats was in excess of 1600 mg/kg bw. Diarrhoea occurred in mice and rats of all groups. In addition, a range of clinical signs were present in rats treated with 1600 mg/kg bw and consisted of dyspnoea, hunched posture, partially closed eyes, decreased activity, chromodacryorrhea and piloerection. Overall, rats appeared to be more sensitive than mice to the effects of selamectin.

The acute oral LD_{50} in rats with the sarolaner and selamectin combined formulation was determined to be greater than 2000 mg/kg bw (this corresponds to a selamectin dose of 141.6 mg/kg bw and a sarolaner dose of 23.6 mg/kg bw). The acute dermal LD_{50} in rats with the sarolaner and selamectin combined formulation was determined to be greater than 2020 mg/kg bw for the formulation (this corresponds to a selamectin dose of 142.8 mg/kg bw and a sarolaner dose of 23.8 mg/kg bw).

Repeat dose toxicity

The repeat-dose toxicities of sarolaner and selamectin alone and in combination were adequately defined in well conducted studies in rats, dogs and/or cats.

The most sensitive repeat-dose toxicity study in rats with sarolaner was determined to be the 90-day oral study where the no-observed-adverse-effect level (NOAEL) was determined to be 2.5 mg/kg bw/day based on partial recovery from the body weight effects in females following the first week of the dosing period. The CVMP did not accept the proposed NOAEL given that an effect on body weight was noted at 25 mg/kg/day and, for females, only a partial recovery of this effect was achieved over the remainder of the study. In addition, at 25 mg/kg/day, effects on food consumption persisted throughout to the end of the study. Furthermore, treatment-related histopathological (dose-dependent) changes in the ovary were noted at lower doses. Therefore, a NOAEL for this study is considered to be 0.25 mg/kg bw/day.

The most sensitive repeat-dose study in dogs was the GLP margin of safety study, in which 10 doses were administered at 28-day intervals with transient tremors observed at 12 and 20 mg/kg bw and convulsions observed in a few dogs at 20 mg/kg bw. Signs appeared to occur in the first 24 hours after dosing. No tremors or convulsions were observed beyond the 5th dose of this 10 dose study. No neurological signs were observed in the 4 mg/kg bw group.

The most sensitive repeat-dose oral toxicity study in rats with selamectin was the 90-day rat where based on the fatty liver changes with correlating increases in mean absolute and relative liver weights and hepatic enzyme levels, and the small intestine lymphangiectasia (lymphatic dilatation), the NOAEL was determined to be 5 mg/kg bw/day. A 90-day oral toxicity study in dogs revealed a no-observed-effect level (NOEL) of 15 mg/kg bw based on emesis and salivation. In the 4-week topical safety study in dogs at weekly doses up to 80 mg/kg bw/dose, there was no toxicity observed at any dose and no signs of local irritation at the application site.

Repeat dermal doses of the combined formulation for 21 days was well tolerated in rabbits at doses up to 1000 mg/kg bw/day. Local irritation at the test site consisted of very slight to well- defined erythema and very slight oedema as well as skin scaling, which had a microscopic correlate (hyperkeratosis) at 250 mg/kg bw/day and higher. However, it is unclear if the local irritation observed in the test article-treated groups was due to the vehicle alone, as this vehicle was different than the control article. Based on these results, a local NOEL was not determined. However, the systemic NOAEL was considered to be 1000 mg/kg bw/day, corresponding to 12 mg/kg bw/day sarolaner and 72 mg/kg bw/day selamectin.

Tolerance in the target species of animal

See Part 4.

Reproductive toxicity

Studies to evaluate the effects of sarolaner on reproduction were not conducted. In developmental toxicity studies with sarolaner, no embryo-foetal development effects were observed below doses that were maternally toxic; the observed developmental effects were considered secondary to maternal toxicity. The NOAEL for maternal toxicity and embryo/foetal development was determined to be 3.2 mg/kg bw/day in rats and 3.0 mg/kg bw/day in rabbits. Sarolaner does not appear to have a direct embryolethal or teratogenic effect in rats or rabbits. In conclusion, sarolaner is not considered to be a developmental toxicity concern.

In a study to evaluate the effects of selamectin on reproduction, the high dose of 60 mg/kg bw/day was maternally toxic (decrements in food consumption and gestational bodyweight, decreased litter size, increased number of resorptions, increased incidence of dead foetuses). The NOAEL for fertility and reproduction, gestation, lactation, foetotoxicity and early postnatal development for F0 females was 10 mg/kg bw/day based on prolonged gestational length and foetotoxicity in the groups treated at 25 mg/kg bw/day or higher. The NOAEL for F0 males was 60 mg/kg bw/day, the highest dose tested. There were no treatment-related effects in the Functional Observational Battery conducted in F1 rats. In studies conducted to evaluate the effect of selamectin on embryotoxicity/foetotoxicity including teratogenicity, selamectin was not maternally toxic embryolethal or teratogenic at oral doses up to 10 mg/kg bw/day when administered during the period of gestation; cardiac effects were observed from 40 mg/kg bw/day. Selamectin was present in the milk of exposed females at similar concentrations to those in maternal plasma.

In the absence of reproductive toxicity studies on the combination, the SPC includes a statement that the safety of the product has not been established during pregnancy and lactation or in animals intended for breeding.

Genotoxicity

The genotoxic and mutagenic potential of both sarolaner and selamectin were adequately assessed in a standard battery of genetic toxicology assays recommended in VICH GL23. Based on the results of these assays, it was concluded that neither test article is considered to be of mutagenic or genotoxic concern.

Carcinogenicity

Carcinogenicity studies were not conducted with sarolaner or selamectin, however, firstly, neither test article was mutagenic or genotoxic and, secondly, there were no proliferative changes in the 90-day oral rat toxicity studies conducted for each compound. In addition, there were no structural alerts for genotoxicity for sarolaner.

Carcinogenicity studies were not conducted with the combined selamectin plus sarolaner formulation or with the individual components, however, based on the information provided on the individual components, the CVMP considers the absence of carcinogenicity studies with the combined selamectin plus sarolaner formulation or with the individual components as justified.

Studies of other effects

Sarolaner was minimally-irritating in an ocular irritation study in rabbits and non-irritating in a dermal irritation study in rabbits. Sarolaner is not considered a sensitizer based on results of a mouse local lymph node assay.

Selamectin was slightly irritating in an ocular irritation study in rabbits and did not produce irritation or corrosion in a dermal irritation study in rabbits. Selamectin is not considered a sensitizer based on results of a skin sensitisation study in guinea pigs.

The combination formulation was moderately irritating in an ocular irritation study in rabbits, was non-irritating in a dermal irritation study in rabbits, and was not considered a sensitizer based on results of a local lymph node assay in mice.

No specific neurotoxicity studies have been conducted with sarolaner, selamectin or both active substances combined. However, in tolerance studies in dogs and cats conducted with sarolaner, adverse neurological effects were observed at doses of respectively 50 mg/kg bw in cats and from 12 mg/kg bw in dogs.

In a dislodgeable residue study conducted with the final formulation, the higher amounts of drugs were measured in cotton gloves used to pet cats 4 hours or 24 hours after topical application. Percent dislodgeable residues were lower than 4.7% of administered product for both substances.

Excipients

The product is formulated in a vehicle which contains dipropylene glycol monomethyl ether (DPGMME), isopropyl alcohol plus 0.2 mg/ml butylated hydroxytoluene. The excipients used in the formulation are those used in approved veterinary or human marketed pharmaceutical products and a quantitative risk characterisation revealed that they are not a human user safety concern (are of low toxicity and/or present at low concentrations) if the user is dermally exposed including subsequent hand-to-mouth contact. However, isopropyl alcohol may cause serious eye irritation and is highly flammable.

User safety

The user safety assessment provided has been conducted in accordance with the CVMP Guideline on user safety for pharmaceutical veterinary medicinal products (EMEA/CVMP/543/03-Rev.1). Based on the information provided, there is no human user safety concern anticipated if the user is dermally exposed to the product including subsequent hand-to-mouth contact, when used in accordance with the product information. The exposure scenarios for which a risk was identified were:

- Ocular irritation, and
- A risk to children associated with ingestion of the final product.

The risks relating to contact with the treated animal - dermal exposure and subsequent hand-to-mouth contact for the acute and prolonged exposure - have been adequately addressed and are generally acceptable. In relation to the potential for indirect user exposure via the environment, in the absence of environmental data it is unknown what additional exposure may occur. However, based on the available information, it is unlikely that indirect exposure will exceed direct exposure. Assuming that the potential environmental exposure is equivalent to direct exposure, it is concluded that exposure via environmental contamination, in addition to exposure via direct contact with the treated animal will not lead to an unacceptable risk for the user.

Risk mitigation measures to address the identified risks have been proposed, including the use of child-resistant packaging. The user safety statements proposed are acceptable.

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

Environmental risk assessment

The applicant conducted a phase I environmental risk assessment in accordance with VICH GL6 on environmental impact assessment for veterinary medicinal products - Phase I (CVMP/VICH/592/98-FINAL).

In accordance with question number three of the phase I decision tree, the product will only be used in non-food producing animals. Consequently, no further risk assessment in respect of the environment is required and the assessment may stop in phase I.

The product is not expected to pose a risk for the environment when stored, handled, administered and disposed of, in accordance with the recommendations proposed for inclusion in the SPC.

The SPC proposes the following risk mitigation measures:

"Stronghold Plus should not enter water courses as this may be dangerous to aquatic organisms. Containers and residual contents should be disposed of along with collected domestic refuse to avoid contamination of any water courses."

Whilst no specific data has been provided in support of such a warning, it is noted that the same warning is already included in the EU authorised product 'Stronghold', which contains selamectin only. The same proposed risk mitigation advice already approved for 'Stronghold' can also be accepted for 'Stronghold Plus'.

Overall conclusions on the safety documentation

The toxicology profiles for the selamectin-sarolaner combination consists of studies conducted with the individual actives, sarolaner and selamectin, and studies conducted with the combination product. All pivotal studies were conducted in accordance with GLP and the relevant OECD guidelines. Based on the suite of studies conducted, it is accepted that the toxicological profile of the product has been adequately characterised.

The user safety assessment provided has been conducted in accordance with relevant CVMP guidance. Based on the information provided, the exposure scenarios for which a risk was identified were:

- Ocular irritation, and
- A risk to children associated with ingestion of the final product.

Risk mitigation measures to address the identified risks have been proposed. The user safety statements proposed are acceptable.

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

Part 4 – Efficacy

Pharmacodynamics

The candidate formulation is a new combination of the active substances selamectin and sarolaner.

Selamectin:

Selamectin has antiectoparasitic and anthelmintic properties and is already authorised as sole active substance as a spot-on solution for use in cats (Stronghold) with the following indications:

- Treatment and prevention of flea infestations caused by *Ctenocephalides* spp. for one month following a single administration. This is as a result of the adulticidal, larvicidal and ovicidal properties of the product. The product is ovicidal for 3 weeks after administration. Through a reduction in the flea population, monthly treatment of pregnant and lactating animals will also aid in the prevention of flea infestations in the litter up to seven weeks of age. The product can be used

as part of a treatment strategy for flea allergy dermatitis and through its ovicidal and larvicidal action may aid in the control of existing environmental flea infestations in areas to which the animal has access.

- Treatment of ear mites (*Otodectes cynotis*).
- Treatment of biting lice infestations (*Felicola subrostratus*).
- Prevention of heartworm disease caused by *Dirofilaria immitis* with monthly administration. Stronghold may be safely administered to animals infected with adult heartworms, however, it is recommended, in accordance with good veterinary practice, that all animals 6 months of age or more living in countries where a vector exists should be tested for existing adult heartworm infections before beginning medication with Stronghold. It is also recommended that dogs should be tested periodically for adult heartworm infections, as an integral part of a heartworm prevention strategy, even when Stronghold has been administered monthly. This product is not effective against adult *D. immitis*.
- Treatment of adult roundworms (*Toxocara cati*).
- Treatment of adult intestinal hookworms (*Ancylostoma tubaeforme*).

Selamectin is a semi-synthetic avermectin which exerts its effect through potentiation and/or agonist action at invertebrate glutamate-gated chloride channels.

Study data was provided that evaluated *in-vitro* pharmacological profile of selamectin and glutamate on *Dirofilaria immitis* glutamate-gated chloride channels using voltage-clamp electrophysiology experiments and *in vitro* pharmacological profile of sarolaner at insect and human GABA-gated chloride channels using a fluorescent imaging plate reader (FLIPR) based membrane potential assay.

Data provided suggest that selamectin preferentially exerts its effect at invertebrate glutamate-gated chloride channels whereas sarolaner exhibits a preference for GABA-gated chloride channels over glutamate-gated chloride channels. In addition, both substances exhibit preferential selectivity of invertebrate over human channels and, consequently, higher functional potency to block insect/acarine receptors compared to mammalian receptors.

Sarolaner:

Sarolaner is an ectoparasiticide substance belonging to the isoxazoline family. The primary mode of action in insects and acarines is functional blockade of ligand-gated chloride channels (GABA-receptors and glutamate-receptors). Sarolaner as sole active substance has been authorised in 2015 for use in dogs as a chewable tablet (Simparica) for the treatment of tick infestations (*Dermacentor reticulatus, Ixodes hexagonus, Ixodes ricinus* and *Rhipicephalus sanguineus*), flea infestations (*Ctenocephalides felis* and *Ctenocephalides canis*), as part of a treatment strategy for the control of Flea Allergy Dermatitis (FAD) and for the treatment of sarcoptic mange (*Sarcoptes scabiei*).

Development of resistance

There is no information available to suggest that resistance to selamectin is of concern. In dogs and cats, anthelmintic resistance to macrocyclic lactones is thought to mainly concern heartworm (*Dirofilaria immitis*) in specific regions in the US. In the EU, heartworm resistance against treatment with selamectin has not been reported at the time of publication of this assessment report. Since 1999, there have been no reports within the EU of a lack of efficacy or development of anthelmintic resistance for selamectin against gastro-intestinal nematodes or heartworm in cats or dogs. Similarly in the scientific literature there are no indications of lack of efficacy against any of the claimed parasites.

Due to limited use thus far of sarolaner, no resistance issues have become apparent.

Given the different sites of action on chloride channels between selamectin and sarolaner, the combination of both active substances in this combination product is not expected to increase the risk for resistance development in any of the proposed target parasites.

Pharmacokinetics

The recommended minimum dosage for the product is 1 mg sarolaner/kg bodyweight and 6 mg selamectin/kg bodyweight. The product is intended for topical application at monthly intervals.

A series of studies (seven in total) was undertaken to determine the pharmacokinetics of sarolaner and selamectin when administered as single substance and when administered in combination.

Studies were conducted for at least 28 days in order to characterise the pharmacokinetics over the intended dosing interval. Repeat dose pharmacokinetics following 8 consecutive monthly administrations was also determined as part of the target animal safety study. The intended commercial combination formulation was used in the GLP topical PK study and for the TAS study.

In all studies that included a PK component, blood samples were analysed for sarolaner and selamectin content by high performance liquid chromatography (HPLC) with tandem mass spectrometry detection (LC-MS/MS). The method was appropriately validated, with a lower LOQ of 1.0 ng/ml for both sarolaner and selamectin.

It can be concluded from these studies that:

- Both sarolaner and selamectin are absorbed following topical administration to the cat with bioavailability mean values of 57.9% and 40.5%, respectively.
- There is large between animal variability in pharmacokinetic parameters, in particular for selamectin.
- Plasma binding of sarolaner in cats was found to be high (99.9%).
- Both compounds are also low clearance compounds with long plasma elimination half-lives. The clearance of sarolaner and selamectin is less than 1% of liver blood flow in cats.
- In cats, the primary route of elimination for selamectin is via the faeces (~90%), with the majority of the radioactivity eliminated in bile as parent compound. Metabolic clearance also contributes to the elimination of selamectin. The primary route of elimination for sarolaner is biliary elimination of parent sarolaner, with contributions by metabolic clearance. The decline of sarolaner drug-related residue concentrations from tissues and biofluids was consistent with the plasma elimination half-life.
- Following topical dosing at 1x, 3x, 3.75x, and 5x the maximum possible exposure (2 mg/kg sarolaner and 12 mg/kg selamectin) when administered at the recommended minimum treatment dose, the sarolaner and selamectin $AUC_{24\text{-}t}$ and C_{max} increased less than proportionally with dose from 1x to 5x. Accumulation of sarolaner and selamectin was observed for all dose levels, with C_{max} plateauing following the 6th dose for sarolaner and following the 3rd dose for selamectin.

Data has also been provided to characterise the pharmacokinetics of selamectin in cats when administered alone and in combination with sarolaner, in order to investigate any possible interaction of sarolaner on the bioavailability of selamectin.

Selamectin pharmacokinetics following topical administration with and without sarolaner, do not show

interference based on the similar exposure (C_{max} and AUC) of selamectin following topical administration alone and in combination with sarolaner (totality of data considered).

Additionally, in the pivotal PK study, the comparisons of $t_{1/2}$ and selamectin PK profiles following administration of identical formulations indicate that there is not a sarolaner induced effect on selamectin clearance. Whilst some study data suggest a possible increase in selamectin plasma concentration when administered in combination with sarolaner, overall, it can be accepted that there is no indication that sarolaner negatively impacts (i.e. decreases) the selamectin exposure.

Based on the totality of data provided, it can be accepted that the administration of sarolaner in combination with selamectin does not appear to result in a decrease in bioavailability of selamectin. Dose proportionality at overdose could not be confirmed despite a dose-response effect up to 3.75x recommended treatment dose (RTD). It is noted that systemic exposure (as measured by C_{max} and AUC) of both sarolaner and selamectin were in general lower following administration of 5xRTD when compared to 3.75xRTD and was most noticeable following the first two dose applications, but persisted to variable degrees up to the final (8^{th}) dose application. Although this may have arisen due to saturation of absorptive capacity, this cannot be confirmed. Notwithstanding the fact that the exact reason for this observation remains unknown, it can be accepted that this finding has little significance in terms of safety or efficacy of the product.

The CVMP accepted that the pharmacokinetics of both sarolaner and selamectin in cats was adequately characterised following both single and repeated (monthly) applications at the proposed treatment dose. The information proposed for inclusion in section 5.2 of the SPC is considered to accurately reflect the findings from the study data.

Justification of combination

Selamectin has been authorised since 1999 in the EU as a topical formulation for the treatment and prevention of fleas (*Ctenocephalides* spp.), the prevention of heartworm disease caused by *Dirofilaria immitis*, the treatment of *Felicola subrostratus*, *Otodectes cynotis*, *Toxocara cati* and *Ancylostoma tubaeforme* in cats. Selamectin has no efficacy claims against ticks.

Sarolaner has been authorised since 2015 in the EU as an oral formulation for the treatment of tick infestations (*Dermacentor reticulatus, Ixodes hexagonus, Ixodes ricinus* and *Rhipicephalus sanguineus*) and flea infestations (*Ctenocephalides felis* and *Ctenocephalides canis*) and the treatment of sarcoptic mange (*Sarcoptes scabiei*) in dogs. Sarolaner has no efficacy claims against biting lice infestations and nematodes (heartworm, roundworms and hookworms).

In line with the CVMP Guideline on Pharmaceutical fixed combination products (EMEA/CVMP/83804/05), the principal advantage claimed for the combination is the broadening of the activity spectrum. Sarolaner and selamectin are combined in order to add an indication against ticks compared to the mono-active product containing selamectin only. The broadening of spectrum appears justified based on the results of recent European surveys on mixed infestations in cats (Beugnet *et al*, 2014; Knaus *et al*, 2014).

The proposed combination of sarolaner and selamectin provides efficacy against a wide spectrum of ectoparasites (fleas, ticks, ear mites and biting lice) and nematodes (gastrointestinal nematodes and heartworm), whereas a similar spectrum of activity appears unavailable from a single authorised product and therefore the use of combinations of currently authorised products would be required to obtain the same spectrum of activity. Thus, as an additional benefit, it is more convenient to use a combination product than to use more than one medicinal product and it is expected to thereby improve the compliance of the user. In addition, the absence of interference of the active substances

in terms of both safety and efficacy has been adequately demonstrated.

The justifications provided appear reasonable and are considered to be in line with the potential advantages for such combinations as set out in the CVMP Guideline on pharmaceutical fixed combination products (EMEA/CVMP/83804/2005).

Dose justification/determination

The tick species against which a treatment claim is proposed for this combination product are: *Ixodes ricinus, Ixodes hexagonus, Dermacentor reticulatus* and *Rhipicephalus sanguineus*.

A total of five studies have been provided in order to justify the dose selection of sarolaner in the final formulation. These studies were conducted in order to evaluate efficacy against ticks (*Amblyomma maculatum*, *Dermacentor reticulatus*, *Dermacentor variabilis* and *Ixodes scapularis*), and fleas (*Ctenocephalides felis*) of a single or repeated application of sarolaner, at dose rates ranging from 0.5 to 2.0 mg sarolaner/kg bodyweight. One of the above studies also investigated possible interference of selamectin on the efficacy of sarolaner against ticks (*Dermacentor reticulatus)*.

Study	Tick species studied	Dose rates of sarolaner investigated	Interference investigated?	Conclusion on dose
Study 1	*Dermacentor variabilis*	1 mg/kg 2 mg/kg	No	Administration of 2 mg/kg bodyweight offers no advantage in terms of persistent efficacy when compared to a dose rate of 1 mg/kg.
Study 2	*Dermacentor variabilis*	0.5 mg/kg 0.75 mg/kg 1 mg/kg	No	Results suggest a dose-response in terms of acaricidal effect. Persistent efficacy was reduced to 21 days at 0.75 mg/kg and insufficient efficacy was demonstrated at 0.5 mg/kg.
Study 3	*Amblyomma maculatum and Ixodes scapularis*	0.75 mg/kg 1 mg/kg	No	Results suggest a dose-response in terms of acaricidal effect. Insufficient efficacy was demonstrated at 0.75 mg/kg.
Study 4	*Dermacentor reticulatus*	1 mg/kg	Yes	Acceptable level of efficacy not demonstrated until after Day 7. Persistent efficacy not consistently demonstrated with percentage reduction falling to below 90% on study days 7, 28 and 49. No interaction of selamectin on acaricidal effect of sarolaner was observed.
Study 5	*Dermacentor variabilis and Ixodes scapularis*	0.75 mg/kg 1 mg/kg	No	Results suggest a dose-response in terms of acaricidal effect. Persistent efficacy was reduced to 7 days at 0.75 mg/kg.

Doses of sarolaner investigated ranged from 0.5 mg/kg to 2 mg/kg. Results from these studies suggest that improved acaricidal effect was found with increasing dose rate of sarolaner. Only limited improvement in efficacy was observed between dose rates of 1 and 2 mg/kg (although only one study included a dose rate of 2 mg sarolaner/kg).

The proposed dose rate of 1 mg sarolaner/kg bodyweight is considered to have been sufficiently

supported for the purpose of carrying forward to pivotal dose confirmatory and field studies. Doses of 0.75 and 0.5 mg/kg were shown to provide reduced efficacy whereas a dose of 2 mg/kg was not shown to provide additional benefit.

The dose rate of selamectin (6 mg/kg) is the same as that already approved for the mono-active product 'Stronghold'. Given that PK data has shown that sarolaner does not negatively reduce systemic bioavailability of selamectin when administered in combination, the same dose rate of selamectin is considered appropriate for this combination product.

The CVMP can agree that even though the final formulation has not always been used, results from the dose determination studies can be accepted in the light of the objective. No interference between both active substances was observed. Only limited improvement in efficacy against *Dermacentor variabilis* (i.e. not *D. reticulatus*) was observed between dose rates of 1 and 2 mg/kg. Efficacy against *D. reticulatus* after infestation on day 7 as well as efficacy on day 28 appeared to be below 90% at the minimum dose of 1 mg sarolaner/kg bw. However, dose confirmation studies with *D. reticulatus*, using 1 mg sarolaner/kg bw in the final formulation demonstrated efficacy higher than 90% up to day 28.

Dose confirmation studies

Ectoparasites:

Fleas

Two-dose confirmatory studies to support immediate and persistent pulicidal efficacy of the product have been provided. Both counted fleas 24 hours after treatment and infestation. Whilst one study marginally failed to demonstrate an acceptable level of immediate efficacy against an existing flea infestation (92.4% versus the required 95%), the second study did demonstrate an acceptable level of immediate efficacy.

The first study was a GCP-compliant study and investigated immediate and persistent efficacy against *C. felis* (EU strain). The final formulation was used and administered at the proposed (minimum) dose rate (1 mg sarolaner/kg and 6 mg selamectin/kg) to sixteen (8 males and 8 females) domestic cats of European mixed breeds, aged 7–74 months and in the bodyweight range of 2.2–5.2 kg. Each animal was artificially infested with approximately 100 unfed adult *C. felis* fleas (sex ratio 1:1) on study days -6, -1, 6, 13, 20, 27 and 34. An adequate level of infestation (> 50 fleas) was achieved in the control group at all time-points. Fleas were counted and removed on study days 1, 7, 14, 21, 28 and 35 (24 hours after treatment application on day 0 and 24 hours after each subsequent infestation). A single topical treatment resulted in 92.4% efficacy at 24 hours (immediate pulicidal effect/ treatment) and at least 97.7% efficacy against weekly re-infestations (persistent effect) with *C. felis* for 35 days after treatment.

Another GCP-compliant study investigated immediate and persistent efficacy against *C. felis* (non-EU strain). The final formulation was used and administered at the proposed (minimum) dose rate (1 mg sarolaner/kg and 6 mg selamectin/kg) to sixteen (8 males and 8 females) domestic short-haired cats, aged 7–8 months and in the bodyweight range 2.8–5.5 kg. An adequate level of infestation (>50 fleas) was achieved in the control group at all time-points. Each animal was artificially infested with approximately 100 unfed adult *C. felis* fleas (sex ratio 1:1) on study days -8, -1, 6, 13, 20, 27 and 34. Fleas were counted and removed on study days -7, 1, 7, 14, 21, 28 and 35 (24 hours after treatment application and 24 hours after each subsequent infestation). A single topical treatment resulted in 100% efficacy at 24 hours (immediate pulicidal effect/treatment) after treatment and against weekly re-infestations (persistent effect) with *C. felis* for up to 35 days after treatment.

The proposed treatment and prevention claim against *Ctenocephalides* spp. for up to 35 days is considered to have been adequately supported.

Another GCP-compliant study was conducted to investigate the speed of kill against adult *C. felis*. Speed of kill was investigated at 6, 12, 24 and 48 hours after treatment application of the final formulation and at 3, 6, 12 and 24 hours after re-infestation using the minimum recommended treatment dose (1 mg sarolaner and 6 mg selamectin per kg bodyweight) for sixty-four (31 males and 33 females) domestic short-haired cats, aged 7–46 months and in the bodyweight range 2.1–6.7 kg. Each cat was infested with 100 unfed viable adult *C. felis* fleas on study days -1, 7, 14, 21, 28, and 35.

An acceptable level of efficacy was achieved 24 hours after treatment application and re-infestation for 35 days. While the data presented appear to support the proposed claim for elimination of fleas within 24 hours with faster (12 hours) between weeks 1 and 3 after treatment, CVMP previously concluded that the time to elimination of fleas stated on the SPC should be relevant for the entire period of the claimed treatment duration/re-treatment interval. Further, the revised guideline (EMEA/CVMP/EWP/005/2000- Rev.3 - due to come into effect on 1st February 2017) states that veterinary medicinal products may only be characterised with one figure for the parameter 'speed of kill'. Therefore, for this product the following text can be accepted for inclusion in section 5.1 of the SPC: "For fleas, the onset of efficacy is within 24 hours for 5 weeks after product application".

Another GCP-compliant study investigated pulicidal effect in addition to effect of the candidate formulation on egg production, hatchability and adult flea emergence. The final formulation was used for twenty (10 males and 10 females) domestic short hair cats, aged 12–35 months. Each cat was infested with 100 unfed viable adult *C. felis* fleas on study days -1, 5, 12, 19, 26 and 33. Flea counts were conducted on days 2, 8, 15, 22, 29 and 36 (that is, 48 hours after treatment and 72 hours after each re-infestation). Up to 200 flea eggs collected from each cat were randomly selected and evaluated for egg hatch (100 eggs) and for adult flea emergence (100 eggs).

Although no ovicidal or larvicidal effect is considered to have been demonstrated, based upon the results of this study, the proposed claim for the product to kill adult fleas before they lay eggs for up to 5 weeks is considered to have been adequately supported.

Ticks

Seven GCP-compliant dose confirmatory studies investigating acaricidal effect have been provided. Six of these studies were conducted within the EU (Ireland).

In general, the studies followed the same design and are accepted as having been conducted in accordance with guideline requirements and included a suitable number of study animals and achieved adequate infestation in controls.

Each study included sixteen (both male and female) domestic cats of European mixed breeds, aged 9–107 months and in the bodyweight range 2–5.8 kg. The intended final formulation was used. Two treatment groups of 8 cats each were administered 0.1 ml/kg of the test articles on study day 0. Animals were infested with ticks on study days -9/-7, -2, 5, 12, 19, 23/26 and 33 and ticks were counted on study days -9 to -7,-7 to -5, 2, 7, 14, 21, 28 and 35.

Conclusions are based upon arithmetic mean live tick counts only; that is, excluding category 6 ticks (dead, attached and engorged). Such an approach (exclusion of category 6 ticks) has previously been accepted by the CVMP when determining efficacy for systemically acting acaricidal products and is in line with the newly revised guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestations in dogs and cats (EMEA/CVMP/EWP/005/2000-Rev.3).

Rhipicephalus sanguineus:

One study demonstrated immediate efficacy (100%) within 48 hours against existing infestations following treatment application and demonstrated persistent efficacy (≥96.9%) for up to 28 days.

Another study demonstrated immediate efficacy (100%) within 48 hours against existing infestations following treatment application and persistent efficacy (≥93.8%) for up to 35 days.

Immediate efficacy (treatment) and persistent acaricidal efficacy for up to 28 days, is considered to have been adequately supported by two dose confirmatory studies.

The proposed claims for this tick species are considered to have been adequately supported by dose confirmation studies.

Ixodes ricinus:

Two dose confirmation studies demonstrated immediate efficacy (treatment) against existing infestations following treatment application and persistent efficacy for up to 35 days. While the immediate efficacy was 100% and 97.2% at 48 hours against an existing *I. ricinus* infestation, the persistent acaricidal efficacy was ≥99.4% and 100% against weekly re-infestations in two studies. Another study investigated the speed of onset of acaricidal efficacy against *I. ricinus*.

An acceptable level of immediate efficacy (99.3%) against existing tick infestations following application of the product was observed at 24 hours (but not at 8 or 12 hours).

An acceptable level of persistent efficacy against new infestations was observed for up to 21 days at 24 hours (but not at 8 or 12 hours). Percentage reduction in live tick counts was lower at 28 days (89%) and at 35 days (67.7%) than the minimum acceptance criteria (≥90%). Based on the data provided, a claim for onset of efficacy within 24 hours of attachment during the month following product application can be accepted. This information should be included in section 5.1.

Ixodes hexagonus:

One study demonstrated immediate efficacy (97.3%) at 48 hours against existing infestations following treatment application and demonstrated persistent efficacy (≥97.4%) for up to 35 days.

Although efficacy has only been adequately supported by one dose confirmatory study, the results of the field study confirmed that no difference in efficacy might be expected with *I. hexagonus* compared to *I. ricinus*. Comparison of the efficacy results from studies against *I. ricinus*and a study against *I. hexagonus*suggests that whilst a marginally lower efficacy against *I. hexagonus* (compared to *I. ricinus*) was observed at the day 7, 14 and 21 counts, an equivalent or higher efficacy was observed at the day 2, day 28 and day 35 counts. In the pivotal field study, the product was demonstrated to be 100% effective against *I. hexagonus* at all counts, which was higher than that observed against *I. ricinus*.

Moreover, according to PK/PD predicted plasma concentrations of sarolaner, Ixodes spp (*I. ricinus* and *I. scapularis*) are one of the more sensitive tick species to the acaricidal effect of sarolaner when administered orally to dogs. In addition, dose confirmatory study data suggests that the acaricidal effect of sarolaner following oral administration to dogs does not differ between *I. ricinus*, *I. hexagonus* or *I. scapularis*.

Based upon the totality of data provided, evidence would suggest that no difference in susceptibility to sarolaner between *I. hexagonus* and *I. ricinus* is to be expected when the product is administered topically to cats. In light of the above, the CVMP is of the opinion that the omission of a second dose confirmatory study investigating efficacy against *I. hexagonus* can be accepted in this instance.

Dermacentor reticulatus:

One dose determination study failed to demonstrate an acceptable level of immediate efficacy until after Day 7 and persistent efficacy was not consistently demonstrated, with percentage reduction falling to below 90% on study days 7, 28 and 49. However, two subsequent dose confirmation studies demonstrated an acceptable level of immediate efficacy (treatment) and persistent acaricidal efficacy for up to 28 days.

Two studies demonstrated immediate (treatment) efficacy against existing infestations (100% and 95.9%, respectively), and demonstrated persistent efficacy for up to 28 days (≥94.4% and ≥99.3%).

It can be accepted that the proposed claims for this tick species have been adequately supported by these dose confirmation studies.

Ear mites

Otodectes cynotis:

Results of two GCP-compliant dose confirmation studies were provided in support of the proposed claim. The same indication against *O. cynotis* is already approved for the mono-active (selamectin) product 'Stronghold'.

Cats were artificially infected and the final formulation was used and was applied on a single occasion in accordance with the recommendations included in the proposed SPC. Total live ear mites (larvae, nymphs and adults) were counted on study Day 30. Percentage efficacy was appropriately investigated using a controlled test.

Results of two studies showed an acceptable level of efficacy (≥90%) against *O. cynotis* ear infestation, 30 days after single topical application of the final formulation.

In line with a recommendation in the SPC for 'Stronghold', a recommendation was included in section 4.9 of the SPC to seek veterinary re-examination 30 days after treatment in order to determine whether a second administration is necessary.

Biting lice

Felicola subrostratus:

The results of a single dose confirmatory study were provided to support the efficacy of the product against *Felicola subrostratus* infestation. This study was conducted in 1999 and used a mono-active (selamectin) formulation with cats treated at the recommended treatment dose of 6 mg/kg. This study has been already reviewed and accepted by the CVMP as being adequate for the purpose of supporting an indication against *Felicola subrostratus* for the mono-active (selamectin) product 'Stronghold'.

Based on the totality of data provided in this application, it can be accepted that the addition of sarolaner in combination with selamectin does not reduce the bioavailability or pharmacodynamic effect of selamectin. Efficacy of the combination product against *Felicola subrostratus* can therefore be taken as given and no proprietary study data with the combination product is required for this indication.

Nematodes:

Roundworms *(Ancylostoma tubaeforme and Toxocara cati)*

Scientific advice from the CVMP addressed an approach to support efficacy against *Ancylostoma tubaeforme* and *Toxocara cati*.

The supporting data comprised *in vivo* data to justify the identification of *Ancylostoma tubaeforme* as the dose-limiting helminth in cats, the demonstration that there is no evidence of significant pharmacokinetic or pharmacodynamic interaction between sarolaner and selamectin and laboratory efficacy data against *Ancylostoma tubaeforme*.

A subsequent request for clarification from the applicant led to the provision of further scientific advice from the CVMP regarding the use of experimentally induced infections in dose confirmatory studies.

Justification for *Ancylostoma tubaeforme* as the dose-limiting helminth

The results of a total of 13 dose confirmatory studies (7 using the mono-active (selamectin) product and 6 using the proposed combination (sarolaner plus selamectin) product). The dose rate of selamectin is reported to have been the same in all of the studies (6 mg/kg - equivalent to the minimum exposure when administered at the RTD).

Results indicate lower percentage efficacy against *Ancylostoma tubaeforme* when compared with *Toxocara cati* in both natural and experimental infections, supporting the applicant's claim that *A. tubaeforme* would appear to be the target helminth in cats that is least-sensitive to selamectin. Whilst it is acknowledged that only one study investigating efficacy against *T. cati* has been performed using the combination formulation, results of that study concur with the findings from previous studies using the mono-active (selamectin) product.

It can therefore be concluded that *A. tubaeforme* would appear to be the least-sensitive target helminth in cats and is considered appropriate for use in dose confirmatory studies using the combination product to demonstrate efficacy against both *A. tubaeforme* and *T. cati*.

Justification for non-interference between sarolaner and selamectin

Results of an exploratory pharmacokinetic study indicate that the pharmacokinetic parameters C_{max}, AUC and $t_{1/2}$ were similar for selamectin whether administered alone or in combination with sarolaner. However, the extent to which this study can be reliably interpreted is limited due to the fact that it included only three animals per test group.

Plasma concentration/time profiles from another study provide evidence that mean plasma concentrations of sarolaner and selamectin do not appear to differ when administered alone or in combination with each other; that is, no evidence of interference between sarolaner and selamectin when administered in combination was observed. Plasma concentrations of selamectin did not appear to be reduced when administered in combination with sarolaner.

That said, the results of the pivotal pharmacokinetic suggest that the estimate of relative bioavailability of selamectin following topical application in combination with sarolaner was higher compared to topical application of selamectin alone. Whilst the results suggest that combination of selamectin with sarolaner may increase systemic bioavailability following topical application to cats, there is no evidence to suggest that bioavailability of selamectin is reduced when administered in combination with sarolaner.

Ancylostoma tubaeforme:

The existing mono-active (selamectin) product already includes an indication for the treatment of adult roundworms (*Toxocara cati*) and the treatment of adult intestinal hookworms (*Ancylostoma tubaeforme*).

Results of four GCP-compliant dose confirmatory studies have been provided in support of the proposed indication.

A GCP-compliant study using the final formulation and was conducted outside the EU (in US). The number of infective larvae (200) used to induce experimental infection satisfies VICH Topic GL20 requirements (100–300). A suitable number (8) of adequately infected cats (at least 43 adult *A. tubaeforme* per cat) were included in the study. Results of this study demonstrated only 84.1% efficacy against *A. tubaeforme* worms.

A second GCP-compliant study using the final formulation and was conducted outside the EU (in US). The number of infective larvae (150) used to induce experimental infection satisfies VICH Topic GL20 requirements (100–300). A suitable number (8) of adequately infected cats (at least 24 adult *A. tubaeforme* per cat) were included in the study. The candidate formulation was shown to be at least as efficacious (99.2%) against *A. tubaeforme* when compared with the control formulation that included selamectin alone (98.6% efficacy based on geometric means). Based on the results of this study, it can be accepted that there was no evidence of interaction of sarolaner on the efficacy of selamectin against *A. tubaeforme* at the minimum exposure (6 mg selamectin/kg bodyweight) when the product is administered at the RTD.

A GCP-compliant study using the final formulation and was conducted within the EU (Ireland). The number of infective larvae (150) used to induce experimental infection satisfies VICH Topic GL20 requirements (100–300). It can be accepted that an adequate level of infection was achieved in this study. The isolate used in this study is described as originating from Albania and subsequently maintained under laboratory conditions in Germany. Consequently, it can be accepted as being adequately representative of the target helminth in Europe. Results of this study demonstrated 94.3% efficacy against *A. tubaeforme* worms.

A fourth GCP-compliant study using the final formulation and was conducted outside the EU (in South Africa). The number of infective larvae (150) used to induce experimental infection satisfies VICH Topic GL20 requirements (100–300). A suitable number (8) of adequately infected cats (at least 12 adult *A. tubaeforme* per cat) were included in the study. Results of this study demonstrated only 42.11% efficacy against *A. tubaeforme* worms.

In summary, results from two of dose confirmatory studies support efficacy of the candidate formulation against adult *A. tubaeforme* whereas two do not.

The findings from the South African study are unexpected and do not support previous study findings. The isolate used is the same as that used in the study in Ireland where an acceptable level of efficacy was demonstrated. Whilst the interval between inoculation and worm enumeration may have contributed to the low efficacy findings, it is noted that an almost identical (one day longer) interval between inoculation and worm enumeration was used in the second US study, yet an acceptable level of efficacy (99.2%) was demonstrated in that study.

Review of relevant study data suggests that the most likely explanation for this difference was an inadequate interval between inoculation and treatment for the EU isolate used in the study in South Africa and the study in Ireland.

The interval between inoculation and treatment was longer in the Ireland study (42 days after inoculation) compared with the South African study (30 days after inoculation).

Further, a notable difference in faecal egg output from Day -4 (pre-treatment) to Day 10 (necropsy) between studies, with a much greater increase (135%) observed in the study in South Africa compared to 17% in the study in Ireland was evident. In addition, maximum number of infected cats and maximal mean faecal egg output in the study in Ireland did not occur until 34 and 39 days post inoculation, respectively, suggesting that the EU isolate used in the study in Ireland (the same isolate used in the

study in South Africa) requires more than 30 days for maturation into ovipositioning adults. Whilst a similar interval between inoculation and treatment was used in the second study in the US, it is accepted that the isolate used in that study was different.

Consequently, it was accepted that the design of the study in South Africa (in terms of interval between inoculation and treatment) was inappropriate (too-short) for the isolate used, accounting for the low efficacy observed in that particular study and it was concluded the product has been shown to be suitably efficacious against *Ancylostoma tubaeforme* (and consequently *Toxocara cati*). The existing mono-active (selamectin) product already includes an indication for the treatment of adult intestinal hookworms (*Ancylostoma tubaeforme*) and study data provided to date indicates that sarolaner does not reduce bioavailability of selamectin.

Heartworm *(Dirofilaria immitis)*

The mono-active (selamectin) product is already approved for the prevention of heartworm disease caused by *Dirofilaria immitis* infections.

The efficacy of the candidate formulation at the minimal dose level of 6.0 mg/kg selamectin in combination with sarolaner was evaluated against induced *Dirofilaria immitis* infections in cats in one exploratory non-interference study and in one pivotal laboratory dose confirmation study.

The exploratory study was non-GCP compliant and conducted outside the EU (in US), using a different formulation to that intended for marketing. The dose rate of sarolaner administered was twice the minimum dose rate proposed for the candidate formulation. The number of infective larvae (100) used to induce experimental infection satisfies VICH Topic GL20 requirements (30–100). In this study, only 5 out of 10 cats in the control group had a minimum of 5 (range 0–23) adult *D. immitis* worms detected at necropsy i.e. less than the required minimum number of adequately infected study animals.

Results from this study suggest that the test formulation administered on three occasions at monthly intervals was 100% effective in reducing adult *D. immitis* counts.

The pivotal study was GCP-compliant and conducted outside the EU (in the US), using the final formulation. The candidate formulation was administered in accordance with the recommendations included in the proposed SPC. The number of infective larvae (100) used to induce experimental infection satisfies VICH Topic GL20 requirements (30–100) and the required minimum number of adequately infected study animals were included.

Results from this study suggest that the test formulation was 100% effective in reducing development of L_3 larvae to adults in artificially infected animals after treatment application on a single or three repeated occasions. However, as there was no re-challenge aspect to this study, insufficient data has been provided to conclude specifically on the duration of effect and thus the prevention is only indicated for one month.

No field study data has been provided for this indication. Given the scientific advice provided by the CVMP in respect of the proposed extrapolation of efficacy data against nematodes from the mono-active product to the combination product, the same approach can be accepted for the proposed indication against *D. immitis*.

Both studies demonstrated an acceptable level of efficacy against *D. immitis* when administered monthly on three consecutive occasions. Study data also indicates that sarolaner does not reduce bioavailability of selamectin or reduce its efficacy against *D. immitis*. Consequently, the proposed indication (Prevention of heartworm disease caused by *Dirofilaria immitis* with monthly administration) is considered to have been adequately supported.

Target animal tolerance

The recommended minimum treatment dose is 1 mg sarolaner/kg and 6 mg selamectin/kg bodyweight applied topically once monthly.

Based on the widest proposed dose band, the maximum possible exposure dose is 2 mg sarolaner/kg and 12 mg selamectin/kg bodyweight. Consequently, the applicant has investigated target animal tolerance with reference to the upper possible exposure dose rates.

The following studies have been provided in support of target animal tolerance:

Selamectin alone:

A topical margin of safety study (MOS) in kittens,

An oral safety study in cats,

A safety study in heartworm positive cats.

The proposed dose rate of 6 mg selamectin/kg in this combination product is identical to that approved for the existing mono-active (selamectin) product. All three studies have been previously reviewed by the CVMP in the context of the application for the mono-active (selamectin) product 'Stronghold' (EMEA/V/C/000050). It can be accepted that target animal tolerance to selamectin when administered on its own to cats has been adequately supported.

Sarolaner alone:

An exploratory topical escalating dose tolerance study investigating tolerance to three doses (10x, 20x and 25x maximum exposure at the RTD) has been provided. Based upon the data provided, acceptable tolerance to a dose of 50 mg sarolaner/kg bodyweight was not shown, however, tolerance to dose rates of up to 40 mg sarolaner/kg bodyweight (20x maximum exposure at the RTD) appeared acceptable.

Sarolaner plus selamectin in combination:

Two GLP-compliant and two non GLP-compliant tolerance studies were provided. In general, the studies included study animals of a suitably representative age and breed. The formulations used were either the final formulation or one sufficiently representative of it.

The pivotal TAS study was a GLP-compliant, repeat-dose margin of safety study, investigated tolerance to the final formulation following monthly topical application at 1x, 3x, 3.75x and 5x the maximum exposure from the RTD on eight consecutive occasions in cats from 8 weeks of age.

One animal (3.75xRTD group) died on study day 115 as a result of haemorrhage in multiple tissues (thought to be due to a low platelet count (cause unknown)). Although the possibility of a drug-induced thrombocytopoenic-type event cannot be excluded, it can be accepted that the platelet count for this particular animal remained within the normal reference range for at least 83 days following monthly application of the product and although a reduction in mean platelet counts was observed in the control group, a similar reduction was not apparent in the 1xRTD group. Furthermore, on study day 210, the least square mean values for platelet counts were within the laboratory reference range for all treatment groups. It was accepted that there is insufficient evidence to suggest a direct causal effect of the candidate formulation for this particular finding.

In a GLP-compliant oral tolerance study the safety to the final formulation was investigated following oral administration (by syringe) of the maximum possible exposure from the RTD (2 mg sarolaner and 10 mg selamectin per kg bodyweight). Findings from this study suggest that following oral ingestion of

the full RTD, cats may exhibit reduced food consumption, salivation, emesis, soft faeces and less frequently, tremor or reduced motor activity. However, it was evident from PK data that the animal reported with tremor had in fact the lowest systemic exposure to the formulation. Consequently, it was accepted that this observation was most likely to be associated with repeated emesis rather than a true symptom of toxicity.

The SPC was revised to include information on the tolerance findings given that oral ingestion of the product may arise following self/mutual grooming. No margin of safety was demonstrated following oral ingestion of the full dose.

In one non-GLP compliant exploratory repeat-dose margin of safety study. Dose proportionality was not confirmed. Lower haematocrit, red blood cell (RBC) count and haemoglobin concentration in male animals in the 3.75x and 5xRTD groups during the latter half of the study was noted. Given the limited number of study animals and the fact that similar observations were not reported in the females, no firm conclusions can be drawn on this particular finding, although similar observations in terms of reduction of RBC were also observed in another study.

Another non-GLP compliant exploratory study in adult cats investigated tolerance to different dose rates (5, 15, 25 and 50 mg/kg – equivalent to 2.5x, 7.5x, 12.5x and 25x the maximum possible exposure at the RTD) of sarolaner when applied topically, on a single occasion, in combination with a fixed dose of selamectin (6 mg/kg). One animal (out of two) from each of the treatment groups (2.5x, 7.5x, 12.5x) was observed to have a statistically significantly reduced RBC count (below the minimum reference value) six days after application of the test article.

Although similar findings of a possible effect of the product on reduction of RBC and/or platelet count were reported, these effects were reported in non-GCP compliant exploratory acute and repeat-dose tolerance studies with a limited number of study animals. However, similar observations were not reported in the pivotal tolerance study that included a larger study population, or in safety study. It was therefore concluded that there is insufficient evidence to suggest any particular effect of the candidate formulation on feline red cell indices.

In addition to the above, safety data from the dose confirmatory and clinical field studies were presented. From those studies, the candidate formulation appears to have been well tolerated in general. However, similar findings were reported from a number of those studies which include:

- reactions of a cosmetic nature (temporary white deposits, greasiness, spiking/stiffness and matting of fur) were reported which in general resolved within 5 days;
- alopecia at the application site;
- erythema at the application site;
- mild and transient pruritus;
- drooling saliva.

The information proposed for inclusion in the SPC is in line with the study findings. Information on reactions of a cosmetic nature that are to be expected are included as well as information relating to clinical symptoms observed following accidental oral ingestion (via self-grooming) of the product.

Studies to evaluate the effects of the sarolaner/selamectin combination in pregnant/lactating cats or cats intended for breeding have not been conducted and section 4.7 of the SPC has been worded accordingly.

The information proposed for inclusion in section 4.10 (overdose) is considered appropriate and

reflects the findings of the pivotal target animal tolerance study which investigated overdose of up to 5x the maximum exposure from the RTD following monthly topical application on eight consecutive occasions to kittens from 8 weeks of age.

Of particular note in some of these (and other studies) was the detection of sarolaner and selamectin in plasma of control (untreated) study animals. Concentrations of sarolaner and selamectin detected were found to be considerably lower than those in the 1xRTD groups and the applicant believes that this resulted from possible cross-contamination of study animals. Despite specific measures being taken in some studies to minimise/eliminate possible cross-contamination, concentrations of both active substances were still detected in the plasma of control animals, albeit at lower concentrations. That said, this finding is considered to have little significance in terms of target animal tolerance due to the comparatively low concentrations detected.

Clinical field trials

Two GCP-compliant field trials were conducted to confirm the efficacy findings from the dose confirmation studies in respect of ticks and fleas.

The first one was a GCP-compliant field study to demonstrate the efficacy and safety of a combination of selamectin and sarolaner administered topically three times at monthly intervals, for the treatment and prevention of natural infestations of fleas on cats. The study was conducted in four European countries (France, Germany, Hungary and Italy) at selected veterinary practices and therefore meets the guideline criteria of having been conducted in at least two different geographic regions. Study animals included a sufficient variety of age, breed, body weight and hair-coat length to be considered sufficiently representative of the target animal population.

The final formulation was applied in accordance with the recommendations included in the proposed SPC. A positive control group was included. The number of study animals was calculated based upon a non-inferiority design approach to demonstrating efficacy when compared with the control product Advocate. The comparator product (Advocate) is authorised for the treatment and prevention of flea infestation (*Ctenocephalides felis*) and can be used as part of a treatment strategy for flea allergy dermatitis in cats.

Ctenocephalides felis was the only species identified on study animals.

Compared to pre-treatment flea counts, >95% efficacy of the candidate formulation was observed at each of the post-treatment flea counts (days 14, 30, 60 and 90), confirming the level of persistent efficacy demonstrated in the dose confirmatory studies. Non-inferiority of the candidate formulation to Advocate was demonstrated at all time-points.

Fourteen cats administered the candidate formulation presented with clinical signs of flea allergic dermatitis (alopecia, dermatitis, erythema, pruritus and scaling) which are reported to have improved following treatment with the candidate formulation.

Two cats administered the candidate formulation are reported to have had mild to moderate alopecia at the application site and a further 6 cats to have had mild and transient pruritus recorded after treatment application.

It can be accepted from the results of this study that the candidate formulation was generally well tolerated when administered on three occasions at monthly intervals to healthy cats aged ≥3 months. The percentage reduction in fleas exceeded ≥95% at all flea count time-points.

The second was a GCP-compliant field study to demonstrate the efficacy and safety of a combination

of selamectin and sarolaner administered topically three times at monthly intervals, for the treatment and prevention of natural infestations of ticks on cats. The study was conducted in four European countries (France, Germany, Hungary and Italy) at selected veterinary practices and therefore meets the guideline criteria of having been conducted in at least two different geographic regions. Study animals included a sufficient variety of age, breed, bodyweight and hair-coat length to be considered sufficiently representative of the target animal population.

The final formulation was applied in accordance with the recommendations included in the proposed SPC.

A positive control group was included. The number of study animals was calculated based upon a non-inferiority design approach to demonstrating efficacy when compared with a spot-on product containing fipronil. The comparator product is authorised for the treatment and prevention of ticks in cats.

The majority of cats were infested with *I. ricinus* (74.8%) and *R. sanguineus* (22.1%) whereas only 5.3% and 7.6% of cats were infested with *D. reticulatus* and *I. hexagonus*, respectively.

Compared to pre-treatment tick counts, >90% efficacy of the product was observed at each of the post-treatment tick counts (days 14, 30, 60 and 90) based on live tick counts. Non-inferiority of the candidate formulation to the comparator product was demonstrated at all time-points.

Four cats administered the candidate formulation are reported to have had reactions - pruritus (n=2; pruritus lasting no longer than 15 minutes in one cat on each treatment day and only following initial treatment application in the other cat), drooling saliva (n=1) and localised alopecia at the application site (n=1).

It can be accepted from the results of this study that the candidate formulation was generally well tolerated when administered on three occasions at monthly intervals to healthy cats aged ≥3.5 months and that the percentage reduction in ticks exceeded ≥90% at all tick count time-points.

Overall conclusion on efficacy

Pharmacodynamics:

Selamectin is a parasiticide substance with killing activity against fleas, mites, lice and nematodes. Sarolaner is an ectoparasiticide substance with killing activity against fleas and ticks.

The mode of action has been sufficiently described. Selamectin is a semi-synthetic avermectin which exerts its effect through potentiation and/or agonist action at invertebrate glutamate-gated chloride channels. The primary mode of action of sarolaner in insects and acarines is functional blockade of ligand-gated chloride channels (GABA-receptors and glutamate-receptors).

Both substances exhibit preferential selectivity of invertebrate over human channels and, consequently, higher functional potency to block insect/acarine receptors compared to mammalian receptors.

Resistance:

The risk of resistance development seems unlikely and not highly critical for a product used in individual companion animals.

The risk of resistance development with regard to the use of this product is currently not expected to pose a risk to the population.

Given the different sites of action on chloride channels between selamectin and sarolaner, the combination of both active substances in this combination product is not expected to increase the risk for resistance development in any of the proposed target parasites.

Pharmacokinetics:

The pharmacodynamic and pharmacokinetic characteristics of selamectin and sarolaner are generally well documented and have been satisfactorily evaluated in the target species.

There is large between animal variability in pharmacokinetic parameters, in particular for selamectin. Plasma binding of sarolaner in cats was found to be high (99.9%).

Both selamectin and sarolaner are well absorbed with bioavailability mean values of 40.5% and 57.9%, respectively and distribute systemically. In cats, selamectin and sarolaner are low clearance compounds with long half-life values, 12.5 days and 41.5 days respectively, following topical administration.

In cats, the primary route of selamectin elimination is in faeces and the majority is parent compound. The primary route of elimination for sarolaner is biliary elimination of parent sarolaner, with contributions by metabolic clearance.

Dose determination:

The proposed dose rate of 1 mg sarolaner/kg bodyweight is considered to have been sufficiently supported for the purpose of carrying forward to pivotal dose confirmatory and field studies. Doses of 0.75 and 0.5 mg/kg were shown to provide reduced efficacy whereas a dose of 2 mg/kg was not shown to provide additional benefit.

The dose rate of selamectin (6 mg/kg) is the same as that already approved for the applicant's mono-active product 'Stronghold'. Given that PK data has shown that sarolaner does not negatively reduce systemic bioavailability of selamectin when administered in combination, the same dose rate of selamectin is considered appropriate for this combination product.

Tolerance:

The candidate formulation was well-tolerated in a clinical field study at the recommended dose of 1 mg sarolaner/kg and 6 mg selamectin/kg bodyweight applied topically once monthly.

In the TAS study, the product was shown to be well-tolerated in doses up to 5x the recommended dose.

Reactions of a cosmetic nature are to be expected following topical application of the product.

An oral tolerance study suggests that cats may exhibit reduced food consumption, salivation, emesis, soft faeces and less frequently, tremor (related to emesis) or reduced motor activity.

Studies to evaluate the effects of the sarolaner/selamectin combination in pregnant/lactating cats or cats intended for breeding have not been conducted.

Efficacy:

Ectoparasites:

Fleas

Results of two-dose confirmatory studies support immediate and persistent pulicidal efficacy of the product. The proposed treatment and prevention claim against *Ctenocephalides* spp. for 35 days is

considered to have been adequately supported.

Another study was conducted to investigate the speed of kill against adult *C. felis*. An acceptable level of efficacy is achieved 24 hours after treatment application and re-infestation for 35 days. The proposed claim for the product to kill adult fleas before they lay eggs for 5 weeks is considered to have been adequately supported.

Ticks

The results from seven GCP-compliant dose confirmatory studies investigating acaricidal effect were provided.

Immediate efficacy (treatment) and persistent acaricidal efficacy against *I. ricinus* for 35 days is considered to have been adequately supported. A claim for onset of acaricidal efficacy within 24 hours of attachment during the month following product application has been adequately supported and a statement was included in section 5.1 of the SPC.

Immediate efficacy (treatment) and persistent acaricidal efficacy against *I. hexagonus* for 35 days has only been adequately supported by one dose confirmatory study. However, the omission of a second dose confirmatory study could be accepted in this instance given the additional supportive data/argumentation provided.

Immediate efficacy (treatment) and persistent acaricidal efficacy against *R. sanguineus* and *D. reticulatus* for 28 days is considered to have been adequately supported. Information relating to the need for attachment and feeding of ticks in order to be exposed to sarolaner was included in section 4.2 of the SPC.

Ear mites *(Otodectes cynotis)*

Results of two GCP-compliant dose confirmation studies were provided in support of the proposed claim. An acceptable level of efficacy was demonstrated against *O. cynotis* ear infestation 30 days after single topical application. The SPC includes a recommendation to seek veterinary re-examination 30 days after treatment to determine whether a second administration is necessary.

Biting lice *(Felicola subrostratus)*

Results of a single dose confirmatory study investigating efficacy against *Felicola subrostratus* infestation was provided and which was already accepted by the CVMP for the purpose of demonstrating efficacy of 'Stronghold' against *F. subrostratus*. The proposed claim is considered acceptable.

Nematodes:

Roundworms *(Ancylostoma tubaeforme and Toxocara cati)*

Results of four GCP-compliant dose confirmatory studies have been provided in support of the proposed indication. Two studies support efficacy of the candidate formulation against adult *A. tubaeforme* whereas two do not. The findings from one study (42.11% efficacy against *A. tubaeforme*) are unexpected and do not support previous study findings.

Review of relevant study data suggests that the most likely explanation for this difference was an inadequate interval between inoculation and treatment for the EU isolate (the interval between inoculation and treatment was longer in the study in Ireland (42 days after inoculation) compared with the study in South Africa (30 days after inoculation)) and consequently, it was accepted that the design of the latter (in terms of interval between inoculation and treatment) was inappropriate (too short) for the isolate used, accounting for the low efficacy observed in that particular study. It was concluded that

the product has been shown to be suitably efficacious against *Ancylostoma tubaeforme* (and consequently *Toxocara cati*).

Heartworm *(Dirofilaria immitis)*

Results of one exploratory non-interference study and one pivotal laboratory dose confirmation study were provided. Both studies demonstrated an acceptable level of efficacy against *D. immitis* when administered monthly on three consecutive occasions.

Two GCP-compliant field studies were provided to confirm the efficacy results observed in the dose confirmatory studies against fleas and ticks. Results from the field studies are considered to have supported the efficacy findings from the dose confirmatory studies and demonstrated an acceptable level of tolerance in the target species.

In conclusion, Stronghold Plus, which contains the combination of sarolaner and selamectin, provides efficacy against ectoparasites (treatment and prevention of flea infestations (*Ctenocephalides* spp.); treatment of tick infestations (*Ixodes ricinus*, *Ixodes hexagonus*, *Dermacentor reticulatus* and *Rhipicephalus sanguineus*); treatment of ear mites (*Otodectes cynotis*); treatment of biting lice infestations (*Felicola subrostratus*)) and nematodes (treatment of adult roundworms (*Toxocara cati*); adult intestinal hookworms (*Ancylostoma tubaeforme*); prevention of heartworm disease caused by *Dirofilaria immitis*) with a monthly administration. The treatment dose has been confirmed. The non-interference of the active substances in terms of both safety and efficacy has been adequately demonstrated. The treatment can be repeated at monthly intervals depending on the indication and epidemiological situation.

Part 5 – Benefit-risk assessment

Introduction

Stronghold Plus is a spot-on solution containing a combination of selamectin and sarolaner, both of which are well-known active substances. The product is presented in three different strengths of unit dose pipettes.

The product is a fixed combination product. It contains a new active substance as it contains a new fixed combination of two active substances previously authorised within EU.

The product is intended for use in cats with, or at risk from, mixed parasitic infestations by ticks and fleas, lice, mites, gastrointestinal nematodes or heartworm. The product is exclusively indicated when use against ticks and one or more of the other target parasites is indicated at the same time. The recommended minimum treatment dose is 6 mg selamectin/kg and 1 mg sarolaner/kg.

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

Benefit assessment

Direct therapeutic benefit

The benefit of Stronghold Plus was demonstrated in a number of controlled studies where its efficacy in cats with, or at risk from, mixed parasitic infestations by ticks and fleas, lice, mites, gastrointestinal nematodes or heartworm was suitably demonstrated. The veterinary medicinal product is exclusively indicated when use against ticks and one or more of the other target parasites

is indicated at the same time.

Additional benefits

Stronghold Plus spot-on solution is a fixed combination product which reduces the number and variety of products to be administered (by different routes of administration) to the cat to cover the same spectrum of activity and so reduces the need for their handling. This can also be considered to be an animal welfare benefit. The product is easy to apply by the cat owner and so increases user convenience.

Risk assessment

Main potential risks have been identified as follows:

Quality:

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

Safety:

Risks for the target animal:

An oral tolerance study suggests that cats may exhibit reduced food consumption, salivation, emesis, soft faeces and less frequently, tremor (associated with emesis) or reduced motor activity.

Administration of Stronghold Plus (topically) in accordance with the SPC recommendations is generally well tolerated. Main reported adverse reactions include mild and transient pruritus at the application site. The potential for other adverse effects such as mild to moderate alopecia at the application site, erythema and drooling cannot be excluded and the SPC section 4.6 (and other PI) include this information.

Studies to evaluate the effects of the sarolaner/selamectin combination in pregnant/lactating cats or cats intended for breeding have not been conducted.

Risk for the user:

The user safety assessment provided has been conducted in accordance with relevant CVMP guidance. Based on the information provided, the exposure scenarios for which a risk was identified were ocular irritation and a risk to children associated with ingestion of the final product. The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations.

Risk for the environment:

Stronghold Plus is not expected to pose a risk for the environment when used according to the SPC recommendations.

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.

Evaluation of the benefit-risk balance

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.

The benefit-risk balance of the application is positive.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Stronghold Plus 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.