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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Simparica Trio (EMEA/V/C/004846/0000)

INN: sarolaner / moxidectin / pyrantel

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 17 July 2018 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Simparica Trio, 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 15 June 2017 as Simparica Trio contains a combination of three active substances (sarolaner, moxidectin and pyrantel embonate), one of which (sarolaner) was not authorised in a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004.

The applicant applied for the following indications: for dogs with, or at risk from, mixed external and internal parasitic infestations. The veterinary medicinal product is exclusively indicated when use against ticks or fleas and gastrointestinal nematodes is indicated at the same time. The veterinary medicinal product also provides concurrent efficacy for the prevention of heartworm disease and angiostrongylosis.

Ectoparasites:

- For the treatment of tick infestations. The veterinary medicinal product has immediate and persistent tick killing activity for 5 weeks against *Ixodes hexagonus*, *Ixodes ricinus* and *Rhipicephalus sanguineus* and for 4 weeks against *Dermacentor reticulatus*;
- For the treatment of flea infestations (*Ctenocephalides felis* and *Ctenocephalides canis*). The veterinary medicinal product has immediate and persistent flea killing activity against new infestations for 5 weeks;
- The veterinary medicinal product can be used as part of a treatment strategy for the control of flea allergy dermatitis (FAD).

Gastrointestinal nematodes:

For the treatment of gastrointestinal roundworm and hookworm infections:

- *Toxocara canis* immature adults (L5) and adults;
- *Ancylostoma caninum* L4 larvae, immature adults (L5) and adults;
- *Toxascaris leonina* adults;
- *Uncinaria stenocephala* adults.

Other nematodes:

- For the prevention of heartworm disease (*Dirofilaria immitis*);
- For the prevention of angiostrongylosis by reducing the level of infection with immature adult (L5) stages of *Angiostrongylus vasorum*.

The active substances of Simparica Trio are sarolaner, moxidectin and pyrantel (as embonate). Sarolaner is an acaricide and insecticide belonging to the isoxazoline family, which blocks GABA (gamma amino butyric acid)- and glutamate-gated chloride channels in the central nervous system of insects and acarines, preventing the uptake of chloride ions by GABA- and glutamate-gated ion channels and thus resulting in increased nerve stimulation and death of the target parasite. Moxidectin is a second generation macrocyclic lactone of the milbemycin family; its principal mode of action is interfering with neuromuscular transmission of the glutamate-gated chloride channels and, to a lesser extent, of GABA-gated channels, thus leading to the opening of the chloride channels on the

postsynaptic junction to allow the inflow of chloride ions and this results in flaccid paralysis and eventual death of parasites exposed to the drug. Pyrantel is a nicotinic acetylcholine (ACh) channel receptor (nAChR) agonist; following receptor binding, the channel opens to allow the influx of cations resulting in a depolarization and excitatory effects on nematode muscle, ultimately leading to spastic paralysis of the worm and death. The target species is dogs.

Simparica Trio chewable tablets are available in six different strengths containing: 3 mg sarolaner / 0.06 mg moxidectin / 12.5 mg pyrantel (as embonate), 6 mg sarolaner / 0.12 mg moxidectin / 25 mg pyrantel (as embonate), 12 mg sarolaner / 0.24 mg moxidectin / 50 mg pyrantel (as embonate), 24 mg sarolaner / 0.48 mg moxidectin / 100 mg pyrantel (as embonate), 48 mg sarolaner / 0.96 mg moxidectin / 200 mg pyrantel (as embonate) or 72 mg sarolaner / 1.44 mg moxidectin / 300 mg pyrantel (as embonate).

All strengths are available in packs containing 1, 3 or 6 tablets.

The rapporteur appointed is Rory Breathnach and the co-rapporteur is Bruno Urbain.

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

On 18 July 2019, the CVMP adopted an opinion and CVMP assessment report.

On 17 September 2019, the European Commission adopted a Commission Decision granting the marketing authorisation for Simparica Trio.

Scientific advice

Not applicable.

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, as amended. Based on the information provided, the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture and packaging are performed in the EEA. Batch release takes place at Corden Pharma GmbH, Germany. The site has a GMP certificate issued on 23 May 2018, which confirms the date of the last inspection and shows that the site is authorised for the manufacture and batch release of such veterinary dosage forms. The certificate is available on EudraGMP.

GMP declarations for the active substance manufacturing sites were provided from the Qualified Person (QP) at the EU batch release site. The declarations were based on audits of the sites within the last three years.

Overall conclusions on administrative particulars

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

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

Part 2 - Quality

Composition

Simparica Trio chewable tablets are a fixed dose combination chewable tablet of the three active substances sarolaner, moxidectin and pyrantel embonate. Simparica Trio chewable tablets are available in six different strengths containing 3/0.06/12.5 mg, 6/0.12/25 mg, 12/0.24/50 mg, 24/0.48/100 mg, 48/0.96/200 mg or 72/1.44/300 mg of sarolaner/moxidectin/pyrantel (as embonate). The tablets are reddish brown coloured pentagon shaped tablets with rounded edges and have the sarolaner strength descriptor debossed on one face (e.g. "3" on the 3 mg strength tablets, etc.) of the tablet. The tablets are packaged in aluminium-aluminium cold form blisters.

The six tablet strengths are manufactured from a common blend and contain the excipients hydroxypropyl cellulose, hypromellose, meglumine, butylhydroxytoluene (BHT), lactose monohydrate, spray dried pork liver powder, hydrolysed vegetable protein, maize starch, confectioner's sugar, wheat germ, calcium hydrogen phosphate anhydrous, glucose liquid (81.5% solids), gelatin Type A, sodium starch glycolate (type A), pigment blend 018, silica colloidal anhydrous and magnesium stearate.

Containers

Aluminium-aluminium cold formed blisters are used to package the finished product. The tablets are packaged in the blisters in configurations of 1, 3 or 6 tablets per blister. Blister cards will then be secondary packaged in cartons. The product contact layer is PVC which complies with Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food and also with European Pharmacopoeia (Ph. Eur.) 3.1.11.

Development pharmaceutics

All excipients are either well known pharmaceutical ingredients compliant with Ph. Eur. standards, or they have already been approved in the EU for use in veterinary medicinal products. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SPC. Drug-drug compatibility studies with the three active substances and drug-excipient compatibility studies were conducted with commonly used tableting excipients to inform the selection of excipients. The formulation used during clinical studies was slightly different to that intended for marketing. Batches of Simparica Trio chewable tablets were manufactured for clinical studies in 2015 and 2016 while some of the composition and process optimisation studies were still underway. Therefore, there are some minor differences between the batches used in clinical studies and those proposed for commercial production. These differences are in the moxidectin granulation and in the sarolaner-pyrantel granulation but the extragranular components remain the same throughout all clinical and commercial batches. In terms of % w/w per tablet, these changes are very small comprising less than 2% of the total tablet weight. The change to the sarolaner-pyrantel granulation

results in a reduction in sarolaner content, with a corresponding adjustment of lactose concentration and again very small in terms of % w/w. Although the differences in formulation appear relatively minor, comparative dissolution studies did not result in acceptable similarity factor (f2) results across all studies. In order to demonstrate the absence of an *in vivo* impact of observed differences in f2 values and differences in formulations, the applicant has provided the results of an *in vivo* bioequivalence study for moxidectin and sarolaner and an *in vitro* study for pyrantel. The results of the *in vivo* bioequivalence study demonstrated a similar rate and extent of absorption of moxidectin and sarolaner between formulations. Given the low oral bioavailability of pyrantel *in vivo* bioequivalence was not investigated and the applicant has provided *in vitro* dissolution study data to demonstrate similarity between the 2016 Clinical Supplies and the final commercial formulation. In addition to the f2 comparison, the applicant used a tolerance limit approach (Martinez and Zhao, 2018). The tolerance approach involves the determination of 95% confidence intervals for the 99th percentile at each time point for the reference formulation dissolution data. From the tolerance intervals (Q), two limits are determined: S1: Q±5% and S2: Q±15% (i.e., add to the upper limits, subtract from the lower limits). To conclude similarity, at most 1 tablet can exceed the S1 limits and can exceed S1 at multiple timepoints but no tablet can exceed the S2 limits at any time point. Whilst such an approach is not specifically mentioned in the CVMP guidelines, it is noted that the guidelines suggest that alternative methods to the $f2$ statistic to demonstrate dissolution similarity are considered acceptable, if statistically valid and satisfactorily justified. Similarity between the 2016 Clinical Supplies and the final formulation intended to be marketed was demonstrated using this approach. The data provided is considered adequate to conclude that, despite the low f2 values observed, the minor difference between the 2016 Clinical formulation and the final formulation will not have any significant clinical relevance and that the safety and efficacy aspects of the studies conducted using the 2016 Clinical formulation may be extrapolated to the final formulation.

Development of the dissolution method occurred alongside formulation and process development and underwent 3 iterations identified as Generation 1, Generation 2 and Generation 3. In the Generation 1 and 2 methods, two different dissolution methods were used, one for moxidectin and the other for sarolaner and pyrantel. The Generation 3 dissolution method is a single method for all three active substances. The method has been demonstrated to be sufficiently discriminatory for all three actives.

Method of manufacture

A single tablet blend is used for manufacturing of all tablet strengths of Simparica Trio chewable tablets. Manufacture of the tablets involves manufacture of granules containing the moxidectin active substance and a second granulation containing the sarolaner and pyrantel active substances. A large portion of the excipients are also granulated separately in 'palatable base granulation'. These three granulations are then blended with the extragranular excipients. Manufacture of the moxidectin granules and the palatable base granules are carried out using fluid bed granulation. The sarolaner-pyrantel granulation is manufactured using a high shear mixer granulator. A step by step description of the process is contained within the dossier. The various tableting processes employed are considered to be standard manufacturing processes and appropriate in-process controls are defined for the various manufacturing stages. Holding periods are defined for the granulations and the lubricated blend. A hold time for the bulk tablets is also defined. In accordance with the Note for Guidance on start of shelf-life of the finished dosage form (EMEA/CVMP/453/01), the start of shelf life of the product has been taken as the date of manufacture of the individual active ingredient granulations as this is the date that the first step is performed involving combining the active ingredient with other ingredients.

The manufacture of the palatable base granules and chewable tablets uses conventional tableting processes and information on the process development of the chewable tablet process provided is

adequate. Therefore, provision of process validation data in the dossier is not required for these processes. A validation protocol of the manufacturing process for commercial scale batches in accordance with Annex I of the Guideline on process validation for finished products - information and data to be provided in regulatory submissions (EMA/CHMP/CVMP/QWP/BWP/70278/2012-Rev 1) is provided.

Control of starting materials

Active substance

Moxidectin for veterinary use

Moxidectin for veterinary use with butylhydroxytoluene is supported by a Certificate of Suitability of the European Pharmacopoeia (CEP), copies of which have been provided within the application. The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability. An active substance specification, as applied at the dosage form site, including any additional tests as listed on the certificates of suitability and a test and specification for microbial quality to cover all suppliers for the active substance moxidectin for veterinary use is provided. Batch analysis data of the active substance is provided.

A solution of moxidectin is used in the manufacturing process and, therefore, the physico-chemical characteristics of this active substance are not critical to this dosage form.

Stability data on 3 full scale batches of active substance stored for up to 18 months under long term conditions at 25 °C/60% RH and for up to 6 months under accelerated conditions at 40 °C/75% RH according to the VICH guidelines were provided from one supplier. The samples were tested using all relevant test methods as described in the Ph. Eur. and CEP. The data provided support a retest period of 18 months at 25 °C/60% RH storage conditions.

Stability data on 3 full scale batches of active substance stored for up to 36 months under long term conditions at 2-8 °C ambient RH and for up to 36 months under accelerated conditions at 25 °C/60% RH according to the VICH guidelines were provided from the second supplier. The samples were tested for appearance, moxidectin content, related substances, BHT and water content. The data provided support a retest period of 36 months at 25 °C/60% RH storage conditions.

Pyrantel embonate

The information on the active substance is provided according to the Active Substance Master File (ASMF) procedure. The data provided in the ASMF is in accordance with current guidelines. An active substance specification complying with Ph. Eur., as applied at the dosage form site is provided.

No eritro/treo or optical isomerism is possible however, cis/trans isomerism is a possibility. Photo-isomerization of pyrantel embonate was demonstrated following exposure to direct sunlight. One of the isomers is controlled as an impurity on the active substance specification. DSC and X-ray spectroscopy studies show a consistent single polymorphic form arising from the synthetic process for 3 batches.

Batch analysis data is provided from the active substance supplier for 3 batches of the active substance. All results are well within specification. Batch data from the dosage form site is provided demonstrating compliance with the specification.

Stability data on 14 full scale batches of the active substance stored for up to 60 months under long term conditions at 25 °C/60% RH and on 3 full scale batches stored for up to 13 months under accelerated conditions at 40 °C/75% RH are provided. The samples were packed in commercial

packaging, double polyethylene bags within a cardboard outer package and tested in accordance with the specification and methods as described in the ASMF. The data provided support a retest period of 5 years at 25 °C/60% RH storage conditions for the active substance.

Sarolaner

The active substance sarolaner is a member of the isoxazoline class of parasiticides. It is not monographed in the Ph. Eur. and full data is provided within the dossier. Sarolaner is manufactured in a four step synthetic process using three starting materials. The active substance was previously approved through EMEA/V/C/3991/0000 'Simparica 5 mg, 10 mg, 20 mg, 40 mg, 80 mg and 120 mg Chewable Tablets'. The applicant confirms that the data provided in support of the sarolaner active substance is as currently approved for the existing sarolaner containing products.

The specification for the active substance is acceptable and includes tests for appearance, identification, assay and related substances, chiral purity, residue on ignition, heavy metals, water content, particle size, polymorphic form and residual solvents. Test methods are well described and are validated in accordance with VICH GL2.

Polymorphism of the active substance is controlled during the manufacturing process to produce always the same polymorph. The active substance has one chiral centre at the 5 position and the manufacturing process routinely produces the same isomer. The other isomer is identified as an impurity and controlled on the specification. The structure has been fully elucidated.

Stability studies were initiated on three batches of the active substance. Twelve 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, the 2 batches manufactured at the proposed site, were also placed on stability. Six months data at all three storage conditions are available for these batches. Finally, an additional 18-month supporting stability data at 25 °C/60% RH has been provided on one batch. A stability protocol for the stability testing of sarolaner is provided.

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

Excipients

Many of the excipients used in the formulation are monographed in the Ph. Eur. and comply with their respective monographs (hydroxypropyl cellulose (200-600 MPAS), hypromellose (HPMC), meglumine, butylhydroxytoluene, lactose monohydrate, maize starch, calcium hydrogen phosphate anhydrous, glucose liquid, gelatin Type A, sodium starch glycolate Type A, silica colloidal anhydrous, magnesium stearate). Confectioner's sugar complies with the USP (NF) monograph. Some excipients are not monographed and comply with in-house monographs (spray dried pork liver powder, hydrolysed vegetable protein, wheat germ).

The palatable base granules component is composed of several excipients. An in-house specification, certificates of analysis and stability data have been provided. A shelf-life for this component of 60 months is established based on stability data. The individual excipients are controlled and had been set an in-house specification when non-compendial.

The non-compendial excipients have been previously authorised in the EU within veterinary medicinal products and 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

Materials of animal origin used in the manufacture of the finished product are lactose monohydrate, spray dried pork liver powder and gelatin Type A. The spray dried pork liver powder is sourced from porcine liver and the gelatin is sourced from porcine skin. The Note for Guidance EMEA 410/01 rev. 3 states that "Pigs and birds, which are animal species of particular interest for the production of medicinal products, are not naturally susceptible to infection via the oral route. Therefore, they are not TSE-relevant animal species within the meaning of this Note for Guidance". The lactose is sourced from healthy animals in the same conditions as milk collected for human consumption and it is therefore in compliance with TSE requirements.

A declaration of compliance with the Note for Guidance EMEA 410/01 rev. 3 is provided by the applicant. The CEP for moxidectin states that one of the materials used in its manufacture is of animal origin. The animal origin and its geographic source are unknown to the applicant and are not divulged on the CEP. However, since EDQM has assessed this information and approved the CEP, there is no known TSE risk from this material and/or this source of moxidectin.

Control tests on the finished product

A release specification for the chewable tablets is provided. The specification includes tests for description, identification, water content, uniformity of dosage units, hardness, average tablet weight, butylhydroxytoluene content, assay and degradation products for each of the active substances, dissolution and microbiological quality. The tests included on finished product specification are acceptable. Analytical methods are well described and have been validated when applicable (in-house methods). Validation of analytical methods was conducted using a bracketing approach of the lowest and highest strength of active substance for all methods.

Batch data is provided for several commercial batches of each tablet strength, as well as batches used in clinical studies and stability batches. All results are within specification with low levels or no impurities detected.

Stability

Primary product stability data is presented for studies carried out on 4 batches of each tablet strength manufactured at both the development (24 batches) the proposed dosage form manufacturing site (24 batches), 48 batches in total. The batches are packaged in intended commercial aluminium/aluminium blister with 6 tablets per blister card. Eighteen months data at 25 °C/60% RH and 30 °C/65% RH, and 12 months data at 40 °C/75% RH are currently available for these batches. Results for appearance, assay, water content, degradation products, BHT content, dissolution, hardness, friability and microbiological quality are reported on the stability tables for the primary stability batches. The parameters hardness and friability are not controlled on the shelf life specification and thus are reported for information purposes on the stability tables. The test for microbiological quality is carried out at the initial time-point and at 6 and 12 months. No significant adverse trends are observed in any of the stability studies reported to date. Wider limits are proposed during shelf life for assay for all three active substances and for butylhydroxytoluene. These wider limits can be accepted. In reviewing the results for dissolution in the stability batches it should be noted that the dissolution methods used from time zero to 9 months were a previous iteration of the method. At the 12 and 18 month time points the currently proposed method was used. The Q values proposed by the applicant are

considered acceptable. The proposed shelf life of 30 months when packaged in aluminium/aluminium blisters and stored at temperature below 30 °C is supported by the data.

Overall conclusions on quality

The product is manufactured by a series of standard granulation and tableting manufacturing processes using excipients that are widely used in tablet formulations. Extensive formulation development is described in the dossier, including active substance properties relevant for development, excipient selection/optimisation and manufacturing process optimisation. The formulation used during clinical studies is slightly different to that intended for marketing. Batches of Simparcia Trio chewable tablets were manufactured for clinical studies in 2015 and 2016 while some of the composition and process optimisation studies were still underway. Therefore, there are some differences between the batches used in clinical studies and those proposed for commercial production. These differences are in the moxidectin granulation and in the sarolaner-pyrantel granulation, but the extragranular components remain the same throughout all clinical and commercial batches. In terms of % w/w per tablet, these changes are very small comprising less than 2% of the total tablet. The change to the sarolaner-pyrantel granulation is very small in terms of % w/w with a corresponding adjustment of lactose concentration. Although the differences in formulation appear relatively minor, comparative dissolution studies did not result in acceptable f2 values across all studies. A combination of *in vivo* bioequivalence and analysis of *in vitro* dissolution using a tolerance approach has been provided to conclude that, despite the low f2 values observed, the difference between the 2016 clinical formulation and the final formulation will not have any significant clinical relevance and that the safety and efficacy aspects of the studies conducted using the 2016 clinical formulation may be extrapolated to the final formulation.

Development of the dissolution method occurred alongside formulation and process development and underwent 3 iterations identified as Generation 1, Generation 2 and Generation 3. In the generation 1 and 2 methods, two different dissolution methods were used, one for moxidectin and the other for sarolaner and pyrantel. The Generation 3 method was a single method for all three active substances and is the proposed release test.

Development of the various manufacturing processes is well described and process optimisation has been undertaken at development scale. In-process controls for the various stages of the manufacturing process are described. A process validation protocol is provided.

The three active substances moxidectin, pyrantel embonate and sarolaner are known active substances and no major issues arise on review of the data submitted for them. The tests proposed at release and at the end of shelf-life are appropriate to control the quality of the finished product. Dosage form stability demonstrates the product to be stable with some fluctuations but no adverse trends the parameters investigated. The proposed widening of the specification for active substances and BHT on shelf life is acceptable. The proposed shelf life of 30 months when packaged in aluminium-aluminium blisters and stored at temperature below 30 °C is supported by the data.

Part 3 – Safety

Simparica Trio chewable tablets for dogs are indicated for the treatment and prevention of mixed internal and external parasite infestations on dogs using the oral route of administration. The active substances of the product are sarolaner, moxidectin and pyrantel embonate. When administered by the oral route of administration, sarolaner is a systemically acting ectoparasiticide, moxidectin has endo-parasiticidal activity, while pyrantel embonate is a tetrahydropyrimidine anthelmintic.

When the product is administered as recommended, the following doses of active substance are achieved: 1.2-2.4 mg/kg bodyweight (bw) of sarolaner, 0.024-0.048 mg/kg bw of moxidectin and 5-10 mg/kg bw of pyrantel.

For the sarolaner component of this product, the recommended treatment dose is in the range 1.2-2.4 mg sarolaner/kg bw: that is, less than the recommended therapeutic dose of 2.0-4.0 mg/kg for the mono-substance product Simparica chewable tablets. In the case of moxidectin, in an oral dosing form, it is approved in several European countries for the prevention of heartworm (*D. immitis*) infections at a minimum recommended dose rate of 0.003 mg/kg bw. The efficacious dose rate of moxidectin determined as part of this application is a recommended dose rate of 0.024 – 0.048 mg/kg bw. A topical dosing form, at a minimum dose rate of 2.5 mg/kg bw is approved for the treatment and/or prevention of several extra-and intra-gastrointestinal nematode species.

A full safety file in accordance with Article 12(3) has been provided.

The active substance sarolaner was previously assessed by the CVMP in the context of the authorisation of the veterinary medicinal product Simparica. The active substances moxidectin and pyrantel embonate were previously assessed by the CVMP in the context of the establishment of maximum residue limits.

Safety documentation

Pharmacodynamics

See Part 4.

Pharmacokinetics

The studies reported in this section that concern the target species dogs, relate to single substance administration only. For further information on pharmacokinetics of the fixed combination product in the target species, see Part 4.

Sarolaner

In rats, the oral pharmacokinetics was evaluated as part of toxicology studies. In the 30-day and 90-day toxicity studies, the exposure (AUC and C_{max}) of sarolaner increased with increasing dose from 0, 0.223, 2.233 or 22.33 mg/kg bw/day and 0.025 mg/kg bw/day to 25 mg/kg bw/day. The plasma pharmacokinetics at the end of the studies (Day 29 or 89) appeared to be higher than those following the first day of dosing, indicating some accumulation of sarolaner over the duration of the studies.

Following oral administration of tablets containing sarolaner as a single substance to fed dogs at 2 mg/kg bodyweight dose, the mean C_{max} was 919 ng/ml and was observed at 15 hours after dosing (t_{max}). The mean C_{max} following administration of tablets to fasted animals was 1100 ng/ml, which occurred at 3 hours after dosing. It was concluded that fed and fasted animals have similar total absorption. Sarolaner had low clearance (0.12 ml/min/kg) and a moderate volume of distribution (2.81 l/kg). Half-life was comparable for the intravenous and oral routes at 12 and 11 days, respectively. Bioavailability was high at >85%. It was concluded that sarolaner is well absorbed by animals in either the fed or fasted state, following oral administration.

The extent of protein binding was estimated to be ≥99.9%.

Moxidectin

A single dose of 0.2 mg/kg bw orally administered moxidectin was primarily excreted unchanged in the faeces of rats, with 59.7-91.3% recovered by 7 days. The highest levels of the administered dose were found in fat. The main route of metabolism in the rat was by hydroxylation. The C_{max} occurred 4.8 hours after treatment with 12.8 and 13.5 ng/ml in males and females, respectively. A terminal $t_{½}$ of 22.9 and 44.6 hours was observed in males and females, respectively. This contrasted with a $t_{½}$ of 63.9 hours following IV administration of 0.2 mg/kg bw. The bioavailability of the oral dose was 19%.

Following oral dosing of 0.2 mg/kg bw moxidectin to Beagle dogs with a solution formulation, the C_{max} was 123 ng/ml occurring at 2 hours.

Pyrantel

A comparative metabolism study in dog, pig, sheep, calf and rat showed that the pyrantel that is absorbed is metabolised into many compounds which are excreted mainly in urine. In all species tested, the highest levels of pyrantel were measured in the liver followed by kidney and heart, with lowest levels in muscle and fat.

Administration of a single oral dose of 10 mg base/kg bw radio-labelled pyrantel tartrate to dogs reported a plasma C_{max} of 4.3 µg/ml occurring at 2 h post dose. The plasma concentration was 0.2 µg/ml by 2 days post dose.

Toxicological studies

Single dose toxicity

Sarolaner

Single dose toxicity studies investigating the acute effects of sarolaner when administered by the oral and dermal routes of administration in rats have been provided (both studies were GLP conducted in accordance with OECD guidelines). In addition, a non-GLP study was conducted to evaluate the tolerance of sarolaner in dogs when administered orally as a single dose at 14 day intervals. Another non-GLP study investigating the tolerance of sarolaner in cats when administered as a single dose dermally was also provided.

The acute oral LD_{50} for sarolaner was estimated to be 783 mg/kg bw in female rats (95% CI: 550 – 2000 mg/kg bw). Adverse effects included body tremors. In the dog, neurological (seizures, hypersensitivity, unsteady gait) and gastrointestinal signs (vomiting, soft stool) following a single oral dose at 62.5 mg/kg bw were observed. The acute oral maximum asymptomatic oral dose in dogs was 25 mg/kg bw; however, only two animals received this dose.

An acute dermal toxicity study in rats administered 2020 mg/kg bw sarolaner for 24 hours reported no signs of mortality or dermal irritation. The acute dermal LD_{50} was determined to be greater than 2020 mg/kg bw. In cats, sarolaner was well tolerated when administered topically at up to 40 mg/kg bw. Neurological signs were observed in one animal in the 50 mg/kg bw dose group (tremor, seizure, dilated pupils, a heightened sensitivity to sound, hyperesthesia).

Moxidectin

Single doses of moxidectin have been reported to be toxic by ingestion with acute oral LD_{50} values in chickens, rats and mice ranging from 100 to 300 mg/kg bw.

An acute dermal toxicity study in rabbits with moxidectin reported an LD_{50} greater than 2000 mg/kg bw.

Pyrantel

Acute oral toxicity of pyrantel embonate is low with LD_{50} values in mouse, rat and dog >2000 mg/kg bw.

Sarolaner, moxidectin and pyrantel embonate in combination

An acute oral toxicity study in rats using a sarolaner/moxidectin/pyrantel embonate formulation (1000/12/2500 mg/kg bw) was conducted. No mortality was observed with this combination formulation, although adverse effects were observed in most animals (80%). These included discoloured/wet inguinal fur, loose stool, yellow faeces, red material around nose and eyes and discoloured paws.

These acute studies provide possible effects of acute over dosage of sarolaner, moxidectin and/or pyrantel embonate in the target species as well as possible effects of accidental administration to humans.

Repeat dose toxicity

Sarolaner

The toxicity of sarolaner was profiled in oral repeat-dose 30- and 90-day studies in rats as well as in target animal toxicity (TAS) studies in dogs.

In the 30 day toxicity study in rats, rats were administered sarolaner by oral gavage at doses of 0, 0.223, 2.233 or 22.33 mg/kg bw/day for 30 consecutive days. Test article-related lower body weight gains or body weight losses and corresponding lower food consumption were noted in the 22.33 mg/kg bw/day group males and females from Study Day (SD) 0 to 7. Females showed partial recovery from the body weight effects following the first week of the dosing period. Clinical observations of thin body condition and dermal atonia correlated with the body weight effects were noted in a single female on SDs 7 and/or 8. In males, complete recovery from the body weight and food consumption effects was noted following the first week of the dosing period. No test article-related clinical observations were noted in the males. Changes in clinical pathology parameters were slight. Test article-related gross enlargement of the adrenal glands at 22.33 mg/kg bw/day in both genders was related to higher adrenal gland weights and microscopic hypertrophy of zona fasciculata cells of the adrenal cortex. In this study, there was no evidence of stress being a cause of the adrenocortical cell hypertrophy (i.e., no stress leukogram and no thymic atrophy). At the dose of 2.233 mg/kg bw/day, effects on the liver were observed (dose dependent vacuolation in females accompanied by decreased triglyceride levels in blood). In addition, vacuolation in the adrenal gland was observed in males and adrenal gland weights were increased in females and (dose dependent) histopathological changes were observed in the ovary. These effects are consistent with those observed in the 90 day study and considered adverse reactions. Therefore, a no-observed-adverse-effect level (NOAEL) for this study of 0.233 mg/kg bw was concluded. At this dose, the vacuolation in liver and ovary hypertrophy was considered mild and non-adverse.

In the 90 day oral toxicity study in Crl:WI(Han) rats, sarolaner was administered by gavage at doses of 0, 0.025, 0.25, 2.5 and 25 mg/kg bw/day. Test article-related lower body weight gains and corresponding lower food consumption were noted in the 25 mg/kg bw/day group males and females from Week 0 to 1. Although the lower food consumption persisted through the end of the study, the animals showed complete (males) or partial (females) recovery from the body weight effects following

the first week of the dosing period. Minor changes in the 2.5 and 25 mg/kg bw/day group haematological and clinical chemistry parameters were considered non-adverse. Test article-related increase in incidence of pale adrenal glands were observed in the 25 mg/kg bw/day group males and females, correlating with increased weights of adrenal glands and hypertrophy and vacuolation of the adrenal cortex. In addition, higher ovary/oviduct weights were observed in the 25 mg/kg bw/day group females. Further investigation identified test article-related vacuolation of interstitial cells of the ovary in the 0.25, 2.5, and 25 mg/kg bw/day group females, the incidence and severity showed a dose-response profile. There was no evidence that the changes in the adrenal cortex or ovary affected organ function and therefore were not considered to be adverse. A NOAEL of 25 mg/kg bw/day is not accepted given that an effect on body weight/food consumption was noted at the high dose group. Pathological changes were noted at lower doses (effects on the ovary were noted in the 0.25 and 2.5 mg/kg bw/day group females). As treatment-related histopathological (dose-dependent) changes in the ovary were noted at lower doses, a NOAEL for this study of 0.25 mg/kg bw was considered more appropriate. While it has not been demonstrated that these effects are reversible, it is accepted that they are mild such that they can be considered non-adverse.

In dogs, orally administered 10 doses of sarolaner at 28 day intervals, test article-related neurological effects were detected in the 12 mg/kg bw (transient tremors) and 20 mg/kg bw (convulsions) dose groups. The neurological signs occurred primarily in the first 24 hours were transient and resolved without treatment. No tremors or convulsions were observed beyond the 5th dose of this 10 dose study and no neurological signs were observed in the 4 mg/kg bw dose group. No other dose related adverse effects were noted.

When considering user exposure, the most relevant exposure for this dosage form is acute exposure arising from accidental ingestion. Because treatment-related effects were observed in the 10 dose margin of safety study after 24 hours, these effects are considered relevant to define the user risk for sarolaner, in particular, the risk to a child after accidental ingestion of a sarolaner containing tablet (see user safety assessment).

Moxidectin

Study summaries from the published literature have been provided reviewing the effects of repeated doses of moxidectin on laboratory animals.

A 28 day repeat dose oral toxicity study with moxidectin was conducted in mice. A NOEL of 6.9 mg/kg bw/day was established based on the absence of effects at this dose.

A two year repeat dose oral toxicity study with moxidectin was conducted in mice. A NOEL of 5.1 mg/kg bw/day was established based on observations of hunched-posture, decreased activity, tremors, laboured breathing and coldness to the touch in the next higher group.

A 28 day repeat dose oral toxicity study with moxidectin was conducted in rats. A NOEL was not identified as adverse reactions (hypersensitivity to touch) were identified in the lowest dose group (12 mg/kg bw/day).

A 13 week repeat dose oral toxicity study with moxidectin was conducted in rats. A NOEL of 3.9 mg/kg bw/day was established based on observations of depressed body weights and significant increases in adrenal weights in females, increased testes weights in males and hypersensitivity to touch in the next higher group.

A 2 year repeat dose oral toxicity study with moxidectin was conducted in rats. A NOEL of 5.1 mg/kg bw/day was established. It is noted that the highest dose of 9.8 mg/kg bw/day was reduced to 5.1 mg/kg bw/day eight weeks into the study due to increased mortality in this group. From the limited information available, no adverse reactions in the 5.1 mg/kg bw/day group were identified.

A 28 day repeat dose oral toxicity study with moxidectin was conducted in dogs. A NOEL of 0.5 mg/kg bw/day was established based on nervous system effects observed in the next higher group.

A 90 day repeat dose oral toxicity study with moxidectin was conducted in dogs. A NOEL of 0.3 mg/kg bw/day was established based on dose-dependent reductions in absolute body weights and food consumption in the higher dose groups. This study was used to establish the toxicological ADI for moxidectin by CVMP by applying a safety factor of 100.

A 52 week repeat dose oral toxicity study with moxidectin was conducted in dogs. A NOEL of 1.15 mg/kg bw/day was established based on the absence of adverse reactions at the highest dose tested.

No dermal studies investigating the repeated administration of moxidectin were conducted.

Pyrantel embonate

The repeat dose oral toxicity study with pyrantel embonate was previously assessed by CVMP in the context of the establishment of maximum residue limits. In a 13-week dog oral toxicity study, dogs received capsules containing pyrantel embonate at doses equivalent to 35, 105 and 210 mg pyrantel base/kg bw, five days per week. An oral NOEL of 35 mg/kg bw/day was established based on increased serum aspartate aminotransferase and serum alanine aminotransferase values at higher dose levels.

Sarolaner, moxidectin and pyrantel embonate in combination

Repeat dose oral studies conducted with sarolaner, moxidectin and pyrantel embonate in combination were conducted in dogs as part of the target animal safety studies. For further information on safety of the fixed combination product in the target species, see Part 4.

The available *in vivo* data with the combination administered to the target species compared to the effects observed in the acute rodent studies suggests that the unintended (adverse) effects of the fixed combination do not differ from those of the individual substances when administered alone.

Tolerance in the target species of animal

See Part 4.

Reproductive toxicity

Study of the effect on reproduction

Studies to evaluate the effects of sarolaner on reproduction were not conducted. As this product is intended for use in companion animals and not in food-producing animals, the absence of studies investigating the effects on reproduction can be accepted.

In a reproduction and lactation study in rats, animals were administered pyrantel embonate at dietary doses equivalent to 0, 9, and 90 mg pyrantel base/kg bw/day from Day 14 prior to mating, throughout pregnancy, and until all pups had been weaned. There were no significant differences between treated rats and control rats on fertility, gestation, viability or lactation indices up to the highest dose of 90 mg/kg bw/day.

In the absence of studies on the effect of reproduction for sarolaner, a statement that the safety of the veterinary medicinal product has not been established during pregnancy and lactation or in dogs intended for breeding is included on the product literature.

Study of developmental toxicity

Sarolaner

In order to investigate the potential developmental toxicity of sarolaner, two studies investigating the effects of sarolaner when administered by the oral route of administration to rats and rabbits have been provided.

The definitive developmental toxicity studies in rats and rabbits were conducted in accordance with GLP and OECD 414. These studies were adequate to evaluate maternal and embryo/foetal toxicity and teratogenic potential during the period of organogenesis. The NOAEL for maternal toxicity and embryo/foetal development was determined to be 3.2 mg/kg bw/day when administered orally to Crl:WI(Han) female rats and 3.0 mg/kg bw/day when administered orally to time-mated New Zealand White rabbits. It is considered that the developmental toxicity is secondary to maternal toxicity, that is, sarolaner does not appear to have a direct foetotoxic effect.

Moxidectin

An embryo/teratogenicity study was carried out in CF1 mice. Moxidectin was administered by gavage at doses of 0, 1.5, 3 and 8 mg/kg bw/day for GD (gestation day) 6-15 to four groups of pregnant mice. Two other groups received moxidectin at doses of 0 and 6 mg/kg bw/day for GD 6-15 because of a high level of mortality in the 8 mg/kg bw/day dose group. A significantly increased percentage of malformed foetuses was reported after oral administration of the two highest doses (96.9% and 53.9% at 3 and 6 mg/kg bw/day, respectively) versus the lowest dose and controls (7.2% and 6.3%, respectively). The malformations included manubrium fused, cleft palate and skull palate incompletely ossified. The NOEL for maternotoxicity was 3 mg/kg bw/day and the NOEL for foetoxicity was 1.5 mg/kg bw/day.

In a two-generation rat study, reduced pup survival was observed at doses above 0.4 mg/kg bw. A NOEL of 0.4 mg/kg bw was concluded.

Two teratogenicity studies were performed in rats (0, 2.5, 5, 10, 12 mg/kg bw) and in rabbits (0, 1, 5, 10 mg/kg bw). In rats, moxidectin was maternotoxic at 10 and 12 mg/kg bw. Foetal alterations such as cleft palate, micrognathia, not ossified or incomplete ossified sternebrae were reported for doses higher than 2.5 mg/kg bw. In rabbits, maternotoxicity was mentioned at 5 and 10 mg/kg bw but there was no influence on foetal development. The NOELs for maternotoxicity were 5 mg/kg bw in rats and 1 mg/kg bw in rabbits. The NOELs for the embryotoxic effects were 2.5 mg/kg/day in the rat and more than 10 mg/kg in the rabbit.

Pyrantel embonate

No evidence of teratogenicity, foetotoxicity or maternal toxicity was observed in rats after oral administration of pyrantel embonate at daily oral doses equivalent to 0, 9, and 90 mg pyrantel base/kg bw/day. No statistical and no dose-related incidence of malformations was observed when compared to the control group.

Pyrantel embonate was administered to rabbits at doses equivalent to 0, 9, and 90 mg pyrantel base/kg bw/day from GD 7 to 17. An increase in the incidence of resorptions (8.5% and 12.6% versus 2.5% for controls) was observed. No conclusions could be drawn with regard to a NOEL for maternal toxicity. No adverse effects of toxicological significance were reported for foetuses up to the highest dose of 90 mg/kg bw/day.

Genotoxicity

Sarolaner

The mutagenic potential for sarolaner was adequately assessed in a standard battery of genetic toxicology assays recommended in VICH GL23. All studies were GLP and were conducted in accordance with relevant OECD guidance.

- Sarolaner did not induce mutations either directly or with metabolic activation in *Salmonella typhimurium* or *Escherichia coli* strains at any dose tested in the Ames assays.
- Sarolaner was negative for inducing structural and numerical (polyploidy-inducing endoreduplication) chromosome aberrations in human peripheral lymphocytes in the *in vitro* chromosome aberration assay.
- Sarolaner was negative in the *in vivo* micronucleus assay in male and female rats.

Based on the study results, sarolaner is not considered to be of mutagenic or genotoxic concern.

Moxidectin

The Ames test, CHO/HGPRT test, unscheduled DNA synthesis in primary rat hepatocytes, *in vivo* chromosome aberration test in rat bone marrow cells did not show mutagenic activity. CVMP concluded that moxidectin does not have mutagenic potential based on the negative *in vitro* and *in vivo* genotoxicity tests.

Pyrantel embonate

CVMP considered the genotoxicity of pyrantel within the context of the MRL evaluation (EMEA/MRL/491/98-FINAL). Pyrantel tartrate gave negative results in the *Salmonella* microsomal assay (TA98, TA100, TA1535, TA1537, TA1538) in the absence and presence of metabolic activation at concentrations of 0.75 to 7500 µg/ml. CVMP concluded that pyrantel does not have mutagenic potential.

Carcinogenicity

Sarolaner

Carcinogenicity studies were not conducted with sarolaner. The absence of carcinogenicity studies is justified on the basis that:

1. the test article was not mutagenic or genotoxic,
2. there were no proliferative changes in the 90-day oral rat toxicity study, and
3. there were no structural alerts for genotoxicity.

Moxidectin

Two carcinogenicity studies were conducted with moxidectin. The first was conducted in rats treated with 0, 15, 60 and 100 ppm for 102 weeks, while the second study was in mice treated with 0, 15, 30, 50 ppm for 105 weeks. Neither study showed potential carcinogenicity.

Pyrantel embonate

Carcinogenicity studies were not conducted with pyrantel embonate. CVMP concluded in the context of the establishment of maximum residue limits for pyrantel embonate that the available long term feeding studies with pyrantel tartrate in rats (93 weeks) and dogs (2 years) were not acceptable to

assess the carcinogenic potential of pyrantel. However, based on the absence of mutagenicity, carcinogenicity studies are not considered necessary.

Studies of other effects

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

Moxidectin was non-irritating to rabbit eyes and skin following application of 2% moxidectin equine gel formulation. In a dermal irritation study with guinea pigs, intradermal administration of moxidectin resulted in erythema and oedema at doses ≥2 mg/ml, with no dermal irritation at ≤0.2 mg/ml.

No dermal irritation studies conducted with pyrantel embonate were provided. It is reported that pyrantel embonate can cause eye and skin irritation as well as skin sensitisation.

No specific studies on the immunotoxicity or neurotoxicity of sarolaner, moxidectin or pyrantel embonate were provided.

Excipients

The excipients include lactose monohydrate, sodium starch glycolate, meglumine, butylhydroxytoluene (BHT), hydroxypropyl methylcellulose, colloidal silicon dioxide, magnesium stearate, hydrolysed vegetable protein, maize starch, confectioner's sugar (sucrose), spray dried pork liver powder, gelatin type A, wheat germ, calcium hydrogen phosphate anhydrous, and corn syrup.

The excipients are considered to be of low toxicity and/or present at low concentrations. It is accepted that they will not pose a concern to the user.

User safety

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

Simparica Trio chewable tablets for dogs will be supplied in an aluminium foil/blister package, with 1, 3 or 6 tablets per blister card. It is a hard, non-divisible, flavoured chewable tablet. The maximum strength tablet contains 72 mg sarolaner/1.44 mg moxidectin/300 mg pyrantel embonate. The product is to be administered up to once monthly. The excipients in the product are considered to be of low toxicity and/or present in small amounts. It is accepted that they will not pose a concern to the user and that the systemic and local toxicity of the product to the user will be determined by its active substances.

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely are those of dermal and/or oral exposure.

It is considered likely that adverse events will not occur as a result of dermal contact with these tablets. Sensitisation and irritation studies have confirmed that sarolaner and moxidectin do not cause these effects in the test animals used. This is also the case for eye irritation. It is reported that pyrantel embonate can cause eye and skin irritation as well as skin sensitisation. However, taking into account the low risk of exposure, standard hygiene measures are considered to be adequate in minimising dermal exposure from handling the tablets and any hand-to-mouth or hand-to-eye transfer that might occur.

With regard to accidental oral exposure, the applicant has considered that ingestion of one of the largest (72 mg sarolaner/1.44 mg moxidectin/300 mg pyrantel embonate) tablets by a small child (10 kg) should be used as a worst-case scenario. When comparing this level to the acute oral toxicological reference value, the margins of exposure are below the trigger value of 100.

As a point of departure the applicant has used observations from the exploratory margin of safety study of a combined formulation in dogs. No effects were observed with a combined dose of 12 mg/kg bw sarolaner + 144 µg/kg bw moxidectin + 30 mg/kg bw pyrantel embonate. In the case of moxidectin and pyrantel embonate, points of departure of 144 µg/kg bw and 30 mg/kg bw, respectively, can be accepted. A MOE of 1 for moxidectin and pyrantel embonate in children indicates an unacceptable risk for children.

However, in relation to sarolaner, it is noted that adverse effects have been observed at doses lower than 12 mg/kg bw in another study. A margin of safety study in dogs investigating different doses of sarolaner noted neurological signs following the first dose in the 12 mg/kg bw and 20 mg/kg bw dosing groups. Signs appeared to occur primarily in the first 24 hours after dosing and resolved without treatment, with no adverse effects on the nervous system observed in any animal at 4 mg/kg bw. Accepting a dose of 4 mg/kg bw to be the relevant point of departure for sarolaner would result in an MOE of 0.56. However, the authorised SPC for the sarolaner mono-substance product, Simparica, carries the following warning: "In very rare cases transient neurological disorders such as tremor, ataxia or convulsion may occur." This suggests that neurological AEs have been reported in dogs administered the RTD for that product of 2-4 mg sarolaner/kg bw on a single occasion and, for the purposes of this user risk assessment, the toxicological reference value should be less than 4 mg/kg bw, resulting in a lower MOE. That said, given that the calculated MOE for the sarolaner component is less than 1, aiming to define the MOE with greater precision will not make any material difference to the conclusions of the assessment and the need for risk mitigation measures.

A child-resistant protocol test investigating the ease of opening of the Simparica Trio packaging was provided. The study consisted of a child panel and a senior panel.

- Child panel (50 children; 30% were 42-44 months, 40% were 45-48 months and 30% were 49-51 months). Each child received a set of blister cards. In accordance with EN 14375, access to nine or more unit doses within 10 minutes from the packaging by a child would constitute a failure. As part of this study, no individual child test was considered a failure, although a majority of the participants could access at least one tablet in the time allowed;
- Senior panel (100 senior adults; 25% were 50-54 years, 25% were 55-59 years and 50% were 60-70 years). Each senior adult received a set of blister cards as part of an initial five-minute test period, followed by a second set of blister cards for a one-minute test period. Failure was any senior adult who was unable to open the package within the first five-minute test period, or was unable to open the package within the second one-minute test period, but was able to pass the screening test. Only four of 100 senior adults failed to open the test packaging, indicating a 96% success rate.

The packaging material and 'peel and push' system is the same as that already considered acceptable for the product Simparica (which was demonstrated to be compliant with EN 14375); the main differences between the two products are the orientation of the perforations and the 'peel' angles. Child-resistance in accordance with EN 14375 was demonstrated in this study, indicating that the immediate packaging can be considered suitably resistant to unintended access by children. Considering that the concerned hazard does not consist of life-threatening or long-term effects, and provided clear advice is given to keep the medicine away from children, the user risk relating to the pre-application phase is overall considered acceptable.

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

- Wash hands after handling the product.
- The accidental ingestion of the product may potentially result in adverse effects, such as transient excitatory neurological signs. To prevent children from accessing the product, only one chewable tablet at a time should be removed from the blister pack and only when required. The blister pack should then be returned into the carton immediately after use and the carton should be stored out of the sight and reach of children. In case of accidental ingestion, seek medical advice immediately and show the package leaflet or label to the physician.

Based on the user risk assessment, the CVMP concludes that the product will not present an unacceptable risk to the user when used in accordance with the SPC.

Environmental risk assessment

A Phase I environmental risk assessment (ERA) was provided according to the CVMP/VICH GL6 (CVMP/VICH/592/98-FINAL).

Phase I:

The environmental risk assessment was stopped in Phase I and no Phase II assessment was undertaken because the veterinary medicinal product will only be used in non-food animals.

Conclusions on the environmental risk assessment

An ERA was provided according to the CVMP/VICH guidelines. Based on the data provided, the ERA stopped at Phase I.

Overall conclusions on the safety documentation

Pharmacology/Toxicology

Sarolaner is a member of the isoxazoline class of parasiticides which acts by blocking the insect GABA gated chloride channels. Moxidectin belongs to the milbemycin group of macrocyclic lactones (avermectins being the other) and has parasiticidal activity against a range of internal and external parasites. Pyrantel is an imidazothiazole belonging to the group of tetrahydropyrimidines anthelmintics, targeting the nicotinic acetylcholine receptors (nAChRs) on worm somatic muscle cells.

The excipients are considered to be of low toxicity and/or present at low concentrations, the systemic and local toxicity of this product will be determined by its active substances, sarolaner, moxidectin and pyrantel (as embonate).

Pharmacodynamics and pharmacokinetics of the fixed combination are addressed in Part 4.

<u>Sarolaner</u>

The acute oral LD50 for sarolaner in rats was estimated to be 783 mg/kg bw in rats, while the dermal LD50 was estimated to be greater than 2020 mg/kg bw in rats.

In repeat dose toxicity studies, dogs orally administered 10 doses of sarolaner at 28 day intervals exhibited test article-related neurological effects in the 12 mg/kg bw (transient tremors) and 20 mg/kg

bw (convulsions) dose groups. The neurological effects were transient and resolved without treatment; no other dose related adverse effects were noted. No neurological signs were observed in the 4 mg/kg bw dose group. As the effects were observed on the first day of administration, they are considered to be relevant in determining the risk to the user.

In repeat dose toxicity studies, the NOAEL for sarolaner was 0.223 mg/kg bw by oral administration in rats. Effects in the liver (dose dependent vacuolation in females accompanied by decreased triglyceride levels in blood), adrenal (vacuolation in males and increased adrenal weights in females) and ovary (hypertrophy) were observed at higher dose levels.

The NOAEL for maternal toxicity and embryo/foetal development was determined to be 3.2 mg/kg bw/day when administered orally to Crl:WI(Han) female rats and 3.0 mg/kg/day when administered orally to time-mated New Zealand White rabbits. It is accepted that the developmental toxicity is secondary to maternal toxicity. In the absence of studies on the effects on reproduction the use of the product is contraindicated for breeding animals.

Sarolaner is not considered to be of mutagenic or genotoxic concern. The absence of carcinogenicity studies is justified.

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

Moxidectin

The acute oral LD_{50} of moxidectin in chickens, rats and mice is reported to range from 100 to 300 mg/kg bw.

In repeat dose toxicity studies, an oral NOEL of 0.3 mg/kg bw/day was established in a dog 90-day toxicity study based on dose dependent reductions in absolute body weights and food consumption identified in dogs. Nervous system effects were observed at higher doses in mice, rats and dogs.

In the reproduction studies, a NOEL of 0.4 mg/kg bw was concluded based on reduced pup survival at doses of moxidectin above 0.4 mg/kg bw in rats. In teratogenicity studies in rats and rabbits, the NOEL for maternotoxicity was 5 mg/kg bw in rats and 1 mg/kg bw in rabbits. Foetal alterations such as cleft palate, micrognathia, not ossified or incomplete ossified ribs were reported for doses higher than 2.5 mg/kg bw in rats. No effects on foetal development were observed in rabbits. The NOEL for the embryotoxic effects was 2.5 mg/kg bw/day in the rat and more than 10 mg/kg bw in the rabbit.

Moxidectin is not considered to be of mutagenic concern.

Carcinogenicity studies in rats and mice did not show the potential for carcinogenicity.

Moxidectin was non-irritating to rabbit eyes and skin. In a dermal irritation study with guinea pigs, intradermal administration of moxidectin resulted in erythema and oedema at doses ≥2 mg/ml, with no dermal irritation at ≤0.2 mg/ml.

Pyrantel embonate

The acute oral toxicity of pyrantel embonate is low, with LD50 values in mouse, rat and dog >2000 mg/kg bw.

In dogs orally administered pyrantel embonate in a 13-week repeat dose toxicity study, a NOEL of 35 mg/kg bw/day was established based on increased serum asparatate aminotransferase and serum alanine aminotransferase values at higher dose levels.

Reproduction toxicity was only studied in laboratory animals and not in the target species. Data in rats indicate that pyrantel embonate is not a developmental or reproductive toxicant.

Pyrantel is not considered to be mutagenic. Carcinogenicity studies have not been performed and are not requested.

It is reported that pyrantel embonate can cause eye and skin irritation as well as skin sensitisation.

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

User safety

A user safety assessment in line with the relevant guidance document has been presented. The worst case scenario for user safety is ingestion of a tablet by a child, with an estimated margin of exposure (MOE) of 0.56, 1, 1 for sarolaner, moxidectin and pyrantel embonate, respectively. These margins of exposure are based on the no effect level on the nervous system in the target animal studies. It is accepted that the risk will, in part, be mitigated by the inclusion of appropriate safety advice/warning statements in the product information. The risk will be further mitigated by the fact that the tablets are presented in packaging that is almost identical to that already accepted for the product Simparica and which meets the criteria for child-resistance in accordance with European standard EN14375. It was accepted that the product will not present an unacceptable risk to the user when stored, handled used and disposed of in accordance with the recommendations included in the SPC.

Environmental safety

An environmental risk assessment was provided. Based on the data provided, the ERA stopped at Phase I. The applicant concluded that the product is not expected to pose a risk for the environment when used according to the SPC.

Part 4 – Efficacy

Pharmacodynamics

No new data has been provided but instead, the applicant has provided data from the public domain to outline the pharmacodynamic properties of the three individual active substances sarolaner, moxidectin and pyrantel (as embonate salt) included in the candidate fixed combination product.

Sarolaner is an acaricide and insecticide belonging to the isoxazoline family which blocks GABA (gamma amino butyric acid)- and glutamate-gated chloride channels in the central nervous system of insects and acarines, preventing the uptake of chloride ions by GABA- and glutamate-gated ion channels and thus resulting in increased nerve stimulation and death of the target parasite.

Moxidectin is a second generation macrocyclic lactone of the milbemycin family; its principal mode of action is interfering with neuromuscular transmission of the glutamate-gated chloride channels and, to a lesser extent, of GABA-gated channels, thus leading to the opening of the chloride channels on the postsynaptic junction to allow the inflow of chloride ions and this results in flaccid paralysis and eventual death of parasites exposed to the drug.

Pyrantel is a nicotinic acetylcholine (ACh) channel receptor (nAChR) agonist; following receptor binding, the channel opens to allow the influx of cations resulting in a depolarization and excitatory effects on nematode muscle, ultimately leading to spastic paralysis of the worm and death.

Justification of fixed combination

The applicant has justified the combination of sarolaner, moxidectin and pyrantel on the grounds that the combination will broaden the activity spectrum of the product 'Simparica' (mono-active product containing sarolaner) by adding indications against endoparasites.

The applicant has further justified the combination by providing data on prevalence of helminths, fleas and ticks in the dog as well as prevalence of these parasites combined in dogs in several European countries. Based on the data provided, it is accepted that the parasites targeted by the combination product (fleas, ticks, gastrointestinal nematodes, heartworm and *Angiostrongylus*) are commonly found in dogs in Europe and can be present simultaneously on the same animal.

In further support of the combination of active substances, the following points are noted:

- Sarolaner is included in the fixed combination to target fleas and ticks. Moxidectin and pyrantel provide anthelmintic efficacy against gastrointestinal nematodes through distinct mechanisms of action.
- The three active substances have different spectra of activity against the target parasites. Sarolaner is included in the fixed combination formulation to target ticks and fleas. Moxidectin is included to provide efficacy against L4 and immature adult (L5) stages of *Ancylostoma caninum*, *Dirofilaria immitis* and immature adult (L5) stage of *Angiostrongylus vasorum*. Pyrantel is included to target immature adult (L5) and adult stages of *Toxocara canis*, adult stages of *Ancylostoma caninum*, *Toxascaris leonina* and *Uncinaria stenocephala*.
- Data from non-interference studies confirm that each individual active substance does not interfere with the efficacy of the other two active substances in the combination product when compared to the sole treatment with a mono-active saraloner, moxidectin or pyrantel product.
- Whilst no synergistic or additive activity is being claimed, it can be accepted that compared to the applicant's mono-active product Simparica, the inclusion of moxidectin and pyrantel will result in broadening of the activity spectrum.
- As a consequence (and a secondary justification), the possibility to administer a single treatment instead of more than one product will have the advantage of facilitating animal handling (reduction of the total number of tablets/other products) as well as owner's compliance.

As it is expected that all of the active substances in a fixed combination should be indicated for use at the moment of treatment, the proposed indication highlights that the product is for use in dogs with or at risk of mixed external and internal parasitic infestations, and that it is only indicated when ectoparasites and gastrointestinal nematodes are targeted at the same time. The applicant has provided data on prevalence of the targeted parasites in Europe, and such an approach is considered acceptable.

The CVMP proposed additional statements in the product information to strengthen the advice that in the absence of the risk of mixed co-infection, a narrower spectrum parasiticide should be used.

The CVMP accepts that the applicant has provided adequate justification of the fixed combination in accordance with the CVMP Guideline on pharmaceutical fixed combination products (EMEA/CVMP/83804/2005).

Development of resistance

No additional studies with the candidate formulation have been performed.

Sarolaner was first authorised in the EU for use in dogs in 2015 and in cats (in combination with selamectin) in 2017. No resistance to sarolaner has been reported to date.

It is acknowledged that there have been reports of resistance development of *D. immitis* to macrocyclic lactone use in dogs in the USA. That said, the CVMP is unaware of similar reports originating from within the EU and accepts that at present macrocyclic lactones continue to be effective in the vast majority of situations, and that the appropriate use of macrocyclic lactone products as per label recommendation is the basis for effective heartworm prevention.

Since the introduction of pyrantel as an anthelmintic in dogs, pyrantel resistance in *A. caninum* has been reported in Australia. No resistance to pyrantel has been reported to date in the EU or against other gastrointestinal nematodes.

Section 4.4 of the SPC includes prudent use warnings relating to the potential for resistance emergence.

Pharmacokinetics

The pharmacokinetics of the three active substances, administered in combination in dogs, was investigated in a series of laboratory studies. The studies were generally of good quality, with pivotal studies conducted in accordance with GLP.

All animal studies were conducted with the '2016 Clinical Supplies' formulation except two studies which used the final commercial formulation. Although it is noted that three formulations have been used in the pre-clinical studies, the '2016 Clinical Supplies' formulation was used to investigate the pharmacokinetic behaviour of the active substances and this was most similar to the final formulation. According to the CVMP 'Guidelines for the conduct of pharmacokinetic studies in target species animals' (EMEA/CVMP/133/99-FINAL), 'the principal objectives are to estimate the factors involved in the absorption, distribution, metabolism and elimination (basic pharmacokinetic studies)' of active substances in the product formulation. Consequently, the CVMP accepts the applicant's approach that the final formulation is not used for pre-clinical pharmacokinetic studies, but rather, the active substances and their proposed dose rates are used.

Studies were mostly conducted with animals administered tablets containing 12 mg sarolaner, 0.24 mg moxidectin and 50 mg pyrantel, but the pharmacokinetics following administration of tablets containing 24 mg sarolaner, 0.48 mg moxidectin and 100 mg pyrantel has also been investigated. The majority of studies used laboratory Beagles, but assessments were also made in mixed breed dogs, pure breed MDR-1 mutant collies and in dogs infected with adult *Dirofilaria immitis*. The age of animals ranged from 9 months to 10.8 years, weighed 6.2 to 45.7 kg.

The findings of the pharmacokinetic studies can be summarised as follows:

Sarolaner: PK differences for males versus females were not considered significant or clinically important.

After single oral dosing of 12 mg sarolaner, 0.24 mg moxidectin and 50 mg pyrantel in fasting dogs, sarolaner was well absorbed with an absolute bioavailability value of 86.7%.

Following oral dosing, sarolaner reached a maximum concentration in plasma within 3.5 hours (T_{max}) after administration.

Excluding the outliers of a single study, linear increase with dose for $AUC_{0-\infty}$ and less than proportional increases in C_{max} were observed when dosed from x0.5 to x2 the intended dose.

There is evidence of limited accumulation upon repeat dosing. In animals dosed with tablets containing 12, 0.24 and 50 mg sarolaner, moxidectin and pyrantel, respectively, on four occasions at 30-day intervals, the accumulation ratio was 1.26 for sarolaner based on the mean C_{30d} after each dose.

The effect of feeding on bioavailability was determined. It is accepted that the prandial state of the dogs does not affect the extent of absorption of sarolaner.

PK parameters for subpopulations comprising Beagles, mixed breeds, avermectin-sensitive (MDR-1 gene mutation) collie and dogs infected with adult *Dirofilaria immitis* have been investigated. Weighted mean PK values of sarolaner for Beagles (obtained from five PK/tolerance studies) were slightly lower than for mixed breeds (data obtained from a tick efficacy study), and significantly lower than for avermectin-sensitive (MDR-1 gene mutation) collies (data obtained from a TAS study). Avermectin-sensitive collies had an $AUC_{0-\infty}$ approximately 2.5 times higher and $t_{1/2}$ 1.8 times longer than Beagles, while C_{max} was comparable.

No new studies were conducted to evaluate distribution, metabolism or excretion of sarolaner. Based on study data from the authorisation of Simparica (EMEA/V/C/003991), the primary route of elimination of sarolaner is biliary excretion of the parent molecule, with minor contributions from metabolic clearance.

Moxidectin: PK differences for males versus females were not considered significant or clinically important.

After single oral dosing of 12 mg sarolaner, 0.24 mg moxidectin and 50 mg pyrantel in fasting dogs, moxidectin was relatively well absorbed with an absolute bioavailability value of 66.9%.

Following oral dosing, moxidectin reached a maximum concentration in plasma within 2.6 hours (T_{max}) after administration.

Excluding the outliers of one study, linear increase with dose was observed for $AUC_{0-\infty}$ and C_{max} when dosed from x0.5 to x2 the intended use dose.

There is some suggestion of potential accumulation upon repeat dosing. In animals dosed with tablets containing 12, 0.24 and 50 mg sarolaner, moxidectin and pyrantel, respectively on four occasions at 30-day intervals, the accumulation ratio was 1.13 for moxidectin based on the mean C_{30d} after each dose; however, the 95% CI for the estimated accumulation ratio included one.

The effect of feeding on bioavailability was determined. It is accepted that the prandial state of dogs does not affect the extent of absorption of moxidectin.

PK parameters for subpopulations comprising Beagles, mixed breeds, avermectin-sensitive (MDR-1 gene mutation) collie and dogs infected with adult *Dirofilaria immitis* have been investigated. Weighted mean PK values of moxidectin for Beagles (obtained from five PK/tolerance studies) were comparable with the PK values from mixed breeds (data obtained from the tick efficacy study). However, pharmacokinetic parameters differed significantly between Beagles and avermectin-sensitive (MDR-1 gene mutation) collies. When the weighted mean PK values of moxidectin for Beagles were compared with the PK values from TAS study in avermectin sensitive collies, the $AUC_{0-\infty}$ was approximately 4.5 times higher, C_{max} 2.0 times higher and $t_{1/2}$ 4.7 times longer in avermectin-sensitive (MDR-1 gene mutation) collies compared to Beagles.

No new studies were conducted to evaluate distribution, metabolism or excretion of moxidectin. Based on information available in the public domain, the primary route of elimination for moxidectin is biliary elimination of parent moxidectin, with minor contributions by metabolic clearance.

Pyrantel: Following oral dosing, pyrantel had a mean C_{max} of 44.5 ng/ml, t_{max} of 1.5 hours and $t_{1/2}$ of 7.74 hours.

PK differences for males versus females were not considered significant or clinically important.

The effect of feeding on bioavailability was determined. The absorption of pyrantel is significantly higher in the fed state compared to the fasted state. However, on account of the overall low bioavailability of pyrantel embonate, this is not expected to impact on efficacy of the compound.

A linear increase with dose was observed for $AUC_{0-\infty}$ but not C_{max} when dosed from x0.5 to x2 the intended use dose.

In animals dosed with tablets containing 12, 0.24 and 50 mg sarolaner, moxidectin and pyrantel, respectively, on four occasions at 30-day intervals, the accumulation ratio was 1.13 for pyrantel based on $AUC_{0-t(last);}$ however, the 95% CI for the estimate for the accumulation ratio included one.

Pyrantel shows less than proportional kinetics especially at higher doses (≥30 mg/kg bw).

PK parameters of pyrantel were similar in avermectin-sensitive (MDR-1 gene mutation) collies and in Beagles.

No new studies were conducted to evaluate distribution, metabolism or excretion of pyrantel. Based on information available in the public domain, absorbed pyrantel is metabolised into many compounds which are excreted mainly in urine. In all species tested (dog, pig, sheep, calf, rat), the highest levels of pyrantel were measured in liver followed by kidney and heart, with lowest levels in muscle and fat.

Although the pharmacokinetic properties of sarolaner in combination with moxidectin and pyrantel in the fixed combination product were investigated in a series of laboratory studies, the potential for pharmacokinetic interactions between active substances has not been investigated. That said, it is noted that each proposed claim of efficacy is supported by new efficacy study data generated with the fixed combination product to support the choice of dose rates selected for each of the active substances. Furthermore, the applicant has investigated the absence of any therapeutic interaction (non-interference studies) between the active substances in some of the dose confirmatory and field efficacy studies. In light of the above, the CVMP considers the omission of pharmacokinetic interactions studies for the individual active substances acceptable.

Based on the data package presented, the CVMP accepts that the pharmacokinetics of sarolaner, moxidectin and pyrantel in dogs when administered in the fixed combination formulation were comprehensively investigated and well characterised. However, given the possibility for continued accumulation of sarolaner and moxidectin following repeated oral administration in dogs, the applicant was asked to justify how the safety of repeated administration of the candidate formulation may be considered to have been adequately demonstrated for all dog breeds (but particularly collie breeds) following long term treatment administration, given that repeated use of the product on more than four consecutive occasions is foreseen. In response, the applicant provided a population pharmacokinetic model for sarolaner and moxidectin based on the data from 17 laboratory PK, efficacy or safety studies. The PK model predicted that, with a 28-day dose interval, >98% of the steady state concentrations for both sarolaner and moxidectin would be achieved by the 3rd dose. Based on the justification/argumentation provided by the applicant, it is accepted that the design of the target animal safety study with a treatment duration of 7 months (7 consecutive monthly doses) in 8 weeks old puppies covered the period of expected drug accumulation in dogs. Furthermore, the results of the study indicate a margin of safety of ≥5-fold of the maximum therapeutic dose level (12 mg of sarolaner per kg bw, 0.24 mg moxidectin per kg bw and 50 mg of pyrantel per kg bw) for a treatment period of 7 months.

The applicant also provided a nonparametric superposition for the 1X group to investigate the predicted steady state plasma concentration levels of sarolaner and moxidectin in MDR1 mutant (avermectin-sensitive) collies, in which tolerance after repeated dosing was not assessed. Based on the

nonparametric superposition for the 1X group, it is predicted that, with a 28-day dose interval, it will take 5 doses for sarolaner and 6 doses for moxidectin in MDR-1 mutant collies to reach >90% of the steady state levels.

Taking into account the short half-life of pyrantel ($t_{1/2}$ of 7.74 hours following oral dosing), it is accepted that pyrantel is not expected to accumulate with the 28-day dose intervals.

Notwithstanding the evidence of limited accumulation following administration of the first three doses (first 6 doses in MDR-1 mutant dogs), it can be accepted that an acceptable tolerance to repeated doses in excess of those recommended (as administered in the margin of safety studies) has been demonstrated despite the concentrations of active substances exceeding those considered as steady state concentrations.

Consequently, the limited accumulation observed following administration of the first three doses (first six doses in MDR-1 mutant dogs) is considered to be of limited clinical significance, the safety of the candidate formulation following repeated oral administration has been adequately demonstrated and the target animal safety data can be considered adequate to support safety in dogs (including collie breeds).

Dose determination/justification

Sarolaner: Sarolaner is included in the fixed combination formulation to target fleas and ticks. The applicant proposes a claim for immediate and persistent acaracidal activity against *Ixodes hexagonus, Ixodes ricinus* and *Rhipicephalus sanguineus* for 5 weeks and for 4 weeks against *Dermacentor reticulatus*. The recommended therapeutic dose (RTD) of sarolaner (1.2-2.4 mg/kg bw) in the fixed combination product differs (is lower) to that of the mono-active product Simparica (2-4 mg/kg bw). The applicant proposes the same persistent ectoparasiticidal efficacy (four or five weeks, depending on the targeted ectoparasite) as for the mono-active product Simparica but different speed of kill against ticks. The applicant argues that taking into account the fact that each additional component of a fixed combination product could increase the risk of adverse reactions and interactions between substances, the reduction of the minimum recommended dose of sarolaner in the fixed combination product compared to that included in the mono-active product Simparica contributes to reduce the overall risk of the fixed combination by widening the sarolaner margin of safety. However, no data to investigate possible interactions of the three active substances has been provided with this application.

A GCP dose determination study against *Dermacentor reticulatus* is presented. The minimum proposed RTD of 1.2 mg sarolaner/kg bw in the candidate formulation was selected as the central dose (1X), which was based on previous exploratory data presented in the marketing authorisation dossier of Simparica (EU/2/15/191/001-018). The CVMP considered for Simparica that "*the susceptibility of parasites tested could be divided into three clusters: "highly susceptible" to 20–30 ng/ml (I. ricinus, C. felis and R. sanguineus), "medium susceptible" to approximately 50 ng/ml (D. variabilis), and "less susceptible" to 70–80 ng/ml (A. maculatum and D. reticulatus)* (see EPAR for Simparica, EMA/605662/2015). As no indication against *A. maculatum* is proposed, the CVMP accepts (based upon previous CVMP assessment of the applicant's mono-active product Simparica), that *D. reticulatus* can be considered the dose limiting tick species for sarolaner.

The study evaluated the efficacy of three different sarolaner doses [(placebo), (0.6 + 0.012 + 2.5 mg/kg), (1.2 + 0.024 + 5 mg/kg bw) and 2.4 + 0.048 + 10 mg/kg bw)] of a combination product containing sarolaner, moxidectin and pyrantel embonate, respectively, against induced infestations of *Dermacentor reticulatus* for up to 35 days on dogs following a single oral administration. The study was conducted in South Africa using tick isolates that originated in Europe and the formulation referred to as "2016 Clinical Supplies". The results indicated that 0.6 mg/kg of sarolaner in the fixed

combination test product did not provide sufficient efficacy (≥90%) beyond 14 days after treatment administration and that a single oral dose of 1.2 mg/kg bw of sarolaner in combination with 24 µg/kg bw of moxidectin and 5 mg/kg bw of pyrantel embonate is the minimal efficacious dose required to demonstrate an acceptable acaricidal effect (including immediate acaricidal effect on an existing tick infestation) against adult *Dermacentor reticulatus* infestations on dogs over a period of up to 35 days.

Moxidectin and pyrantel provide anthelmintic efficacy against gastrointestinal nematodes through distinct mechanisms of action (i.e. both moxidectin and pyrantel provide efficacy against gastrointestinal nematodes such as *Ancylostoma caninum* and *Toxocara canis,* although against different developmental stages).

Moxidectin: Moxidectin is included to provide efficacy against L4 and immature adult (L5) stages of *Ancylostoma caninum*, *Dirofilaria immitis* and immature adult (L5) stage of *Angiostrongylus vasorum*. The RTD of moxidectin is 0.024-0.048 mg/kg bw.

Moxidectin is approved, in an oral dosing form, in several European countries for the prevention of heartworm (*D. immitis*) infections at a minimum recommended dose rate of 0.003 mg/kg bw. In the fixed combination product, the minimum RTD is 0.024 mg/kg bw of moxidectin.

A GCP dose determination study was conducted to determine the minimum efficacious dose of moxidectin in a combination product containing sarolaner, moxidectin and pyrantel embonate after a single oral administration for the prevention of induced *Angiostrongylus vasorum* infections in dogs. The study was conducted in Ireland using experimentally infected third-stage *A. vasorum* larvae isolates that originated in Europe. The prototype formulation '2015 Clinical Supplies' was used and dogs were administered the following doses: (placebo), (2 + 0.003 + 5 mg/kg bw), (2 + 0.012 + 5 mg/kg bw) (2 + 0.024 + 5 mg/kg bw) sarolaner, moxidectin and pyrantel embonate, respectively. Treatment efficacy was calculated based on the reduction of geometric mean adult worm counts in each IVP-treated group versus the placebo treatment group. Percent reductions in geometric mean faecal and lung larvae counts in the IVP-treated groups relative to the placebo group were also calculated.

The findings of the study indicated that the 0.003 mg/kg bw standard approved oral dose of moxidectin with a non-significant 7.2% reduction in adult worm counts versus placebo had no efficacy (0%) against the L5 stages of *A. vasorum.* The dose of 0.012 mg/kg bw moxidectin in the fixed combination product achieved a statistically significant (P=0.0234) reduction of 54.5% in adult worm counts versus placebo and 75.4% against the lung larvae of *A. vasorum*. A dose rate of 0.024 mg/kg bw moxidectin in the fixed combination achieved a reduction of 94.7% in adult worm counts versus placebo which was statistically significantly better than all other dose groups (P<0.0001) and 100% effective against the lung larvae of *A. vasorum*.

It is accepted that moxidectin is the only active substance in the fixed combination product to provide efficacy against vascular nematodes (*D. immitis* and *A. vasorum*). In a GCP non-interference study, the test item ("2015 Clinical Supplies") was administered at a dose rate of 2.0 mg/kg bw sarolaner / 0.024 mg/kg bw moxidectin / 5 mg/kg bw pyrantel (i.e. the dose of sarolaner differs from the RTD). Sarolaner showed negligible efficacy (8.2%) against *D. immitis*. Data available in the public domain indicates that pyrantel administered at a single oral dose of 5 mg/kg bw does not provide efficacy for heartworm prevention.

The results of a supportive exploratory laboratory study suggest that moxidectin administered at the dose rate of 0.024 mg/kg bw is the primary active substance in the fixed combination product against L4 and L5 stage *A. caninum*. Variable efficacy of moxidectin against adult stages *T. canis* was observed in the studies provided. In a GCP non-interference study, the mono-active moxidectin at a dose of 0.024 mg/kg bw achieved satisfactory efficacy (92.8%) against adult *T. canis*; however, inconsistent

efficacy was observed when moxidectin was administered at the same dose (0.024 mg/kg bw) in exploratory studies. These findings suggest that although moxidectin contributes with pyrantel to the efficacy against adult GI nematodes, pyrantel is considered to be the primary active substance in the combination product with activity against adult GI nematodes. No claim of additive efficacy is made.

Pyrantel: Pyrantel is included to target immature adult (L5) and adult stages of *Toxocara canis,* and adult stages of *Ancylostoma caninum, Toxascaris leonina* and *Uncinaria stenocephala.* The RTD of pyrantel is 5-10 mg/kg bw, which is the same dose used in all authorised oral formulations in the EU. Therefore, no dose determination study data is provided for pyrantel with regard to the efficacy against GI nematodes. The applicant justifies the omission of a dose determination study investigating a lower dose of pyrantel in the combination product with reference to the CVMP guideline on pharmaceutical fixed combination products on the basis that, to select a different (lower) pyrantel dose in the combination, a constant and reliable baseline of moxidectin efficacy on GI nematodes would have been required. Furthermore, pyrantel embonate has a very wide safety margin (the LD_{50} is 2000 mg/kg bw for dogs after oral administration).

The applicant states that pyrantel's activity spectrum is broadened by moxidectin to include immature hookworms (L4 larvae and immature adults of *Ancylostoma caninum*). Pyrantel (as embonate salt) is only poorly absorbed from the gastrointestinal tract and therefore it has no efficacy against extra-intestinal nematode species (*D. immitis* or *A. vasorum*).

The efficacy of pyrantel against adult hookworms and roundworms is well-established. Findings from studies provided by the applicant suggest that moxidectin contributes with pyrantel to the efficacy against adult GI nematodes (see above moxidectin); however, pyrantel is considered to be the primary active substance in the combination product against adult GI nematodes. No claim of additive efficacy is made. In the GCP non-interference study, sarolaner administered as a monovalent substance at the RTD provided minimal efficacy (36.7%) against adult *T. canis.*

Based on the totality of data provided (pharmacodynamic, pharmacokinetic and preliminary clinical efficacy studies, including the non-interference studies that follow), the CVMP accepts that the minimum proposed treatment doses of 1.2 mg/kg bw for sarolaner, 0.024 mg/kg bw for moxidectin and 5 mg/kg bw for pyrantel embonate are reasonable to be taken forward for confirmation in dose confirmatory and clinical field studies.

Target animal tolerance

The target animal tolerance for Simparica Trio has been investigated in one pivotal target animal safety (TAS) study and several other TAS studies using the fixed combination product formulation. In addition, the applicant presents proprietary data for the individual active substance sarolaner (previously assessed by the CVMP in the context of the Simparica dossier), and publically available scientific literature for each of the active substances moxidectin and pyrantel.

Sarolaner: the proposed RTD in Simparica Trio is 1.2–2.4 mg/kg bw. The RTD of the mono-active product Simparica (EU/2/15/191/001-018) containing sarolaner is 2–4 mg/kg bw orally at a minimum of monthly intervals. Oral tolerance of sarolaner as a single ingredient was previously assessed by the CVMP (see the European Public Assessment Report (EPAR) for Simparica (EMA/605662/2015)). In general, Simparica chewable tablets were well tolerated when administered at the recommended treatment dose of 2–4 mg/kg bw. However, neurological signs were seen in dogs treated at 3X the maximum RTD (i.e. 12 mg/kg bw), generally manifested as tremors.

Moxidectin: The RTD of moxidectin in the combination product is 24–48 µg/kg bw (0.024–0.048 mg/kg bw). Based on information provided from the published literature, it is evident that, at sufficiently high

doses, avermectins and milbemycins (moxidectin) may be toxic to dogs. Signs of toxicity are indicative of neurotoxicity and include lethargy, hypersalivation and ataxia. However, the main concern with these compounds is the increased sensitivity shown by some animals in the collie population and in some other breeds of dog. This sensitivity arises from the MDR-1 gene deletion and accompanying defective P-glycoprotein which permits increased intestinal and brain permeability for some xenobiotics, thus resulting in increased gastrointestinal absorption and higher exposure to the brain.

Pyrantel: The RTD of pyrantel in Simparica Trio is 5–10 mg pyrantel base/kg bw. Based on information provided from the published literature, it is accepted that pyrantel embonate is a compound with a high margin of safety. Overall, the most common adverse effects appear to be gastrointestinal and some of those may be related to the rapid killing of the intestinal parasites (e.g. worm impaction, especially in young animals).

Fixed combination product (combination of sarolaner, moxidectin and pyrantel): the applicant submitted the results of several target animal safety (TAS) studies.

Pivotal TAS study

In the pivotal TAS study, the safety of sarolaner in combination with moxidectin and pyrantel was studied in 32 Beagle puppies of 8 weeks of age. A developmental formulation ("2016 Clinical Supplies") was used. Based on data provided, it is accepted that the "2016 Clinical Supplies" formulation is sufficiently representative of the final formulation intended to be marketed to permit extrapolation of tolerance data to the final formulation. Animals weighed ≥1.8 kg bw, which is slightly higher than the minimum bodyweight (1.25 kg) of animals to which it is proposed to recommend product administration (i.e. the animals in which the recommended dose would not be exceeded when using the lowest strength available). This study was GLP-compliant and conducted in accordance with VICH GL43. Noting that the guideline recommends that the animals in the TAS studies should generally be the youngest age for which product approval is sought, a statement has been included in section 4.5 of the SPC indicating that *"in the absence of available data, treatment of puppies less than 8 weeks of age and/or dogs less than 1.25 kg bodyweight should be based on a benefit-risk assessment by the responsible veterinarian".* There were 4 treatment groups with 8 puppies per group. Treatment group T01 was a non-treated control (sham dosed). Treatment groups T02, T03 and T04 were given the prototype formulation "2016 Clinical Supplies" in a fed state at the following doses respectively: 1X (2.4, 0.048 and 10 mg/kg bw of sarolaner, moxidectin and pyrantel, respectively), 3X (7.2, 0.144 and 30 mg/kg bw), or 5X (12, 0.24 and 50 mg/kg bw) of the maximum RTD for 7 doses at monthly intervals.

In this study, the product was generally well tolerated at doses up to 5 times the maximum recommended treatment dose, with no evidence of adverse effects on clinical, clinic-pathological or pathological parameters. Muscle fasciculation (shivering) was observed in 4, 5, 5, and 7 dogs in groups T01, T02, T03, and T04, respectively. Given that the majority of these observations were sporadic and occurred at the early stage of the study when the puppies were very young and also in the placebo group, the applicant considers that these signs may have been related to shivering in response to cold/stress. Mydriasis was observed in 4, 2, 1, and 1 dog in groups T01, T02, T03, and T04, respectively, and salivation was observed in 4, 2 and 1 dog in groups T02, T03, and T04, respectively, which were not considered to be related to treatment. Gastrointestinal disturbances (emesis, abnormal faeces) were noted in some pups during the course of the study. Twenty-six emetic events occurring within 0-48 hours of dosing at a frequency of 2, 7, 11, and 6 cases in groups T01, T02, T03, and T04, respectively. Cases of soft faeces/mucoid faeces/diarrhoea were also observed, which the applicant considered likely to be associated with stress resulting from frequent handling/single housing of young puppies. Based on the results of this study, the CVMP concludes that the fixed combination product,

when administered orally at 1X, 3X, and 5X the maximum intended clinical dose once monthly over a 6-month period (7 consecutive doses) to 8-week old Beagle puppies, was well tolerated.

Other TAS studies

In a second TAS study, moxidectin in combination with sarolaner and pyrantel was investigated in 31 sensitive collies. In this non-GLP compliant study, there were 4 treatment groups with 8 dogs per group (except group T04 (5X) in which one dog has a seizure prior to treatment on Day -7 and was not replaced). The final commercial formulation was used and avermectin-sensitive (MDR-1 gene mutation) collie dogs were administered the test item in a fed state based upon their body weights at 1X (2.4 mg/kg bw sarolaner, 0.048 mg/kg bw moxidectin and 10 mg/kg bw pyrantel), 3X (7.2 mg/kg bw sarolaner, 0.144 mg/kg bw moxidectin and 30 mg/kg bw pyrantel), or 5X (12 mg/kg bw sarolaner, 0.24 mg/kg bw moxidectin and 50 mg/kg bw pyrantel) the maximum RTD. Mild to moderate neurological signs associated with avermectin sensitivity in MDR negative collies (including mild ataxia, mild depression, mild and moderate muscle fasciculations, mild mydriasis, and mild salivation) were observed in animals in all four study groups. Given that the incidence of neurological signs observed in the placebo group was higher than that observed in the 1X group (T02) and the 3X group (T03) combined, the CVMP questioned the sensitivity of the assessment conducted in terms of differentiating nervous behaviour from possible avermectin toxicosis. In response to a question from the CVMP, the applicant reviewed the neurological signs observed based on the total incidence and the number of dogs affected and concluded that there is a clear 3X margin of safety in avermectin-sensitive collies and signs associated with test article are observed at 5X (ataxia and muscle fasciculations). Having considered the data provided in the revised assessment, the CVMP accepts that there is insufficient evidence to conclude that the clinical signs observed following single oral administration at 1X and 3X the recommended dose were product-related.

In another exploratory tolerance study in MDR1-/- collies, a non-final formulation containing only moxidectin and sarolaner was used. The dogs (5 per group) were administered 12 mg/kg bw sarolaner and 144, 240, or 360 µg/kg bw moxidectin (i.e. 3X, 5X, or 7.5X maximum RTD of moxidectin). Signs consistent with avermectin toxicosis were not observed at 3X the recommended moxidectin dose, but occurred at 5X and 7.5X that dose (ataxia, hypersalivation, depression, and muscle fasciculation, all resolving within two days without treatment).

The CVMP accepts that an acceptable safety margin in MDR1-/- collies has been demonstrated following single oral administration at 1X and 3X the maximum recommended treatment dose and a warning statement has been included in section 4.5i indicating that the recommended dose should be strictly observed in collies or related breeds. Information on the effects observed in collies following administration of the test item at 5X the maximum recommended treatment dose has been included in SPC section 4.10. Sarolaner and moxidectin have been shown to be substrates of the efflux transporter PGP and avermectin-sensitive dogs have no PGP efflux capability due to a mutation in their ABCB1 genome. The CVMP concludes that the safety profile of the combination product when administered to avermectin sensitive dogs with no PGP efflux capability due to a mutation in their ABCB1 genome has been adequately characterised.

In another study, tolerance to the fixed combination candidate formulation was also investigated in 24 (10 month old) Beagle dogs experimentally infected with adult stage *Dirofilaria immitis.* In this GLP compliant study, there were 3 treatment groups with 8 dogs per group. Dogs were experimentally infected via surgical transplantation and infection status was verified prior to the study. Dogs were administered the prototype formulation "2016 Clinical Supplies" in a fed state at 1X (2.4 mg/kg bw sarolaner, 0.048 mg/kg bw moxidectin and 10 mg/kg bw pyrantel) or 3X (7.2 mg/kg bw sarolaner, 0.144 mg/kg bw moxidectin and 30 mg/kg bw pyrantel) the maximum RTD three times at 28-day intervals. A significant reduction in microfilariae was observed in both treatment groups at all time

points following dosing. No severe anaphylactic reactions were reported. However, pyrexia was observed in 2 dogs on Day 1 (one dog in 1X group and one dog in 3X group). The applicant considers that these symptoms are likely to be related to a mild systemic inflammatory reaction secondary to microfilaria reduction associated with treatment.

Based on the findings of this study, the CVMP accepts that the test article was generally well tolerated when administered at doses up to 3X the maximum proposed dose (7.2 mg/kg bw sarolaner + 144 µg/kg bw moxidectin + 30 mg/kg bw pyrantel) in dogs experimentally infected with adult *D. immitis.*

Other clinical signs observed during this study included emesis, decreased appetite and abnormal stools (soft stool or mucoid diarrhoea or haemorrhagic colitis).

In response to a question from the CVMP relating to a possible association between the gastrointestinal disturbances (vomiting and soft faeces/mucoid faeces/diarrhoea) and treatment, the applicant reviewed all relevant safety data and concluded that whilst gastrointestinal signs were observed on occasion in animals administered the prototype formulation 2016 Clinical Supplies or the final formulation intended for marketing, such signs are unlikely to be related to treatment. It is noted that the dataset reviewed includes 56 dogs in three target animal safety studies, 234 dogs treated in the 21 laboratory efficacy studies and 1969 dogs which received the prototype formulation 2016 Clinical Supplies in 14 clinical field studies. Taking account of all available information, the CVMP accepted that there is insufficient evidence to conclude that the administration of Simparica Trio is associated with gastrointestinal adverse events.

In conclusion, based on the totality of data obtained from target animal safety studies and various confirmatory and field efficacy studies conducted, the CVMP accepts that Simparica Trio was generally well tolerated.

Dose confirmation studies

The applicant provided a large number of laboratory dose confirmation studies, with at least 2 studies per parasite for most target parasite species, which were conducted in the EU, US and/or other regions. The confirmatory efficacy studies are grouped by parasite and claim type into four groups: flea efficacy, tick efficacy, gastrointestinal (GI) nematode efficacy, and vascular nematode efficacy. With the exception of the supportive tick efficacy study against *Dermacentor reticulatus* and the supportive gastrointestinal nematode efficacy studies against L5 stage of *Toxocara canis* and L4 and L5 stages of *Ancylostoma caninum*, all studies were conducted according to the standards of VICH GCP.

Although it is noted that three formulations have been referred to in the clinical studies used to investigate the safety and efficacy of the candidate formulation, the majority used the '2016 Clinical Supplies' formulation which was most similar to the final formulation. In the opinion of the CVMP, it would normally be expected that the formulation used to demonstrate safety and efficacy would be the final formulation. Given that a non-final formulation has been used, a number of questions have been raised concerning the data provided in support of similarity of the final formulation compared to the developmental formulation. In order to address this concern, the applicant provided *in vivo* bioequivalence study data comparing systemic bioavailability between the final formulation intended to be marketed and the two developmental formulations. This was also supported by *in vitro* dissolution study data. Based on the data provided, it can be accepted that the "2016 Clinical Supplies" and the "2015 Clinical Supplies" formulation is sufficiently representative of the final formulation intended to be marketed to permit extrapolation of the findings from the dose confirmation studies to the final formulation.

In the dose confirmation studies, dogs were fasted overnight before dosing and were not fed again until at least four hours after treatment administration. All flea and tick efficacy studies were conducted using the same basic design as detailed in the CVMP Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats (EMA/CVMP/EWP/005/2000-Rev.3). Group allocation was by ranking the dogs by descending parasite infestation rates and random allocation to the study groups, including at least 8 dogs (adults, usually Beagle dogs or purpose-bred cross-breeds) in each treatment group. The assessment of efficacy was based on the percent reduction in the arithmetic mean (live parasite counts relative to control) using the recommended Abbott's formula.

Ticks

All pivotal tick efficacy studies used the prototype formulation referred to as "2016 Clinical Supplies". The product was administered in accordance with the proposed SPC and a dose as close to the minimal proposed treatment dose was administered. Fifty unfed ticks were used, with an appropriate sex ratio. Timing of infestation and counting was in accordance with the guideline recommendations. Group allocation was by ranking the dogs by descending parasite infestation rates and random allocation to the study groups; >6 dogs (adult Beagle and mixed breed dogs) per group were included. Tick strains originating from within the EU were used in both dose confirmatory studies for each of the tick species studied apart from *R. sanguineus* for which only one study used an EU originating tick. Arithmetic mean counts have been used to calculate efficacy (using Abbott's formula).

Efficacy studies against the proposed tick species including *Dermacentor reticulatus, Rhipicephalus sanguineus, Ixodes ricinus and Ixodes hexagonus* are presented below. A supportive laboratory efficacy study was also submitted for the tick species *A. maculatum* for which an indication is not claimed.

Given that parasites need to start feeding on the host to become exposed to the active substance, a statement is included in the product literature that the risk of the transmission of parasite-borne diseases cannot be excluded.

It is the opinion of the CVMP that the dose confirmation studies are adequate to support efficacy against:

Dermacentor reticulatus: One dose determination study and one dose confirmation study are presented. An exploratory non-GCP study was also provided. The prototype formulation "2016 Clinical Supplies" was used in both the dose determination and dose confirmation studies. The dose determination study was conducted in South Africa using tick isolates that originated in Europe. Based on the results of this study, a single oral dose of 1.2 mg/kg bw sarolaner in combination with 24 µg/kg bw moxidectin and 5 mg/kg bw pyrantel embonate demonstrated satisfactory immediate efficacy and persistent efficacy for up to 35 days (≥95.0% efficacy). The dose confirmation study was conducted in Europe using tick isolates that originated in Europe. The study demonstrated satisfactory immediate efficacy and persistent efficacy for up to 28 days (≥97.2% efficacy). Although efficacy was not demonstrated at Day 35 (84.3%), the data are considered adequate to support the applicant's claim for 4 weeks against *D. reticulatus.* Adequate field data are available to support the proposed indication against *D. reticulatus.*

Ixodes ricinus: Two dose confirmation studies are presented. Both studies were conducted using the prototype formulation "2016 Clinical Supplies" in Europe using tick isolates that originated in Europe. Both studies demonstrated satisfactory immediate efficacy and persistent efficacy for up to 35 days (≥97.2% efficacy). Adequate field data are available to support an indication against *I. ricinus*.

Ixodes hexagonus: Two dose confirmation studies are presented. However, the results of one of these studies are considered invalid due to inadequate infestation of control animals. Both studies used the

prototype formulation "2016 Clinical Supplies". One study was conducted in Europe using tick isolates that originated in Europe. The study demonstrated satisfactory immediate efficacy and persistent efficacy for up to 35 days (≥98.6% efficacy).

Although efficacy has only been adequately supported by one dose confirmatory study, the results from one study against *I. hexagonus* demonstrated a higher efficacy than against the least susceptible tick species (*D. reticulatus*) and the results of this study are comparable to that of the two dose confirmation studies against *I. ricinus* (≥97.2%) for 35 days after treatment administration.

Moreover, it is evident from the EPAR for Stronghold Plus (EMEA/V/C/004194/0000) that the CVMP has previously accepted that, 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 and that 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.*

The similar or higher *in vivo* susceptibility of *I. hexagonus* compared to *I. ricinus* is confirmed by the results of the dose confirmation studies conducted in cats with Stronghold Plus (in which the sarolaner component provides the efficacy against ticks). These studies (one with *I. hexagonus* and two with *I. ricinus*) show in particular that the efficacy level at the 5-week time point is 100% against both *I. hexagonus* and *I. ricinus*; the efficacy figures obtained against *D. reticulatus* show that the comparison at the time point of 5 weeks is sensitive, because at that time efficacy has become insufficient in this dose-limiting tick species.

Based upon the totality of data provided, evidence would suggest that the susceptibility of *I. ricinus* and *I. hexagonus* to sarolaner following oral administration to dogs is likely to be similar. In light of the above and in consideration of the 'three Rs', the CVMP accepts the omission of a second dose confirmatory study for *I. hexagonus* in this instance and that the data are considered adequate to support the applicant's claim for 5 weeks against *I. hexagonus.* Although the field data provided for *I. hexagonus* are limited (only 3 dogs in the Simparica Trio group, i.e. 2.4%, were infested with that species), it is considered that the overall data package provided is adequate to support the proposed indication.

Rhipicephalus sanguineus: Four dose confirmation studies are presented. However, the results of one of these studies are considered invalid by the applicant due to a change in management of ticks. All four studies used the prototype formulation "2016 Clinical Supplies". One study was conducted in South Africa using tick isolates that originated in Europe. The study demonstrated satisfactory immediate efficacy and persistent efficacy for up to 35 days (≥97.2% efficacy). Another was conducted in USA using tick isolates that originated in USA. The study demonstrated satisfactory immediate efficacy and persistent efficacy for up to 35 days (≥94.2% efficacy) except on Day 14 when efficacy was marginally below the threshold (89.7%). One study was conducted in USA using tick isolates that originated in USA. The study demonstrated satisfactory immediate efficacy and persistent efficacy for up to 35 days (≥94% efficacy). While two studies were conducted using tick isolates that originated outside the EU, taking into account the results of one dose confirmation study and field study which provides adequate data to support the indication against *R. sanguineus* found on dogs under natural conditions within the EU, the CVMP is of the opinion that further dose confirmatory data is unnecessary. The CVMP concludes that adequate laboratory efficacy data has been provided to support the indication against *Rhipicephalus sanguineus*.

Taking all of the above into account, the CVMP is of the opinion that a claim against *Dermacentor reticulatus, Ixodes hexagonus, Ixodes ricinus* and *Rhipicephalus sanguineus* has been adequately supported (that is, tick species for which there are adequate dose confirmation data and for which efficacy has been confirmed in the field).

No speed of kill studies against ticks were submitted. Based on the results of the dose confirmation studies, the CVMP accepts that ticks on the animal prior to administration or from new infestations after product administration are killed within 48 hours. Consequently, the information proposed for inclusion in SPC section 5.1 ("Ticks on the animal prior to administration or from new infestations after product administration are killed within 48 hours") is considered acceptable.

Fleas

All pivotal flea efficacy studies against *Ctenocephalides felis* and *C. canis* were conducted in accordance with GCP and, in general terms, the design of each study was in line with guideline requirements. All studies used the prototype formulation referred to as "2016 Clinical Supplies". The product was administered in accordance with the proposed SPC and a dose as close to the minimal proposed treatment dose was administered. Flea infestations were conducted by placing approximately 100 viable, adult unfed fleas with an appropriate sex ratio 1:1 directly on each dog. Timing of infestation and counting was in accordance with the guideline recommendations. More than 6 dogs (Beagle and mixed-breed dogs) per group were used. Flea strains originating from within the EU were used in one dose confirmatory study for each of the flea species studied. Arithmetic mean counts have been used to calculate efficacy (using Abbott's formula).

Ctenocephalides felis: Two dose confirmation studies are presented. One study was conducted in South Africa using flea isolates that originated in Europe and the study demonstrated satisfactory immediate efficacy and persistent efficacy for up to 35 days (≥99.9% efficacy). Another study was conducted in the USA using flea isolates that originated in the USA. The study demonstrated satisfactory immediate efficacy and persistent efficacy for up to 35 days (≥99.7% efficacy) when the test product was administered at a dose of 1.2 mg/kg bw sarolaner + 0.024 mg/kg bw moxidectin + 5 mg/kg bw pyrantel). While the latter study was conducted using flea isolates that originated outside the EU, taking into account the results of field study which demonstrated effectiveness against the claimed flea species (*C. felis* and *C. canis*) found in dogs under natural conditions within the EU, and given that differences in susceptibility of fleas to sarolaner isolated in the US and Europe are not anticipated in view of current knowledge, the CVMP is of the opinion that further dose confirmatory data is unnecessary. The CVMP concludes that adequate laboratory efficacy data has been provided to support the indication against *C. felis* and *C. canis*.

Ctenocephalides canis: One dose confirmation study conducted in the EU using flea isolates that originated in the EU is presented. The study demonstrated satisfactory immediate efficacy and persistent efficacy for up to 35 days (100% efficacy). It is noted that the relevant CVMP guideline (7AE17a - Demonstration of efficacy of ectoparasiticides) recommends two dose confirmation studies per claimed target parasite; however, it is not expected that there will be a marked difference in sensitivity between the two flea species. In addition, it is acknowledged that the predominant flea species is *C. felis* and that the applicant's mono-active product 'Simparica' has been approved for use against both *C. felis* and *C. canis*.

Speed of kill study: A single speed of kill study against *C. felis* is presented. This study was conducted within the USA and the isolate used to induce artificial infestation originated from the USA. Given that differences in susceptibility of fleas to sarolaner isolated in the US and Europe are not anticipated, this study can be accepted as sufficiently representative of the EU. Greater than 95% kill within 8 hours was achieved on the day of treatment and this (>95% kill within 8 hours) was maintained until Day 7. Greater than 95% kill within 12 hours was maintained until Day 28, with a reduction to 85.3% efficacy by Day 35. Based on the results of the dose confirmation studies, the CVMP accepts that fleas on the animal from new infestations after product administration are killed within 24 hours of attachment for 5 weeks after product administration. In line with guideline EMEA/CVMP/EWP/005/2000-Rev.3 requirements, the CVMP accepts that the onset of efficacy is within 12 to 24 hours of attachment for

five weeks after product administration and fleas are killed within 8 hours on the animal prior to administration.

Prevention of pre-adult stages of fleas on dogs: One study was presented to demonstrate efficacy in the prevention of flea infestations (C. felis) with pre-adult stages. The study was conducted within the USA and the isolate used to induce artificial infestation originated from the USA. The test product killed adult fleas before they had an opportunity to lay eggs, thus reducing the risk of environment contamination with fleas. The applicant proposes to include the following wording in SPC section 5.1: "The veterinary medicinal product kills newly emerged fleas on the dog before they can lay eggs and therefore it prevents environmental flea contamination in areas to which the dog has access" and this is considered acceptable.

Gastrointestinal nematodes

All gastrointestinal nematode studies were conducted using the same basic design as detailed in the VICH GL7 Efficacy of Anthelmintics: General Requirements (CVMP/VICH/832/99-Corr.) and generally in accordance with VICH GL19 Efficacy of Anthelmintics: Specific Recommendations for Canines (CVMP/VICH/835/1999). The prototype formulation referred to as "2016 Clinical Supplies" was used in all GI nematode efficacy studies except three studies where the final formulation intended for marketing was used. Study animals were orally inoculated with embryonated eggs/larvae at various time points depending on the species and developmental stage. In the studies against adult stages, healthy dogs with the highest geometric mean faecal egg counts between Days -9 and -4 were selected and allocated randomly to treatment groups according to a randomised complete block design based upon pre-treatment geometric mean faecal egg counts. In the non-interference study against adult *T. canis*, randomisation was based upon the egg counts on Day -3. In the studies against immature stages, the dogs were ranked based upon their body weight by gender and in each gender the same number of dogs with the highest body weight were selected. The selected dogs were allocated randomly to treatment groups according to a randomised complete block design based upon body weight. Dogs were administered the IVP or placebo at Day 0 and faecal egg counts were performed at enrolment, -8, -6, -4, -3 and at necropsy on Day 7 (except one study when faecal egg counts were performed at enrolment, -9, -7, -5, -4 and at necropsy on Day 7). In another study, necropsy and worm counts were conducted on Day 10. At necropsy, the entire gastrointestinal tract was removed, the mucosa was scraped twice to remove the worms and the worms were enumerated. The assessment of efficacy was based on the percent reduction in the geometric mean (worm counts at necropsy relative to control) using the recommended Abbott's formula. The worm counts of the treated group and the control group was compared using a mixed linear model. Testing was two-sided at the significance level P=0.05.

It is the opinion of the CVMP that the data provided are adequate to support efficacy against:

Toxascaris leonina (adults): Two valid dose confirmation studies against adult stage *Toxascaris leonina* are presented. Both studies were conducted in South Africa using worm isolates that originated from two different sources in the EU. It is accepted that test animals in both studies were adequately infected. One study demonstrated satisfactory efficacy (99.6%) against adult *T. leonina* and the efficacy demonstrated in another study was marginally below the threshold (89.7%). Although only 16 dogs in the European field study were infected with *T. leonina* at enrolment, the CVMP is prepared to accept that the confirmatory studies are adequately supported by field data (98.2% reduction in faecal egg output) against *T. leonina.* The CVMP concludes that adequate laboratory efficacy data has been provided to support the indication against adult stage *Toxascaris leonina.*

Uncinaria stenocephala (adults): Two dose confirmation studies against adult stage *Uncinaria stenocephala* are presented. Both studies were conducted in South Africa using worm isolates that originated from different sources in the EU. It is accepted that test animals in both studies were

adequately infected. Both studies demonstrated satisfactory efficacy (100%) against adult *U. stenocephala*. Although it is acknowledged that the positive control product used in the European field trial is not authorised for the treatment of *Uncinaria stenocephala* in the EU, the CVMP is prepared to accept that the confirmatory studies are adequately supported by field data (99.79% reduction in faecal egg output) against *U. stenocephala.* The CVMP concludes that adequate laboratory efficacy data has been provided to support the indication against adult stage *Uncinaria stenocephala.*

Toxocara canis (adults): Two dose confirmation studies against adult stage *T. canis* are presented. One study was conducted in South Africa using worm isolates that originated in the EU. Another study was conducted in the USA using worm isolates that originated in the USA. It is accepted that test animals in both studies were adequately infected. The first study demonstrated satisfactory efficacy (97.3%) against adult *T. canis*. The second demonstrated satisfactory efficacy (99.2%) against adult *T. canis* using a single oral dose of the test product administered at 1.2 mg/kg bw sarolaner, 0.024 mg/kg bw moxidectin and 5 mg/kg bw pyrantel. While the second study was conducted using worm isolates that originated outside the EU, taking into account the results of field study which demonstrated an adequate level of efficacy (99.0% reduction in faecal egg output) against *T. canis* found in dogs under natural conditions within the EU, the CVMP is of the opinion that further dose confirmatory data is unnecessary. The CVMP concludes that adequate laboratory efficacy data has been provided to support the indication against adult stage *T. canis.*

Ancylostoma caninum (adults): Two dose confirmation studies investigating efficacy against adult stage *A. caninum* are presented. One study was conducted in South Africa using worm isolates that originated in the USA. Another study was conducted in Morrocco using worm isolates that originated in EU. It is accepted that test animals in both studies were adequately infected. Both studies demonstrated satisfactory efficacy (100%) against adult *A. caninum*. While one study was conducted using worm isolates that originated outside the EU, taking into account the results of field study which demonstrated an adequate level of efficacy (98.7% reduction in faecal egg output) against *A. caninum* found in dogs under natural conditions within the EU, the CVMP is of the opinion that further dose confirmatory data is unnecessary. The CVMP concludes that adequate laboratory efficacy data has been provided to support the indication against adult stage *A. caninum.*

Toxocara canis (immature adults (L5)): Two dose confirmation studies are presented. An exploratory non-GCP study was also provided. One study was conducted in the USA using worm isolates that originated in the USA. In this study, the CVMP accepts that the placebo treated negative control animals maintained adequate GI nematode infections (range of 4 to 24 and a geometric mean of 10.6). This study demonstrated efficacy of 95.2% against L5 stage *T. canis* using a single oral dose of the test product administered at 1.2 mg/kg bw sarolaner, 0.024 mg/kg bw moxidectin and 5 mg/kg bw pyrantel. The second dose confirmation study was conducted in South Africa using worm isolates that originated in the EU. In this study, the adequacy of infection with *T. canis* (sum of L4, L5 and adult) was justified. The required efficacy of the IVP against the total worm count (sum of L4, L5 and adult) was achieved (97.9%). While one of the studies was conducted using worm isolates that originated outside the EU, taking into account the results of the EU dose confirmation study and the EU field study and given that differences in susceptibility of immature adult (L5) stages *T. canis* to pyrantel isolated in the US and Europe are not anticipated, the CVMP is of the opinion that further dose confirmatory data is unnecessary. The CVMP concludes that adequate laboratory efficacy data has been provided to support the indication against immature adults (L5) stage *T. canis.*

Ancylostoma caninum (L4 larvae): Two pivotal studies and one supportive study against L4 stage *A. caninum* are presented. In the supportive study, moxidectin and pyrantel were administered as mono-active substances. One study was conducted in South Africa using worm isolates that originated in the USA. In line with VICH GL19, study animals were aged 7 to 11 weeks and 8 to 10 weeks on Day -14, the range of infective stages used to produce adequate infections in dogs was 200±50, time of

treatment after infection and the time period from the termination of treatment until necropsy were both 7 days. In both studies there was an adequate level of infection. The required efficacy of the IVP against the total worm count (sum of L4, L5 and adult) was achieved in both the first (98.4%) and second (100%) study. While one study was conducted using worm isolates that originated outside the EU, taking into account the results of the EU dose confirmation study and the EU field trial and given that differences in susceptibility of L4 stage *Ancylostoma caninum* to moxidectin isolated in the US and Europe are not anticipated, the CVMP is of the opinion that further dose confirmatory data is unnecessary. The CVMP concludes that adequate laboratory efficacy data has been provided to support the indication against L4 stage *A. caninum.*

Ancylostoma caninum (immature adults (L5)): Two pivotal dose confirmation studies and one supportive exploratory laboratory study against L5 stage *A. caninum* are presented. One study was conducted in South Africa using worm isolates that originated in the USA and one study using isolates that originated from EU. In both studies there was and adequate level of infection. The required efficacy of the IVP against the total worm A166C-ZA-18-959, the total *A. caninum* was achieved (99.8%). In the second study (with EU isolate) the required efficacy of the IVP against the total worm count was achieved (100%). While one study was conducted using worm isolates that originated outside the EU, taking into account the results of the dose confirmation study and the EU field trial and given that differences in susceptibility of L5 stage *Ancylostoma caninum* to moxidectin isolated in the US and Europe are not anticipated, the CVMP is of the opinion that further dose confirmatory data is unnecessary. The CVMP concludes that adequate laboratory efficacy data has been provided to support the indication against immature adult (L5) stage *A. caninum.*

Vascular nematodes

All vascular nematode studies were conducted using the same basic design as detailed in the VICH GL7 Efficacy of Anthelmintics: General Requirements (CVMP/VICH/832/99-Corr.) and generally in accordance with VICH GL19 Efficacy of Anthelmintics: Specific Recommendations for Canines (CVMP/VICH/835/1999). The prototype formulation referred to as "2016 Clinical Supplies" was used in the *Angiostrongylus vasorum* efficacy studies and three of the *Dirofilaria immitis* field efficacy studies. The prototype formulation referred to as "2015 Clinical Supplies" was used in three of the *Dirofilaria immitis* efficacy studies. Study animals were inoculated with infective L3 larvae at various time points depending on the species and developmental stage.

In the dose confirmation studies for the prevention of angiostrongylosis, the dogs that did not vomit after inoculation were ranked by increasing age and were allocated randomly to treatment groups according to a randomised complete block design based upon age. Dogs recruited to these studies were administered the IVP or placebo at Day 0 and faecal egg counts were performed at enrolment and at several time points between Day -8 and Day 58. Necropsy was carried out on Day 63/64 or 64/66.

Dogs recruited to the dose confirmation studies for the prevention of heartworm disease were ranked by body weight and were allocated randomly to treatment groups according to a randomised complete block design. Dogs were administered the IVP or placebo at Day 0 and microfilariae and antigen testing were performed at Day -32 and 90. Necropsy was carried out on Day 118 or 122.

The assessment of efficacy was based on the percent reduction in the geometric mean (worm counts at necropsy relative to control) using the recommended Abbott's formula. The worm counts of the treated group and the control group was compared using a mixed linear model. Testing was two-sided at the significance level $P<0.05$.

It is the opinion of the CVMP that efficacy against the following parasites has been sufficiently supported:

Angiostrongylus vasorum: Two dose confirmation studies against L4 and L5 larval stages *A. vasorum* are presented. Both studies were conducted in Europe using European isolates. It is accepted that test animals in both studies were adequately infected. Day 28 post inoculation was identified as the time point to differentiate between efficacy of L4 and L5 larvae. At the time point chosen for necropsy (Day 63 or 64 in the first study and Day 64 or 66 in the second study) only adult worms were identified. Using these time points, the applicant claims that the first study demonstrated satisfactory efficacy against L4 (90.6%) and L5 (94%) *A. vasorum*, and the second study demonstrated satisfactory efficacy against L5 (92.9%) but not L4 (73.5%) *A. vasorum* using a single oral dose of the test product administered at 1.2 mg/kg bw sarolaner, 0.024 mg/kg bw moxidectin and 5 mg/kg bw pyrantel. In response to a question raised by the CVMP, the applicant has provided evidence that efficacious moxidectin levels to eliminate lung worms are expected to persist for only a few days after treatment administration. Data regarding the lifecycle and prepatent period has been provided supporting the timing of treatment (Day 28 post-inoculation) and necropsy during the dose confirmation studies, to target the L5 stages of *A. vasorum*. The CVMP concludes that adequate laboratory and field efficacy data has been provided to support the indication against immature adult (L5) stage *A. vasorum.*

Dirofilaria immitis: Two valid dose confirmation studies to support prevention of heartworm disease are provided, one of which also investigated non-interference. It is accepted that test animals in both studies were adequately infected and that the studies can be accepted as valid. Both studies demonstrated satisfactory efficacy (100%) against *D. immitis* as 100% efficacy against development of dirofilariasis due to adult *D. immitis* following artificial infection with L3 larvae was achieved in both studies. One study also supports the non-interference of the efficacy of moxidectin by the addition of sarolaner and pyrantel in the fixed combination and supports the inclusion of moxidectin in the combination formulation. Field studies evaluated the efficacy of *D. immitis* outside the EU. Efficacy of 100% against *D. immitis* in dogs under natural conditions within the USA, Japan and Australia was demonstrated. Taking the sum of the data provided into account and given that differences in susceptibility of *D. immitis* to moxidectin isolated in three countries, on three different continents are not anticipated to differ with *D. immitis* isolates in Europe, the CVMP is of the opinion that further dose confirmatory data is unnecessary. The CVMP concludes that adequate laboratory and field efficacy data has been provided to support the indication against *D. immitis*. In addition, it is noted that although resistance of *D. immitis* to moxidectin has been reported in the Mississippi River Delta region of the USA, no evidence of resistance in Europe has been reported. In both dose confirmation studies, as no re-challenge was investigated, it cannot be determined for how long the effect against *D. immitis* lasts. That said, the fact that the product was administered 30 days after artificial infestation with L3 larvae in both studies and given the findings of the studies, it can be accepted that even if re-infestation occurs after product administration, then the subsequent administration of the product will prevent development to adult stages. Given that only efficacy against prior infection has been investigated, the SPC section 4.9 highlights that treatment should continue for at least one month after last anticipated exposure to mosquitoes.

Clinical field trials

Ticks

The tick efficacy claim was supported by a European multicentre field study conducted in Germany, Hungary and Portugal. The primary objective was to demonstrate the efficacy and safety of Simparica Trio in the treatment and control of natural infestations of ticks on dogs presented as veterinary patients in Europe. The secondary objective of the study was to evaluate product's palatability. The study can be considered representative of the European situation, in terms of geographical region, age, breed and gender of animals. The study design is considered appropriate and in line with

guideline requirements. The study used the prototype formulation referred to as "2016 Clinical Supplies".

Dogs (154 purebred and 126 crossbreeds, 132 females and 148 males, 0.2 to 15.0 years of age at enrolment, 2.1 to 66.1 kilograms body weight at enrolment) that were infested with at least 3 live ticks were enrolled, and treated either with the recommended dose of 1.2-2.4 mg/kg bw sarolaner, 0.024-0.048 mg/kg bw moxidectin and 5-10 mg/kg bw pyrantel (n=189) administered orally as a single dose, or a positive control containing afoxolaner/milbemycin oxime (n=91). The positive control used is authorised in the EU for the treatment of tick infestations (*Dermacentor reticulatus, Ixodes ricinus, Rhipicephalus sanguineus*) in dogs for 4 weeks; however, it is not authorised for the treatment of *Ixodes hexagonus.*

Tick counts and clinical signs were monitored at Day 0, 7, 14, 21 and 30. The primary efficacy end point was the percentage reduction in live tick counts from baseline at the post-treatment time points over all tick species combined.

Based on the primary efficacy parameter, the test item was confirmed to be non-inferior to the control product. The CVMP accepts that the product, when administered to dogs under field conditions of use, was effective against the ticks (*Ixodes ricinus, Rhipicephalus sanguineus, Dermacentor reticulatus*). However, given that only 3 dogs (2.4%) in the sarolaner/moxidectin/pyrantel group were infested with *Ixodes hexagonus* and the positive control product is not authorised against this particular tick species, the field data available for *I. hexagonus* was considered to be very limited. However, the applicant submitted additional data to demonstrate that *I. hexagonus* has a similar or greater susceptibility profile to the active substabce sarolaner compared to *I. ricinus*.

Regarding safety, the IVP appears to have been well tolerated. There was one adverse event which the applicant considers that may have been treatment related in which a dog showed weakness, apathy and loss of appetite on Day 1. The condition was considered moderate and resolved without treatment by Day 3. There were three cases of abnormal faeces (loose stools, mild diarrhoea, haemorrhagic gastroenteritis) in animals administered the IVP which the applicant suggests are unlikely to be treatment related.

Based on the primary efficacy parameter, sarolaner was confirmed to be non-inferior to the control product. The study demonstrated that a single oral dose of the IVP is safe and efficacious for the treatment of tick infestation (*Ixodes hexagonus, Ixodes ricinus, Rhipicephalus sanguineus, Dermacentor reticulatus*) under natural conditions, with a duration of acaricidal effect of up to 30 days having been supported in this study.

It is evident from the EPAR for the applicant's mono-active product Simparica that the field study conducted with that product measured acaricidal effect at 30 days post-treatment administration yet a similar duration of persistent acaricidal efficacy was granted for that product as is proposed for Simparica Trio.

On that basis, the CVMP accepts that the effectiveness of Simparica Trio against ticks reported in the field trial is adequate for the purposes of confirming the findings from the dose confirmation studies.

Fleas

The flea efficacy claim was supported by a European multicentre field study conducted in Germany, Hungary and Portugal. The primary objective was to demonstrate the efficacy and safety of Simparica Trio in the treatment and control of natural infestations of fleas on dogs presented as veterinary patients in Europe. Secondary objectives were to evaluate the efficacy of the IVP in the reduction of clinical signs associated with flea allergy dermatitis (FAD) and the palatability of Simparica Trio. The

study can be considered representative of the European situation in terms of geographical region, age, breed and gender of animals.

The study used the formulation referred to as "2016 Clinical Supplies". Dogs (156 purebred and 305 crossbreeds, 205 females and 256 males, 0.2 to 16.0 years of age at enrolment, 2.1 to 74.2 kilograms body weight at enrolment) that were infested with at least 5 live fleas were enrolled, and treated either with the recommended dose of 1.2-2.4 mg/kg bw sarolaner, 0.024-0.048 mg/kg bw moxidectin and 5-10 mg/kg bw pyrantel (n=297) administered orally as a single dose, or an authorised positive control containing afoxolaner/milbemycin oxime (n=164). Flea counts and clinical signs were monitored at Day 0, 14 and 30.

Efficacy results showed that Simparica Trio was demonstrated to be non-inferior to a positive control product. Results showed that the product, when administered to dogs under field conditions of use, was effective against the claimed flea species (*Ctenocephalides felis* and *C. canis*), with a duration of pulicidal effect of up to 30 days having been supported in this study. Based on the primary efficacy parameter, the test item was confirmed to be non-inferior to the control product. Over the course of the study, there was a reduction in the numbers of animals with clinical signs of FAD in both study groups. Regarding safety, the IVP was well tolerated: no adverse events attributable to treatment were reported.

Based on the results of this study, the CVMP accepts that a single oral dose of Simparica Trio is safe and efficacious for the treatment of flea infestation (*C. canis* and *C. felis*) for 30 days under natural conditions and is supportive of the treatment claim for FAD.

It is evident from the EPAR for the applicant's mono-active product Simparica that the field study conducted with that product measured pulicidal effect at 30 days post-treatment administration yet a similar duration of persistent pulicidal efficacy was granted for that product as is proposed for Simparica Trio.

On that basis, the CVMP accepts that the effectiveness of Simparica Trio against fleas reported in the field trial is adequate for the purposes of confirming the findings from the dose confirmation studies.

Gastrointestinal nematodes

One pivotal European multicentre GCP field trial and one supportive US field study have been submitted in support of the safety and efficacy of the IVP when administered for the treatment of gastrointestinal nematodes in dogs under field conditions.

The pivotal European nematode study was performed in Germany, Hungary and Portugal involving 291 client-owned dogs of different ages, genders and breeds. The study can be considered representative of the European situation in terms of geographical region, age, breed and gender of animals.

The study design is considered appropriate and is in line with guideline requirements. The study used the formulation referred to as "2016 Clinical Supplies". The positive control product used in this study is authorised in the EU for the treatment of infestations with adult gastrointestinal nematodes of the investigated species *Toxocara canis, Toxascaris leonina*, *Ancylostoma caninum*. It is noted that the control product is not authorised for the treatment of *Uncinaria stenocephala.*

Prior to treatment (days from –7 to 0), all dogs were confirmed positive for natural infections of gastrointestinal nematodes using faecal counts; *T. canis, T. leonina, U. stenocephala* and *A. caninum* were demonstrated in 200, 16, 36 and 80 dogs, respectively. On day 7 (+3), a second faecal sample was examined and compared with the pre-treatment counts.

The dogs were divided in 2 treatment groups. Treatment group 1 (194 dogs) received orally 1.2-2.4 mg sarolaner/kg bw + 0.024-0.048 mg moxidectin/kg bw + 5-10 mg pyrantel/kg bw.

Treatment group 2 (97 dogs) received 2.50–5.36 mg afoxolaner/kg bw and 0.50–1.07 mg milbemycin oxime/kg bw orally.

The IVP was demonstrated to be non-inferior to the positive control product for the primary parameter treatment success. Efficacy in the Simparica Trio group was 99.0%, 98.2%, 98.7%, and 99.7% for *T. canis, T. leonina, A. caninum,* and *U. stenocephala*, respectively (as compared to 99.3%, 99.9%, 97.3%, and 98.7% in the positive control). Statistical comparison in the two treatment groups by species was only conducted for *T. canis* because more than 50% of dogs in both groups were infected by this species. For *T. canis*, 85.8% and 91.1% of the dogs were considered treatment success in the sarolaner/moxidectin/pyrantel and the afoxolaner/milbemycin oxime groups, respectively.

Regarding safety, the test product appears to have been well tolerated. There were two cases of vomiting on Day 0 in animals administered the IVP, which the applicant suggests are unlikely to be treatment related.

Based on the results of this study, the CVMP accepts that a single oral dose of Simparica Trio is safe and efficacious for the treatment of gastrointestinal roundworm (*Toxocara canis* and *Toxascaris leonina)* and hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala)* infections in dogs.

Angiostrongylus vasorum

One pivotal European multicentre GCP field trial has been submitted in support of the safety and efficacy of the IVP when administered for the prevention of angiostrongylosis in dogs under field conditions.

The pivotal study was performed in Denmark and Italy involving 622 client-owned dogs of different ages, genders and breeds. A placebo control product was used in this study containing no active ingredient.

Dogs were randomly allocated in the ratio 1:1 to treatment groups according to a randomisation plan. The IVP (1.2-2.4 mg sarolaner/kg bw + 0.024-0.048 mg moxidectin/kg bw + 5-10 mg pyrantel/kg bw) and CP (placebo) were administered every 30 days from Day 0 to Day 270. *A. vasorum* antigen, antibody and faecal samples were tested every 30 days from Day 0 to Day 300. Dogs which tested positive to *A. vasorum* on or before Day 60 were considered to have been infected prior to the study start date and were excluded from data analysis.

Efficacy in the Simparica Trio group was concluded to be 100% based on the fact that there were no positive tests for *A. vasorum* in the IVP group. Although only two dogs (out of the 262 dogs in the placebo-treated group that completed the study) were diagnosed with *A. vasorum* infection after Day 60, it is noted that a further 37 animals tested positive for *A. vasorum*. This included 32 animals that received a treatment but were removed from the study because they were considered to have been infected before enrolment and five more dogs that have not received any study treatment because they tested positive at the first study visit and therefore did not meet the inclusion criteria to the study. This represents approximately 6% of all study dogs. In the opinion of the CVMP, the data from another study in which an infection pressure of 12.2% was determined, and that from previous published studies indicate that it can be accepted that this can be considered an endemic region for *A. vasorum*.

Regarding safety, the test product appears to have been well tolerated. There were a number of adverse events. Two dogs were withdrawn due to gastrointestinal events. The applicant suggests all adverse events are unlikely to be treatment related. The CVMP is in agreement with the applicant.

Based on the results of this study, efficacy of the combination product when administered as single oral treatment to dogs in order to prevent angiostrongylosis is considered to have been adequately supported.

Dirofilaria immitis

One supportive USA multicentre GCP field trial, two Australian studies and one study conducted in Japan have been submitted in support of the safety and efficacy of the IVP when administered for the prevention of *Dirofilaria immitis* infection in dogs under field conditions.

The study performed in the USA involved 410 client-owned dogs of different ages, genders and breeds. A positive control product containing ivermectin and pyrantel was used in this study.

Dogs were randomly allocated in the ratio 2:1 to one of the treatment groups according to a randomisation plan. The IVP (1.2-2.4 mg sarolaner/kg bw + 0.024-0.048 mg moxidectin/kg bw + 5-10 mg pyrantel/kg bw) and CP (positive control) were administered every 30 days from Day 0 to Day 300. Serum samples were collected on days -1 or 0, 120, 240 or 330 and evaluated efficacy using an antigen detection test and the modified Knotts test to test for microfilariae. Blood and urine tests for clinical pathology were taken on days 0 and 330. All dogs tested negative to both antigen and microfilariae tests at Day 120 and 240 confirming that none had pre-existing heartworm infection.

Regarding safety, the applicant claims there were no adverse events which were attributable to the IVP.

Testing for antigen and microfilariae at day 330 was used as the primary efficacy endpoint. Efficacy in the Simparica Trio group was concluded to be 100% based on the fact that there were no positive tests for *D. immitis* in the IVP group. Two dogs tested positive for *D. immitis* antigen and one of those for microfilaria in the positive control group. To justify the absence of a placebo control group, the applicant provided detail regarding the infection pressure in the areas in which the study A161C-US-13-211 was conducted. Macrocyclic lactone resistant strains of *D. immitis* have been identified in the Lower Mississippi Delta in a number of studies provided by the applicant. Thirty-eight percent of the population recruited resided in this area. The CVMP is of the opinion that this information, in addition to the data provided in the American Heartworm Society 2016 Incidence Map, provides sufficient support that the infection pressure in this area was likely to be satisfactory and that it can be considered a heartworm-endemic region. Hence, the findings that the monthly oral administration of the sarolaner/moxidectin/pyrantel combination product was 100% efficacious in the prevention of heartworm disease can be accepted.

In addition, field studies conducted in two further countries, Australia and Japan, concluded a high level of efficacy in each study (100%) against naturally occurring *D. immitis* infection. The applicant provided information to support the endemic status of *D. immitis* in the regions in which these two studies were conducted. The methodology and analysis is comparable to the study from the USA.

The CVMP accepts that the combined efficacy demonstrated in the four field studies, from three countries on three different continents, indicates that any genetic diversity does not appear to influence the efficacy of the product. In addition, as highlighted earlier, the active substance moxidectin is currently authorised in the EU at a lower dose for the prevention of heartworm with no apparent issues with efficacy/resistance or safety. In view of this and the "3Rs", the CVMP accepts that the product can be considered efficacious in EU and the findings of the studies may be extrapolated to the EU.

As the product was re-administered at monthly intervals, it is not considered possible to know if that frequency of re-administration is required or if a longer re-treatment interval may have been adequate. As no re-challenge was investigated in the dose confirmation studies, it cannot be determined for how long the effect against *D. immitis* lasts. That said, the fact that the product was administered 30 days after artificial infestation with L3 larvae in both studies and given the findings of the studies, it can be accepted that even if re-infestation occurs after product administration, then the subsequent administration of the product will prevent development to adult stages. Given that only

efficacy against prior infection has been investigated, the SPC section 4.9 highlights that treatment should continue for at least one month after last anticipated exposure to mosquitoes.

Based on the data presented, efficacy of the combination product when administered as monthly oral treatment to dogs in order to prevent infection of *D. immitis* in Europe can be accepted.

Other studies

Palatability

The tick field study and flea field study also aimed to demonstrate the palatability of the test product taking into account the recommendations of the CVMP Guideline on the demonstration of palatability of veterinary medicinal products (EMA/CVMP/EWP/206024/2011). In the tick field study, the IVP was voluntarily and fully consumed within two minutes on 79.9% of all 189 occasions offered, which, when rounded to the nearest percentage, meets the minimum threshold to permit a palatability claim. However, in the flea field study, the IVP was voluntarily and fully consumed within two minutes on 77.7% of all 296 occasions offered and does not therefore meet the requirements (≥80% in dogs) outlined in the guideline. Given the findings of the pivotal field study against fleas, the CVMP considered that a palatability claim had been inadequately supported. In response to the concern raised, the applicant provided the results of four additional field studies conducted in Japan in which the secondary objective of the studies was to evaluate its palatability. The assessment of palatability was conducted in line with the design of the tick field study and flea field study. Based on the combined results of all six field studies, Simparica Trio tablets were voluntarily and fully consumed within two minutes on 81.0% of all 1000 occasions offered to dogs, which meets the requirements (≥80% in dogs) outlined in the relevant guideline. Based on the totality of data provided, the proposed new claim for palatability of the candidate formulation can be accepted.

Overall conclusion on efficacy

A comprehensive efficacy dataset has been provided.

Pharmacodynamics

It is accepted that the product is an ecto- and endoparasiticide.

Justification of the fixed combination

It is accepted that the applicant has provided adequate justification of the fixed combination in accordance with the CVMP Guideline on pharmaceutical fixed combination products (EMEA/CVMP/83804/2005).

Development of resistance

No resistance to sarolaner has been reported to date. Pyrantel resistance in *A. caninum* has been reported in Australia; however, no resistance to pyrantel has been reported to date in the EU or against other gastrointestinal nematodes. Lack of efficacy of macrocyclic lactones in the prevention of heartworm disease in dogs has been reported outside Europe. Given the concerns regarding resistance development, section 4.4 of the SPC includes warnings relating to the potential for resistance emergence.

Dose determination/finding

Based on the totality of data provided (pharmacodynamic, pharmacokinetic and preliminary clinical efficacy studies, including the non-interference studies), it is accepted that the minimum proposed

treatment dose of 1.2 mg/kg bw for sarolaner, 0.024 mg/kg bw for moxidectin and 5 mg/kg bw for pyrantel embonate has been adequately justified.

Target animal tolerance

Based on the totality of data obtained from target animal safety studies and various confirmatory and field efficacy studies conducted, the CVMP accepts that Simparica Trio was generally well tolerated.

Clinical studies

Ticks

The CVMP is of the opinion that a claim against *Dermacentor reticulatus, Ixodes hexagonus, Ixodes ricinus* and *Rhipicephalus sanguineus* has been adequately supported (that is, tick species for which there are adequate dose confirmation data and for which efficacy has been confirmed in the field).

No speed of kill studies against ticks were submitted. Based on the results of the dose confirmation studies, the CVMP accepts that ticks on the animal prior to administration or from new infestations after product administration are killed within 48 hours.

Fleas

The CVMP accepts that there is adequate dose confirmation data and field efficacy data to support a claim against *Ctenocephalides felis* and *Ctenocephalides canis*.

The data support an onset of effect within 8 hours for pre-existing flea infestations and within 12 to 24 hours of attachment for five weeks after product administration.

Gastrointestinal nematodes

The CVMP accepts that there is adequate dose confirmation data to support a claim against *Toxascaris leonina* (adults), *Uncinaria stenocephala* (adults), *Toxocara canis* (immature adults and adults) and *Ancylostoma caninum* (L4 larvae, immature adults and adults).

The overall conclusions of the gastrointestinal nematode field study are accepted: when administered to dogs under field conditions of use, the product was effective against the claimed nematode species (*Toxocara canis*, *Toxascaris leonina, Ancylostoma caninum* and *Uncinaria stenocephala).*

Vascular nematodes

Based on the efficacy data presented by the applicant, the CVMP is of the opinion that the proposed claims relating to *A. vasorum* and *D. immitis* can be accepted.

Part 5 – Benefit-risk assessment

Introduction

Simparica Trio is a chewable tablet containing a fixed combination of three active substances: sarolaner, moxidectin and pyrantel (as embonate).

Sarolaner is a systemically acting acaricide and insecticide belonging to the isoxazoline family, whilst moxidectin, a second generation macrocyclic lactone of the milbemycin family, and pyrantel, a nicotinic acetylcholine channel receptor agonist, act against endoparasites (gastrointestinal and vascular).

The product is intended for use in dogs with, or at risk from, mixed external and internal parasitic infestations. The product is exclusively indicated when use against ticks or fleas and gastrointestinal

nematodes is indicated at the same time; the product also provides concurrent efficacy for the prevention of heartworm disease and angiostrongylosis. The product can be used as part of a treatment strategy for the control of flea allergy dermatitis.

The proposed dose is 1.2–2.4 mg/kg bw of sarolaner, 0.024–0.048 mg/kg bw of moxidectin and 5-10 mg/kg bw of pyrantel.

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

Benefit assessment

Direct therapeutic benefit

The combination of three active substances is justified on the basis that the parasites targeted by the combination product (fleas, ticks, gastrointestinal nematodes and/or heartworm and angiostrongylosis) are commonly found in dogs in Europe and can be present simultaneously on the same animal; the three active substances (sarolaner, moxidectin and pyrantel) included in the combination product have different spectra of activity and, as a consequence, the spectrum of activity is broadened.

The CVMP is of the opinion that a claim against the following target parasites has been adequately supported:

- Tick species: acaricidal activity for 5 weeks against *Ixodes hexagonus*, *Ixodes ricinus* and *Rhipicephalus sanguineus* and for 4 weeks against *Dermacentor reticulatus*;
- Flea species: pulicidal activity for 5 weeks against *Ctenocephalides felis* and *Ctenocephalides canis*;
- Gastrointestinal nematodes: treatment of immature adult (L5) and adult stages of *Toxocara canis*, L4 larvae, immature adults (L5) and adults stages of *Ancylostoma caninum* and adult stages of *Toxascaris leonina* and *Uncinaria stenocephala*;
- *Dirofilaria immitis*: for the prevention of heartworm disease (*Dirofilaria immitis*);
- *Angiostrongylus vasorum*: for the prevention of angiostrongylosis by reducing the level of infection with immature adult (L5) stages of *Angiostrongylus vasorum*.

Additional benefits

The effective control of fleas on treated dogs will directly reduce the risk of infestation of other animals in contact with infested dogs.

The product, being a fixed combination, facilitates dog handling by reducing the total number of tablets given.

Risk assessment

Quality:

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

Safety:

Risks for the target animal:

Administration of the combination product in accordance with label recommendations is generally well tolerated. However, information on target animal tolerance in SPC sections 4.5 and 4.10 has been recommended.

Risk for the user:

The risk to the user is considered acceptable noting in particular that the pharmaceutical form (chewable tablet) limits the potential for the user to be exposed to the active substance when removing the product from the packaging and administering the tablet to the animal.

As Simparica Trio can pose a risk to children accidentally ingesting a tablet, specific measures are necessary to mitigate the risk; the tablet is presented in suitable child-resistant packaging, and the product information includes a warning advising of the potential for adverse effects in case of accidental ingestion and specific instructions to remove tablets from the packaging only when required, and to store the product out of the sight and reach of children.

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

Risk for the environment:

Simparica Trio is not expected to pose a risk for the environment when used according to the SPC. Standard advice for the disposal of any unused product or waste material is included in the product literature.

Risk of anthelmintic resistance:

At present, no resistance of the claimed parasites in dogs to the individual active substances has been reported for Europe. However, there are reports of resistance development of *D. immitis* to macrocyclic lactones in dogs in the USA and pyrantel resistance in *A. caninum* in Australia. Given the concerns regarding resistance development, section 4.4 of the SPC includes warnings relating to the potential for resistance emergence.

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.

User safety:

User safety risks have been identified, mainly the risks associated with exposure in children. These risks are mitigated, in part, by the safety warnings in the SPC, together with the use of suitable child-resistant packaging.

Target animal tolerance:

Information on target animal tolerance in SPC sections 4.5 and 4.10 has been recommended.

Evaluation of the benefit-risk balance

Based on the data presented to date, the overall benefit-risk balance is considered positive.

The benefit of Simparica Trio is its efficacy in the treatment of flea and tick infestations, gastrointestinal nematodes, heartworm disease and angiostrongylosis in dogs.

The product indication as initially proposed by the applicant was for dogs with, or at risk from, mixed external and internal parasitic infestations. Following evaluation of the data, the CVMP agreed with the applicant's proposal.

The formulation and manufacture of the product is well described and the proposed specifications ensure that product of consistent quality will be produced. At the recommended dose, 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.

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 Simparica Trio 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.