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Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Trifexis (EMEA/V/C/002635/0000)

Spinosad / milbemycin oxime

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

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Introduction

The applicant Eli Lilly and Company Limited submitted on 23 January 2012 an application for marketing authorisation to the European Medicines Agency (the Agency) for Trifexis chewable tablets for dogs, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004.

The eligibility to the centralised procedure was agreed upon by the EMA/CVMP on 15 September 2011 as Trifexis contains a new combination of two existing active substances (spinosad and milbemycin oxime) which was not authorised as a veterinary medicinal product in the Community on the date of entry into force of the Regulation. Neither spinosad nor milbemycin oxime are new active substances in EU authorised veterinary medicinal products, but inclusion of both in a fixed combination product is a novel presentation.

The following indication was proposed: For the treatment and prevention of flea infestations in dogs, and for the concurrent prevention of heartworm disease and/or treatment of gastrointestinal nematode infections.

The target species is the dog. Trifexis chewable tablets contain spinosad and milbemycin oxime, and are available in five different strengths (to facilitate treatment of different weight dogs). The tablets are packed in blister packs, which are then packed into outer cartons containing either 1, 3 or 6 tablets. The route of administration is oral use.

The dossier has been submitted in line with the requirements for submissions under Article 13(b) (fixed combination) of Directive 2001/82/EC – fixed combination application. The two active substances in this fixed combination product are each authorised individually in veterinary medicinal products in the EU. Spinosad is authorised in a monosubstrate product (Comfortis, Eli Lilly and Company Limited) for the treatment and prevention of flea infestations (*Ctenocephalides felis*) in dogs. Milbemycin oxime is the active substance in a veterinary medicinal product authorised in the EU for the prevention of heartworm disease and the control of roundworms, hookworms and whipworms in dogs. Milbemycin oxime is also included in combination with praziquantel in a product authorised in the EU. The data provided are in accordance with the requirements of the CVMP guideline on pharmaceutical fixed combination products (EMEA/CVMP/83804/2005).

The CVMP adopted an opinion and CVMP assessment report on 17 July 2013.

On 19 September 2013, the European Commission adopted a Commission Decision for this application.

Scientific advice

The applicant did not seek scientific advice at the CVMP.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system was provided which fulfils the requirements of Directive 2001/82/EC. It provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country. However, considering

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the inconsistencies noted during the assessment procedure regarding the pharmacovigilance data provided, an early pharmacovigilance system inspection should be foreseen.

Manufacturing authorisations and inspection status

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

Trifexis chewable tablets for dogs are manufactured in the US and shipped to Europe. Secondary packaging and batch release for the EU will be carried out in the UK.

The site of finished product manufacture and primary packaging in the US has been inspected by an EU competent authority (Irish Medicines Board), found to be GMP-compliant, and current certificates of GMP compliance have been provided.

Regarding the sites for secondary packaging and the site for batch release in the EEA, copies of the current manufacturer's/importer's authorisation been provided.

Overall conclusions on administrative particulars

The Committee considers that the pharmacovigilance system fulfils the necessary requirements and provides adequate evidence that the applicant has both the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country. However, considering the inconsistencies noted during the assessment procedure regarding the pharmacovigilance data provided, an early pharmacovigilance system inspection should be foreseen.

The information provided in regard to the manufacturing sites and their GMP status is complete and no further information is necessary on the manufacturing sites, nor is any inspection necessary or requested.

Part 2 - Quality

Composition

Trifexis tablets are chewable tablets for dogs containing two active ingredients, spinosad and milbemycin oxime. The tablets are mottled tan to brown, round, standard, biconvex, chewable tablets with item codes debossed on one side and dimples on the other side. The number of dimples on the tablet corresponds to its strength.

Trifexis tablets are presented in five different strengths. The strengths range from 270 mg up to 1620 mg spinosad (at a percentage content of 53.33%), and 4.5 mg up to 27 mg milbemycin oxime. The tablets are homothetic, having the same percentage composition and are all compressed from a common blend.

No overages are included in the product.

According to the dosing table in the SPC, the tablets do not need to be divided, therefore the absence of break-lines is justified.

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Spinosad is a product of fermentation derived from strains of *Saccharopolyspora spinosa*. Approximately 90% of spinosad is comprised of spinosyns A and D. Of that 90%, the ratio of spinosyn A to A+D is 0.85 when calculated as spinosyn A/(spinosyn A +D).

The active substance milbemycin oxime (INN) is a mixture of milbemycin A_3 5-oxime and milbemycin A_4 5-oxime with an approximate ratio of 20:80. The manufacture of milbemycin oxime consists of two major stages: firstly, a fermentation stage to produce milbemectin from *Streptomyces hygroscopicus* subsp. *aureolacrimosus*, and subsequently a chemical synthetic stage to manufacture the milbemycin oxime (two synthetic steps).

Well established excipients are used in the manufacture of Trifexis tablets: pharmaceutical grade microcrystalline cellulose and hydroxypropyl cellulose (serving as diluent and binder); croscarmellose sodium (disintegrant); colloidal silicon dioxide (glidant); and magnesium stearate (lubricant).

Artificial powdered beef flavour is used to achieve palatability. This particular flavouring agent has been used previously in several EU authorised veterinary medicinal products.

Container

The finished product is presented in blister packs (1, 3 or 6 tablets per blister) inside a folding cardboard carton. The foil-based blister laminate (polyamide/aluminium/polyvinyl chloride (PA/alu/PVC)) is heat sealed to the foil-based lidding laminate (based on PET/alu/PVC) with a PVC-based coating (on the internal blister surface). The lidding laminate may have an optional printing surface such as paper, polyester, or lacquer. The product contact surface for the tablet is the PVC of the blister laminate and the PVC based heat sealing coating of the lidding laminate.

Development pharmaceutics

The aim was to develop an immediate release palatable and chewable tablet formulation for use in dogs. Different tablet sizes are manufactured using the same bulk mix. The potency of spinosad (amount of spinosyn factors A and D within a given lot of spinosad) of the final blend is adjusted to 53.33% and the potency of milbemycin oxime is adjusted to 0.89% of milbemycin oxime factors A_3 and A_4.

To accommodate the target dog weight range, five tablets strengths (270/4.5, 425/7.1, 665/11.1, 1040/17.4 and 1620/27 mg spinosad and milbemycin oxime respectively) are proposed. The choice of the excipients has been adequately justified.

Method of manufacture

The manufacturing formula is given for the chosen normal batch size. The theoretical number of tablets depends on how the blends are split, which will depend on marketing needs.

A comprehensive description of the equipment utilised and the manufacturing method is given in the documentation. A wet granulation technique, with subsequent blending and compression, is employed and is regarded to be a standard manufacturing process.

Satisfactory in-process controls are in place at each stage.

All validation parameters were satisfactory for all tablet strengths and packaging. The validation data demonstrate that the manufacturing procedure is robust, reproducible and can consistently produce

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tablets of the desired quality. The process does not cause degradation of the active substances. The manufacturing process is considered validated and suitable for routine production.

Control of starting materials

Active substances

Milbemycin oxime (INN) is not the subject of a monograph in either the European Pharmacopoeia (Ph. Eur.), or any other pharmacopoeia of the EU.

The data are presented in the form of an Active Substance Master File (ASMF) which supports the marketing authorisation application. The manufacturing site of the active ingredient manufacturer (ASM) is specified.

Specification for milbemycin oxime:

The specifications distinguishes between and limits milbemycin 5-oxime factors A_3 + A_4, milbemycin oxime D and total related substances (milbemycin oxime factors other than A_3, A_4 and D), which have been described in the dossier, and are appropriate to control its quality.

The proposed re-test period of 24 months has been justified by stability data.

Spinosad is not the subject of a monograph in either the European Pharmacopoeia (Ph. Eur.), or any other pharmacopoeia of the EU.

The data for this active substance are also presented in the form of an ASMF which supports the marketing authorisation application. The manufacturing site of the active ingredient manufacturer is specified.

Specification for pharmaceutical grade spinosad:

The specifications distinguish between spinosyn factors exhibiting greater than 90% activity and the spinosyn factors with less than 90% activity, and are appropriate to control its quality.

The proposed re-test period of 24 months has been justified by stability data.

Excipients

The tablets include the following five excipients, which are all the subject of Ph. Eur. monographs and are typical for a product of this type (that is, chewable tablet): cellulose microcrystalline (Ph. Eur.); hydroxypropylcellulose (Ph. Eur.); croscarmellose Sodium (Ph. Eur.); silica, colloidal anhydrous (Ph. Eur.); magnesium stearate (Ph. Eur.).

The sixth excipient is an artificial powdered beef flavour, the specific type of which has previously been used in several veterinary medicinal products authorised in the EU, and which is the subject of an in-house specification. It is gamma irradiated and contains pork liver powder. Full details have been provided and the specification is considered satisfactory to control its quality. All the ingredients of the flavour are FDA approved human food ingredients (complying with FDA law and regulations). Gamma irradiation of the artificial powdered beef flavour is carried out and the limits established for microbiological purity are sufficient.

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

The magnesium stearate is of vegetable origin.

There is no transmissible spongiform encephalopathy (TSE) risk associated with the use of the artificial powdered beef flavour. A corresponding certificate has been issued by the manufacturer and is supplied.

Additionally, none of the other materials used in the manufacture of Trifexis tablets carries any TSE risk. Compliance of the active substance and all the excipients in the product with the current version of the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) has been confirmed.

Control tests during production

All the in-process controls are defined and are regarded justified to achieve a consistent batch-to-batch quality.

Control tests on the finished product

The specification includes all of the tests expected for a tablet and is in line with VICH guideline GL39 (EMEA/CVMP/VICH/810/04). The specifications proposed at release are suitable to control the quality of the finished product. The specification to be applied during shelf-life differs slightly but the differences are justified.

Adequate specifications and routine tests have been described to ensure appropriate and constant quality of the finished product. The following methods are employed which comply with the Ph. Eur.: Uniformity of Dosage Units; Dissolution; Microbial Limit Tests (in accordance with Ph. Eur. 5.1.4); Moisture content.

The identity of the two active substances, spinosad and MO, are each confirmed by two independent identity tests (^{13}C-NMR chemical shift assignments and HPLC retention time).

The content of spinosyn factors A + D and of related substances is determined by a reversed phase (RP) HPLC method with isocratic elution and UV detection. The method is also stability indicating.

The content of milbemycin oxime factors A_3 and A_4, and of related substances, is determined by a reversed phase (RP) HPLC method with gradient elution and UV detection. The method is also stability indicating.

Analytical methods are fully described and validated in accordance with VICH guidelines.

Stability

Stability studies are performed under VICH conditions. The stability data presented include 24 months data from 9 batches stored at 25 °C/60% RH, and 6 months data from 40 °C/75% RH for all 9 batches. After 24 months of storage at 25 °C/60%RH, all batches comply with their specifications.

Three batches each of two strengths of the tablets were also exposed to light according to the guideline VICH guideline GL5 on photostability testing (CVMP/VICH/901/00).

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The presented stability data are considered to satisfactorily support the claimed shelf life of 36 months with no special storage conditions.

Since the tablets exhibit no score line, no in-use stability data has been provided and this is acceptable.

Overall conclusions on quality

The data on the active substance, spinosad, are presented in the form of an ASMF and include comprehensive information on its manufacture and characteristics. It is not the subject of a monograph of the Ph. Eur. or a pharmacopoeia of any EU member state. The routine tests and specifications are considered sufficient to assure constant quality of the active substance. The proposed re-test period of 24 months is acceptable.

Data on the second active substance, milbemycin oxime, are also presented in the form of an ASMF and include comprehensive information on its manufacture and characteristics. Milbemycin oxime is a very stable molecule and the retest period of 24 months is justified by the data and in accordance with the guidance in VICH guideline GL3.

The excipients used are considered acceptable, as well as the packaging materials. All excipients contained in the tablets, except the artificial powdered beef flavour (containing pig liver powder) are of pharmacopoeial grade quality. The microbiological and virological safety of the beef flavour has been proven. There are no concerns in relation to TSE for any of the ingredients of the product.

The rationale for the choice of the formulation is acceptable.

A production scale batch blend size has been validated. Satisfactory in-process data have been noted during the compression runs and batch analytical results have demonstrated that the manufacture of the product is well controlled. The finished product release specifications are considered acceptable. Control methods have been validated and the specification is considered to be relevant for a product of this type.

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

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

Part 3 – Safety

Both active substances included in the product are present in a number of authorised products and their safety was previously assessed. Full technical details on the safety of spinosad have previously been documented in the European public assessment report (EPAR) for Comfortis. Milbemycin oxime has a well-established use and is presently authorised in the EU in 2 different products for use in companion animals used at a dose range of 0.5 to 2.5 mg milbemycin oxime/kg bw. The pharmacology and toxicity of milbemycin oxime is summarised in publicly available scientific literature. Also, acute toxicity data with the product in respect of its user safety, an *in vivo* study determining the role of trans-membranous transport P-glycoprotein (P-gp) in the disposition of each drug, and genotoxicity studies with milbemycin oxime were submitted. A number of tolerance studies in the target species, including a reproductive safety study, are also described in detail in Part 4.

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For the purposes of this assessment report, the toxicological profile of the individual actives can be considered well established. Consequently, only an overview on relevant data of the active substances is given in each section of Part 3.

Safety documentation

Pharmacodynamics

See Part 4.

Pharmacokinetics

No new study data on laboratory animals were submitted for spinosad and milbemycin oxime in respect to absorption, distribution, metabolism and excretion. Data on pharmacokinetics performed in the dog is described in Part 4.

It had been previously concluded by the CVMP that spinosad was well absorbed after oral administration and that elimination followed a similar pathway in laboratory species. Following extensive hepatic metabolism, the predominant excretion route for metabolites is *via* the faeces, with minor amounts present in the urine. Milbemycin oxime was found to be well absorbed after oral administration and to undergo extensive hydroxylation in the liver in both rats and dogs. Similar to spinosad, the metabolites are mainly excreted in the faeces with minor amounts present in the urine.

Since macrocyclic lactones are known to be substrates for trans-membranous transport P-glycoprotein, which has been linked to neurotoxicity in Collies and related breeds, an *in vivo* study was conducted with wild type and knockout mice to determine if spinosad and/or milbemycin oxime do interact on the level of P-glycoprotein. Mice were orally treated once with milbemycin oxime alone, spinosad alone or with a combination of spinosad/milbemycin oxime. Plasma and brain samples were collected at different time points post-dose (3 mice/time) and were stored frozen prior to being analysed using LC/MS/MS assays, which were validated for use in non-good laboratory practice (non-GLP) studies. Following oral administration of spinosad or milbemycin oxime, the resulting mean plasma concentrations for both spinosad and milbemycin oxime were similar between wild-type and multidrug resistant (MDR1a) knockout mice. In contrast, the resulting mean brain concentrations for both spinosad and milbemycin oxime were notably different. When the test articles were co-administered, the same trend held true that the mean plasma concentrations for milbemycin oxime and spinosad were similar while the brain concentrations were different in wild-type compared to MDR1a knockout mice. It was also demonstrated that the co-administration of spinosad with milbemycin oxime increases the overall exposure (plasma and brain concentrations) of milbemycin oxime in both the wild type and MDR1a knockout mice. The plasma concentrations for both the wild-type and the knockout mice were extremely variable between sampling times and made it difficult to assess if there was a difference in the spinosad plasma concentrations following single versus co-administration of the test articles.

It was concluded that both milbemycin oxime and spinosad are P-glycoprotein substrates in mice.

Toxicological studies

Toxicity studies in different laboratory animals have been evaluated during the application for a community marketing authorisation for the spinosad only containing product from the same company

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(Comfortis). Most of the studies were GLP compliant and conform to the relevant Organisation for Economic Co-operation and Development (OECD) Guidelines.

For milbemycin, toxicity data submitted are published in publicly available scientific literature.

Toxicity studies performed with the combination were performed in respect to target animal safety (Part 4) and in respect to acute toxicity for the assessment of the user risk.

Single dose toxicity

Spinosad has been established to have a low acute toxicity following oral exposure in rats and mice (LD_{50} >2000 mg/kg bw) and intraperitoneal exposure in rats (LD_{50} >800 mg/kg bw). No systemic effects after dermal application were observed in rabbits up to a dose of 5000 mg/kg bw.

Milbemycin oxime has been shown in earlier studies, after oral administration, to be of moderate general toxicity. In rats no specific organ toxicity was seen. No systemic effects after dermal application were observed in rats up to a dose of 5000 mg/kg bw.

The single dose data were considered complete and satisfactory.

Repeat dose toxicity

In the repeated dose toxicity studies from the application for the monocomponent spinosad tablets, the lowest relevant no observed effect level (NOEL) was 5 mg/kg bw per day in dogs based on phospholipidosis, which was the most prominent effect of spinosad in 13-week toxicity studies in laboratory animals, including the dog, and is induced by intracellular accumulation of cationic amphiphilic/lipophilic drugs (CAD). A large variability has been shown among species or strains with respect to the tissue/organ specificity and severity of the condition induced by a particular CAD. No dermal effects and no systemic toxicity were observed in dermal repeat dose toxicity studies in rabbits.

Milbemycin oxime showed haematological changes at 30 mg/kg bw/day and above in earlier studies, and showed liver changes at 100 mg/kg bw/day and bodyweight loss at 300 mg/kg bw/day in repeated dose toxicity studies in rats.

These data were considered to be satisfactory and justified.

Tolerance in the target animal species

See Part 4.

Reproductive toxicity

The studies on reprotoxicity performed in rats and rabbits (from the application for Comfortis, the monocomponent spinosad tablets) provided no evidence for adverse effects of spinosad on male or female reproductive functions at dose levels below parental toxic levels. No developmental effects occurred at maternal toxic levels. The no observed adverse effect levels (NOAELs) for parental and reproductive/developmental toxicity were 10 mg/kg bw/day. The NOAEL for embryonal/foetal toxicity and teratogenicity was 50 mg/kg bw/day both in rats and rabbits.

There are no data available on the effects of milbemycin on fertility or reproductive parameters in laboratory animals, but a reproductive toxicity study with the final formulation was performed in the target animal species. Milbemycin did not show a teratogenic potential when examined in two species (rats and rabbits) up to doses of 30 mg/kg bw.

For reproductive toxicity in the target species, see Part 4.

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Mutagenicity / genotoxicity

A full battery of *in vitro* and *in vivo* genotoxicity tests examining gene mutations and clastogenic endpoints has been conducted for spinosad and assessed previously (for the monocomponent tablets). It was concluded that spinosad did not induce point mutations in bacteria, gene mutations, chromosomal aberrations, or unscheduled DNA synthesis in mammalian cells *in vitro* and was considered to be non genotoxic.

Three GLP-compliant studies on the genotoxic potential of milbemycin oxime have been performed according to appropriate OECD guidelines in 2008. The assays were conducted up to the designated limit doses or up to doses demonstrating induced cytotoxicity. Treatment with positive control chemicals induced clear positive results in the presence and absence of metabolic activation. Milbemycin oxime was negative when tested in the bacterial reverse mutation (Ames) test and an *in vitro* chromosomal aberration test in Chinese hamster ovary cells, and it did not induce micronuclei *in vivo* in the bone marrow of imprinting control region (ICR) mice.

It can be concluded that milbemycin oxime has no mutagenic potential.

Carcinogenicity

Spinosad was previously evaluated for its carcinogenic potential in rats (24 months) and mice (18 months). In mice, the incidence of tumours in both males and females was not increased relative to controls in any of the tested dose groups. The overall NOAEL of both chronic toxicity studies in mice was found to be 11.4 mg/kg bw/day. In rats, a NOAEL after 24 months was set at a dose of 2.4 mg/kg bw/day. There were no increases in tumour incidence noted in any of the tested dose-groups compared to controls. It was concluded, that spinosad did not show any carcinogenic potential.

Carcinogenicity studies were not performed with milbemycin oxime. This is acceptable, since milbemycin oxime did not show a genotoxic potential nor does the molecule possess any structural alerts.

Studies of other effects

The acute toxicity after single intraperitoneal injection or single oral dose of the combination of spinosad and milbemycin was tested in a GLP study in rats at the same dose ratio (60:1) to that in the proposed medicinal product. This study was designed largely according to the older OECD guideline 401 rather to guideline 420, but is acceptable. It was shown that the combination has been of low mammalian toxicity and a NOAEL of 2000 mg spinosad and 34 mg milbemycin oxime per kg bw for both sexes has been established after oral application. This dose exceeds the highest concentration of 1 tablet of the product (1620 mg/27 mg). For the intraperitoneal route, a NOAEL of 300 mg spinosad and 5 mg milbemycin oxime per kg bw was established. Toxicity of the combination was not enhanced compared to the administration of the individual chemicals.

An acute GLP-compliant skin irritation study was conducted in accordance with the OECD guideline 404 in rabbits. Based on the results it can be agreed, that the compounds were not skin-irritating.

In a murine local lymph node assay no signs of systemic toxicity and/or local irritation were seen, when the combination of spinosad and milbemycin (60:1) was administered. It was concluded that the combination is not a contact sensitiser.

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User safety

A comprehensive user safety assessment was submitted according to the CVMP guideline on user safety (EMEA/CVMP/543/03-Rev.1). The user can be identified as dog owners (and children by accidental dermal/oral contact). Acute dermal and oral exposures are considered most relevant due to the intended use of the product.

Acute toxicity studies with the product have shown that the product is not more toxic than the active substances when administered alone. Furthermore, no contact sensitising potential was observed for the product. The concentrations of spinosad/milbemycin used in the study on skin irritation were 10-fold lower than the expected worst case concentration for dermal exposure. However, since the active substances alone did not show irritating properties, it can be considered that the product is also not skin irritating. Eye irritating potential cannot be excluded due the reversible, slight to moderate eye irritant potential of spinosad in rabbits. However, appropriate advice to wash hands after use of the product included in the SPC is considered satisfactory to prevent accidental hand-to-eye contact with the product.

Systemic toxicity of the combination product in the adult user, including pregnant and lactating women, following dermal contact or accidental ingestion arising from hand-to-mouth transmission can be considered negligible. Nevertheless, the user is advised to wash their hands after administration of the product to minimise any risk, including accidental hand to eye contact.

Possible health risks after acute oral exposure has also been discussed for children. Emesis seen after single administration in the target species is also considered more relevant for risk characterisation than the NOAEL derived from the acute toxicity study after intraperitoneal administration. The estimated exposure based on the assumption of the ingestion of a whole tablet is below the calculated dose with a Margin of Exposure (MOE) of 2.8. However, the warning sentences included in the SPC “Children must not come in contact with the veterinary medicinal product. Accidental ingestion may cause adverse reactions.” are considered satisfactory to minimise the risk for children.

In a study complying with the Consumer Product Safety Commission's (CPSC) current standards and protocols for poison prevention packaging, a child-resistant effectiveness of 96% was represented.

In conclusion, the proposed child-resistant packaging and warning phrases are considered satisfactory to minimise the risk for the user, including children. The product does not pose an unacceptable risk to the user when it is used in accordance with the SPC.

Environmental risk assessment

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

Trifexis chewable tablets for dogs are not expected to pose a risk for the environment when used according to the SPC. Adequate advice on disposal of any unused product or waste material is included in the product literature.

Overall conclusions on the safety documentation

The safety of both active substances has been previously assessed. Full supporting data on the safety of spinosad was included in the centralised application for the monocomponent product, Comfortis, and assessed by the Committee as satisfactory. Milbemycin oxime has a well-established use and is already

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authorised in 2 different veterinary medicinal products in the EU for use in companion animals. It has been shown (in the Comfortis application) that spinosad was well absorbed after oral administration and that elimination followed a similar pathway in both the dog and cat. Following extensive hepatic metabolism, the predominant route of excretion for metabolites is via the faeces, with minor amounts present in the urine.

For milbemycin oxime, the metabolism, distribution, and elimination are well-known and have been investigated in rats and dogs. Milbemycin oxime was found to be well absorbed after oral administration and undergoes extensive hydroxylation in the liver in both species. Similar to spinosad, the metabolites are mainly excreted in the faeces with minor amounts present in the urine.

An *in vivo* study was conducted with wild type and knockout mice to determine, if spinosad and/or milbemycin oxime are P-glycoprotein substrates. The concentrations of the substances were determined by validated methods. The findings indicate that both spinosad and milbemycin oxime are substrates for the P-glycoprotein transporter system. Furthermore, co-administration with spinosad increased the plasma and brain concentration of milbemycin oxime.

Spinosad has a low acute toxicity following oral and intraperitoneal exposure. No systemic effects after dermal application were observed in rabbits up to a dose of 5000 mg/kg bw. The most prominent effect of spinosad in repeat dose toxicity studies was phospholipidosis induced by intracellular accumulation of cationic amphiphilic/lipophilic drugs. A large variability has been shown among species or strains with respect to the tissue/organ specificity and severity of the condition induced by a particular cationic amphiphilic/lipophilic drug.

No dermal effects and no systemic toxicity were observed in dermal repeat dose toxicity studies in rabbits. It was concluded that spinosad was not irritating to the skin of rabbits.

The studies on reproduction toxicity performed in rats and rabbits provided no evidence for adverse effects of spinosad on male or female reproductive functions at dose levels below parental toxic levels. No developmental effects occurred at maternal toxic levels. The NOAELs for parental and reproductive/developmental toxicity were 10 mg/kg bw/day. The NOAEL for embryonal/foetal toxicity and teratogenicity was 50 mg/kg bw/day both in rats and rabbits.

Spinosad is not considered to be mutagenic nor carcinogenic. Spinosad was not irritating to the skin of rabbits and not sensitising to the skin of guinea-pigs. It caused reversible, slight to mild irritation in the eyes of rabbits.

Milbemycin oxime has been shown to be of moderate oral toxicity. No systemic effects after dermal application were observed in rats up to a dose of 5000 mg/kg bw. In repeated dose toxicity studies, milbemycin oxime showed haematological changes at 30 mg/kg bw/day and above, liver changes at 100 mg/kg bw/day and bodyweight loss at 300 mg/kg bw/day.

There are no data available on the effects of milbemycin on fertility or reproductive parameters in laboratory animals, but a reproductive toxicity study with the final formulation was performed in the target animal species. Milbemycin did not show a teratogenic potential when examined in two species (rats and rabbits) up to doses of 30 mg/kg bw.

Milbemycin oxime is not considered to be genotoxic nor carcinogenic. Milbemycin oxime was not irritating to the skin of rabbits and not sensitising in a local lymph node assay. Eye irritating potential was not tested, but a slight risk of eye irritation was assumed.

A comprehensive user safety assessment was submitted according to the CVMP guideline on user safety (EMEA/CVMP/543/03-Rev.1). It was concluded that the child-resistant packaging and warning sentences proposed by the applicant are considered satisfactory to minimise the risk for the user

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including children. The product does not pose an unacceptable risk to the user when it is used in accordance with the SPC.

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

Part 4 – Efficacy

Pharmacodynamics

Primary pharmacodynamics

The two actives substances in Trifexis, spinosad and milbemycin oxime, are approved in the EU in mono-substance products, and milbemycin oxime is also approved in the EU in combination with other substances (e.g. praziquantel). The insecticidal activity of spinosad is characterised by nervous excitation triggered by nicotinic acetylcholine receptors, leading to muscle tremors, prostration, paralysis and ultimately death of the fleas. The activity of milbemycin oxime is related to its action on invertebrate neurotransmission. It increases nematodal and tick membrane permeability to chloride ions via glutamate-gated chloride channels. This leads to hyperpolarisation of the membranes followed by paralysis and death.

The combination of both substances in Trifexis broadens the spectrum of anti-parasitic activity over that of the monosubstance products and is, therefore, indicated for the treatment and prevention of flea infestations, when concurrent prevention of heart worm disease and/or treatment of nematode infections is indicated. The combination is sufficiently justified according to chapter 4.3.2 of the CVMP guideline on pharmaceutical fixed combination products (EMEA/CVMP/83804/2005).

Secondary pharmacodynamics

Pharmacological interference must be expected in the target species following observations with the concurrent administration of spinosad and high doses of ivermectin in dogs, which had prompted the US FDA in June 2008 to release a warning on the increased risk of ivermectin-like toxicity following concomitant exposure (FDA, Centre for Veterinary Medicine: Comfortis and ivermectin interaction safety warning notification).

Interaction studies revealed that co-administration of ivermectin and spinosad to Beagle dogs resulted in significantly increased C_{max} and AUC levels of ivermectin when compared to administration of either substance alone. *In vitro* experiments demonstrated that spinosad (like ivermectin) is a substrate and an inhibitor of P-glycoprotein. Although the relevance of these findings for dogs is unproven, it was hypothesised that spinosad reduces ivermectin efflux from mammalian cells, particularly from those constituting the blood-brain barrier, and thereby increases ivermectin toxicity in dogs treated with a combination of both compounds.

Corresponding findings with spinosad and milbemycin oxime demonstrated that the kinetics of milbemycin oxime were significantly changed in terms of an increased exposure, when administered together with spinosad to Beagle dogs. Based on the findings with ivermectin and spinosad, a potential interaction of milbemycin oxime and spinosad on the level of transport P-glycoprotein was discussed, which may reduce the efflux of milbemycin oxime from cells and, by this, increase its concentration in the organism. As milbemycin oxime did not only reach increased plasma concentrations when administered together with spinosad, but was also eliminated from plasma with a prolonged

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elimination half-life, a potential interaction at the cytochrome P450 enzymes was also taken into consideration.

Despite these observations no dose adjustment of milbemycin oxime was considered for the combination product, although the $AUC_{0-\infty}$ of the compound was increased by more than the 3-fold in the presence of spinosad. Specific studies gave evidence that efficacy and safety were not affected by the increased exposure rates of milbemycin oxime following administration of the combination product. It was emphasised in addition that protein binding of both milbemycin oxime and spinosad in canine plasma was high and not affected by concurrent administration, so that no impact on the toxicity arising from the free drug fractions was expected.

The examination of the metabolic degradation of spinosad and milbemycin oxime has demonstrated that milbemycin oxime was transformed to dealkylation, hydroxylation and glucuronidation products and was mainly eliminated with the faeces. It remains unknown whether the metabolism of milbemycin oxime was affected at least quantitatively in the presence of spinosad, because no control group treated with milbemycin oxime alone had been run. It has been speculated that milbemycin oxime and spinosad may interfere at the cytochrome P450-enzymes which trigger the conversion of many lipophilic drugs to hydrophilic degradation products.

The reverse case, i.e. the potential influence of milbemycin oxime on the metabolic degradation of spinosad was not subject of this experiment no pharmacokinetic interaction of milbemycin oxime and spinosad had been identified to the account of the latter in the pharmacokinetic interaction studies.

In summary, spinosad and milbemycin oxime interfere in the mammalian organism as indicated by considerably increased exposure to milbemycin oxime and prolonged elimination. Although not proven, interaction on the level of trans-membranous transport glycoproteins as well as on degradation enzymes of the cytochrome P450-family were considered. Exposure and elimination of spinosad is apparently not notably affected when administered together with milbemycin oxime.

Development of resistance

Spinosad

The spinosyns including spinosad affect the nicotinic acetylcholine receptors (nAChRs) in insects like other neonicotinoids (e.g. imidacloprid, nitenpyram), but they interact with different subunits of the nAChR receptor. Thus, spinosad has a unique mode of action. Fleas on dogs had not been exposed to spinosad until its introduction in 2011 and emergence of resistance would not be expected at this early stage. No resistance of cat fleas to spinosad has been reported to date in the PubMed database.

Milbemycin oxime

Since the introduction of milbemycin oxime there has not been evidence of resistance either within gastrointestinal nematodes or heartworms in Europe, and a recent literature search failed to find references relating to milbemycin oxime and resistance. There is evidence, however, reported from the US FDA (Snyder et al., 2011) that the frequency of reports on lack of efficacy in *D. immitis* heartworm prevention have increased in number in the US. It is also published that a dog collected in New Orleans suffering from class 2 heartworm disease showed continuing high microfilaraemia after oral treatment with 2 mg milbemycin oxime/kg bw daily in two series of 7 and 8 days (Bourguinat et al., 2011). In a laboratory study less than 100% efficacy (98.9–99%) was obtained following a single dose of 0.5–0.75 mg milbemycin oxime per kg bw in combination with 30–45 mg spinosad per kg bw orally on D30 or D45 after artificial infection with the recently isolated US strain "*Georgia*". In turn 100% efficacy against this heartworm strain was obtained by repeated treatments on D45 and D75 or on D30, D60 and D90 post inoculation revealing adequate efficacy of the fixed combination after multiple monthly

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treatments. Recent data did, however, not provide evidence that *D. immitis* strains collected in Europe have reduced susceptibility to macrocyclic lactones like milbemycin oxime.

Pharmacokinetics

Thirteen *in vitro* and *in vivo* studies were conducted to evaluate the pharmacokinetic profile and absorption, distribution, metabolism and excretion of the veterinary medicinal product. Only studies on metabolism, distribution and elimination of ^{14}C-spinosad, major metabolism, and elimination of ^{14}Cmilbemycin oxime and on pharmacokinetics/dose linearity were conducted according to GLP.

Protein binding was high (>90%) for spinosad- and milbemycin oxime-components. There was no interaction between spinosad and milbemycin oxime regarding the protein binding.

Metabolism, distribution and elimination of ^{14}C-spinosad after oral administration: Up to 55% of the administered dose was excreted in the faeces by day 21 post-dose. A further 16–19% is excreted in the urine. Unmetabolised compound was found in tissues, principally skin-associated fat, peri-renal fat and the liver. There was no evidence of accumulation, since concentrations in these tissues had markedly decreased by day 21 post-dose. Spinosyns A and D were extensively metabolised in the bile, urine and faeces of the dogs. Glutathione conjugation and demethylation were defined as the primary pathways of metabolism.

Metabolism and elimination of ^{14}C-milbemycin oxime after oral co-administration of spinosad and ^{14}C-milbemycin oxime: Milbemycin oxime was mainly eliminated with the faeces and underwent extensive degradation with the parent compound being present in plasma only. Some of the metabolites in plasma, urine and faeces were dealkylation, hydroxylation and glucuronidation products of milbemycin oxime. It remains unknown whether the metabolism of milbemycin oxime was affected by the presence of spinosad.

Plasma pharmacokinetics and interactions of the components after oral administration: According to several non-GLP studies key pharmacokinetic parameters may be affected by the pharmaceutical formulation as well as by the physic-chemical properties of the components.

The studies clearly demonstrate that key pharmacokinetic parameters of spinosad are marginally or not affected by co-administration of milbemycin oxime, but the exposure ($AUC_{0\rightarrow inf}$) to milbemycin A_3 and A_4 5-oxime is increased considerably, and also $T_{1/2}$ and T_{max} are affected. However, results from clinical studies show that this has no impact on the clinical efficacy of the fixed combination product.

Plasma pharmacokinetics and dose-linearity after single oral administration: A pilot study showed dose linearity of systemic exposure of spinosyns A and D within the dose range tested (30–120 mg spinosad per kg bw and 0.5–2.0 mg milbemycin oxime per kg bw), and virtually comparable times of maximum plasma concentrations of spinosyns A and D. For milbemycin A_3 and A_4 5-oxime, dose linearity was not clearly shown. A GLP-study was conducted to investigate dose-linearity between doses administered and systemic exposure of the relevant components analysed. The increase in C_{max} was statistically significantly less than proportional for each of the four components. Mean pharmacokinetic results after single oral administration of the recommended treatment dose are as follows:

	Milbemycin A_3 5-oxime	Milbemycin A_4 5-oxime	Spinosyn A	Spinosyn D
Mean AUC_{inf} (ng•h/ml)	1602	16653	102529	16785
Mean C_{max} (ng/ml)	54.1	301	3200	619

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Mean T_{max} (h)	3.6	4.3	4.3	4.3
Mean $T_{1/2}$ (h)	33.9	77.2	131	135

Accumulation after repeated oral administration: There are indications for an accumulation of the substances during repeated administration the impact of which on efficacy and safety is discussed in the safety and efficacy parts.

Absolute bioavailability: The results of the study on absolute bioavailability are invalid because of statistical concerns. Nevertheless, the study indicates that both, spinosad and milbemycin oxime, are highly bioavailable after oral administration in fed dogs.

Gender effect: Studies indicate that dog's sex may impact pharmacokinetics of spinosad and milbemycin oxime. It is noted that a gender effect on pharmacokinetics is also demonstrated in the GLP-compliant TAS study. The gender effect appears to be irrelevant as regards efficacy, since the recommended treatment dose-range is broad and a gender effect on efficacy was not observed in clinical studies, in particular dose-finding studies. The relevance as regards safety is assessed in the safety part of this assessment report.

Interactions - metabolic stability: Metabolic stability of milbemycin oxime and ivermectin was evaluated in the presence and absence of spinosad in *in vitro* incubations with Beagle dog microsomes and Beagle dog hepatocytes. In both test systems the presence of spinosad affected the metabolism of ivermectin and milbemycin A_3 5-oxime. The effect of spinosad on milbemycin A_4 5-oxime could not be assessed. There are interactions between ivermectin and the components of Trifexis. The SPC includes an adequate statement as regards interactions with substrates for P-glycoprotein in section 4.8 of the SPC, which includes interactions with macrocyclic lactones like ivermectin.

Summary: When spinosad and milbemycin oxime are administered in combination, systemic exposure to milbemycin oxime is increased as compared to the administration of this component alone. The exposure to spinosad did not change when co-administered with milbemycin oxime. Repeated administration of the combination resulted in accumulation of both, spinosad and milbemycin oxime, in blood plasma of juvenile dogs and possibly also of adult dogs without reaching steady state within 6 months.

Dose determination / justification

Spinosad

The identification of the minimum recommended dose of 45 mg spinosad/kg bw orally in dogs for the treatment and prevention of flea infestations (*Ctenocephalides felis)* for up to 4 weeks has been previously determined for the monocomponent product, spinosad chewable tablets for dogs.

Milbemycin oxime

The nematocidal active milbemycin oxime is already authorised in EU countries either in combination with the cestocidal active praziquantel, or with the insect growth regulator lufenuron at a minimum recommended oral dose of 0.5 mg milbemycin oxime/kg bw in dogs. To justify the higher minimum recommended dose of 0.75 mg milbemycin oxime per kg bw in Trifexis, both dose determination and non-interference studies were focussed on the dose-limiting hookworm species (L4/L5 of *A. caninum* and adults of *U. stenocephala*). In all studies dogs were fasted overnight prior to the day of treatment and were fed with approximately 25% of their daily ration shortly prior to dosing. Dose determination studies were performed on artificial infections.

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Ancylostoma caninum hookworms (immature L4/L5 and adult stages):

In a pilot non-GCP laboratory study, gelatine capsules containing milbemycin oxime technical powder were administered orally at point doses of 0.25, 0.5 and 0.75 mg/kg bw on D0 to puppies artificially infected with *A. caninum* larvae three weeks before treatment. A negative and positive control group (0.5–1.0 mg milbemycin oxime/kg bw orally) run in parallel. Efficacies were of 33.2%, 98.3% and 100% at necropsy on D7, respectively. The efficacy at the point dose of 0.5 mg milbemycin oxime per kg bw was statistically not different to the commercial milbemycin oxime product (efficacy: 100%).

In a GCP-compliant dose determination study, the efficacy against adult *A. caninum* was evaluated in groups of dogs each dosed orally with 0.15, 0.5, and 1 mg milbemycin oxime/kg bw each in co-administration with 30 mg/kg bw spinosad. A negative and a positive control group (0.5 mg milbemycin oxime/kg bw orally) run in parallel. In contrast to the low dose group (77.3%) the mid and high dose group achieved the threshold of ≥90% efficacy.

Study results revealed that the addition of spinosad did not cause any inhibitory effect on the activity of milbemycin oxime.

In another non-GCP laboratory study, two groups of dogs were artificially infected with *A. caninum* L3 on D0 and treated orally with point doses of 30 mg spinosad/kg bw and 0.5 mg milbemycin oxime/kg bw on D7 and D11. Dogs were necropsied on D12 (L4 stages) and on D17 (L5 stages), respectively. Two groups served as negative controls. Neither the efficacy for L4 (32.4%) nor for immature L5 of *A. caninum* (76.8%) achieved the accepted threshold of ≥90% according to the VICH anthelmintic guidelines.

A GCP-compliant laboratory study was, therefore, conducted at the higher dose rate of 0.75–1.0 mg milbemycin oxime/kg bw in fixed combination with 45–60 mg spinosad/kg bw (final tablet formulation) in 32 dogs artificially infected with 300 3rd stage larvae of *A. caninum*. Groups of dogs were each treated orally on D7 (L4) or D11 (L5). Two groups served as vehicle controls. At necropsy on D12 and D16 efficacies of 98.9% and 97.8% against L4 and L5 stages of *A. caninum* were demonstrated, respectively.

Study results show that the larval stages (L4, L5) of *A. caninum* are dose limiting for milbemycin oxime.

Uncinaria (U.) stenocephala (adult hookworms):

Four laboratory studies were provided to demonstrate efficacy against *U. stenocephala* but adequate efficacy at the minimum oral dose of 0.75 mg milbemycin oxime/kg bw could not be demonstrated.

Toxocara (T.) canis roundworms (immature adult L5 stages):

One GCP-compliant dose determination study was conducted in three groups of puppies artificially infected with *T. canis* eggs on D0. Two groups were treated orally on D24 with point doses of 0.5 mg (group 2) or 0.75 mg (group 3) milbemycin oxime/kg bw each in combination with 45 mg spinosad/kg bw, respectively. A negative control group run in parallel. The calculated efficacies in group 2 and 3 at necropsy on D29 were 96.6% and 99.0% for immature adults (L5), and each 100% for adults of *T. canis*, respectively.

Dose confirmation studies

Fleas *(Ctenocephalides felis):*

Three GCP-compliant dose confirmation studies were provided in flea infested dogs to confirm both the efficacy of spinosad in the fixed combination and the non-interference after administration of the final

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tablet formulations. In all studies dogs were fasted overnight prior to the day of treatment and were then fed with approximately 25% of their daily food ration prior to dosing.

In a GCP-compliant non-interference study two groups of dogs were treated orally on D0 either with a commercial tablet formulation at 45 to 60 mg spinosad/kg bw or with the intended final tablet formulation at the dose range of 45 to 60 mg spinosad/kg bw in fixed combination with 0.75 to 1 mg milbemycin oxime/kg bw, respectively. After each weekly infestation arithmetic mean efficacies with the final tablet formulation were 99.5–100% up to and including D23 and 91.2% on D30. Statistically equivalent results were found for the comparator product revealing that milbemycin oxime does not interfere with the activity of spinosad.

In a GCP-compliant dose confirmation study in the EU a group of dogs was starting on D-1 weekly infested with 100 fleas per animal and was then dosed with the final tablet formulation at 45 to 60 mg spinosad/kg bw and 0.75 to 1 mg milbemycin oxime/kg bw orally on D0. A negative control group run in parallel. Arithmetic mean efficacy of 100–99.1% was calculated up to and including D30, respectively.

A GCP-compliant dose confirmation study in the US was undertaken to demonstrate the efficacy at the lower US dose range of 30 to 45 spinosad mg/kg bw in fixed combination with 0.5 to 0.75 mg milbemycin oxime/kg bw. Group A served as vehicle control while dogs in group B were orally treated with 30–45 mg spinosad/kg bw in combination with 0.5–0.75 mg milbemycin oxime/kg bw on D0. Group C dogs were orally treated on D0 with a product containing spinosad at the dose range of 30 to 60 mg/kg bw. Group D dogs were treated orally with a commercial milbemycin oxime product at a dose range of 0.5–1 mg/kg bw. Dogs of group B showed 99.9 to 100% persistent efficacy against fleas for 4 weeks and 92.3% in the 5th week based on arithmetic mean. The commercial spinosad product was 100% effective during 4 weeks and 91.5% effective in the 5th week. No activity against fleas was found in group D. Results evidenced that milbemycin oxime does not interfere with the activity of spinosad.

Results justify the label claim: “Treatment and prevention of flea infestations (*Ctenocephalides felis*) for up to 4 weeks after a single administration of the veterinary medicinal product”.

To justify the claims “reduction in egg production in fleas” and “the veterinary product can be used as part of a treatment strategy for the control of flea Allergy Dermatitis (FAD)” reference was made to the studies submitted with the Comfortis application (spinosad alone). Thus, no additional studies are deemed necessary.

A*ncylostoma caninum (*4th stage larvae (L4), immature adult (L5) and adult hookworms*):*

A GCP-compliant dose confirmation study was carried out at a dose range of 0.75–1 mg milbemycin oxime/kg bw in combination with 45–60 mg spinosad/kg bw to demonstrate efficacy against immature L4 and L5 stages of *Ancylostoma caninum*. Four groups of puppies were infected each with 300 infective 3rd stages on D0. The calculated efficacy against L4 stages was 99.3% (D7) compared the control group, while the efficacy against immature L5 stages amounted to 98.6% on D11.

Results confirm that the increased dose of 0.75 mg milbemycin oxime/kg bw exhibits adequate activity against L4 and L5 stages of *A. caninum* and thus broadening the spectrum of activity of milbemycin oxime.

In a GCP-compliant dose confirmation study in the US four groups of dogs were experimentally infected with *A. caninum* larvae on D-27. Group 1 served as vehicle control. Group 2 was treated orally on D0 with the approved lower US dose range of 0.5–0.75 mg/kg bw milbemycin oxime in fixed combination with 30–60 mg spinosad/kg bw. Group 3 was treated orally with a commercial product in a dose range of 30–60 mg spinosad/kg bw. Group 4 was treated orally with a commercial product at a

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dose range of 0.5 to 1 mg milbemycin oxime per kg bw. Groups 2 and 4 exhibited ≥ 99.5% efficacy at necropsy on D7. No activity against adult *A. caninum* was found in the spinosad group 3.

A GCP-compliant dose confirmation study was initiated in South Africa on dogs naturally infected with *A. caninum*. One group of dogs was treated with the final tablet formulation at the US dose rate of 0.5–0.75 mg milbemycin oxime/kg bw in combination with 30–45 mg spinosad/kg bw. A vehicle control group was run in parallel. The final tablet formulation showed a reduction of 99.8% at necropsy on D7.

Toxocara canis (immature adult L5 and adult roundworms):

A GCP-compliant dose confirmation study in the US was carried out on dogs artificially infected with 250 larvated eggs of *T. canis* on D0 and treated with the intended final formulation at a dose rate of 45–60 mg spinosad/kg bw and 0.75–1.0 milbemycin oxime mg/kg bw either on D14 (to assess L4) or D24 (to assess immature adult L5). Two groups served as vehicle controls. All dogs were necropsied on day 5 or 6 after each respective treatment. Acceptable infection rates in the controls could only be achieved for L5 larvae of *T. canis* at necropsy and a 96.15% percent reduction in immature adult L5 was calculated for the group treated on D24.

A GCP-compliant dose confirmation study in the US was performed in dogs artificially infected on D-51 with 150 eggs of *T. canis*. A group of dogs was treated with the final tablet formulation at the US dose rate of 0.5–0.75 mg milbemycin oxime/kg bw in combination with 30–45 mg spinosad/kg bw. The final tablet formulation exhibited 99.6% efficacy ($p < 0.0001$) against the adults of *T. canis* at necropsy on D7.

A GCP-compliant dose confirmation study in the EU was conducted on pure and cross breed dogs naturally infected with *T. canis* roundworms. One group of dogs was treated with the final tablet formulation at the lower US dose rate of 0.5–0.75 mg milbemycin oxime/kg bw and 30–45 mg spinosad/kg bw. 100% efficacy was calculated against the adults of *T. canis* at necropsy on D7.

Toxascaris leonina (adult roundworms):

A GCP-compliant dose confirmation study in the US was performed in dogs experimentally infected with *Toxascaris leonina* eggs on D-72 prior to treatment. One group was treated with the final tablet formulation at a dose rate of 0.5–0.75 mg milbemycin oxime/kg bw and 30–45 mg spinosad/kg bw whereas a second group served as negative vehicle control. The final tablet formulation exhibited 93.4% efficacy at necropsy on D7.

A GCP-compliant dose confirmation study in the EU was conducted on pure or mixed bred dogs naturally infected with *Toxascaris leonina*. One group of dogs (A) was treated orally with the final tablet formulation at a dose rate of 0.5–0.75 mg milbemycin oxime/kg bw and 30–45 mg spinosad/kg bw. A second group served as negative vehicle control. The final tablet formulation demonstrated 93.3% efficacy at necropsy on D7.

Trichuris vulpis (adult whipworms):

A GCP-compliant dose confirmation study in the US was performed in dogs experimentally infected with *Trichuris vulpis* eggs on D-86 prior to treatment. A group of dogs was treated orally with the final tablet formulation at a dose rate of 0.5–0.75 mg milbemycin oxime/kg bw and 30–45 mg spinosad/kg bw on D0 and a second group served as negative vehicle control. The final tablet formulation exhibited 96.5% efficacy at necropsy on D7.

A GCP-compliant dose confirmation study in the EU was conducted on adult dogs naturally infected with *Trichuris vulpis*. One group of dogs was treated with the final tablet formulation at a dose rate of

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0.5–0.75 mg milbemycin oxime/kg bw and 30–45 mg spinosad/kg bw on DO. One group served as vehicle control. The final tablet formulation exhibited 100% efficacy at necropsy on D7.

The treatment claims for immature L4, immature adults (L5) and adults of *A. caninum* hookworms, immature adults (L5) and adults of *T. canis* roundworms, adults of *T. leonina* roundworms and for *T. vulpis* roundworms (adults) are justified according to the current VICH anthelmintic guidelines GL7 and GL19.

Dirofilaria immitis (prevention of heartworm infections):

In the EU milbemycin oxime has already been approved for the prevention of heartworm infections in dogs at repeated doses of 0.5 mg/kg bw. The efficacy of this active substance in this indication is, therefore, not in question.

Three GCP-compliant studies performed in the US have been presented investigating the product in dogs experimentally infected with third stage larvae of *Dirofilaria immitis* to confirm the efficacy of milbemycin oxime in Trifexis at a dose of 0.5 mg/kg bw in the prevention of heart worm disease. Results were transferable to the EU, because there are no known differences in the morphology and the genome of *D. immitis* between US and EU strains.

Studies were performed according to the VICH anthelmintic guidelines GL7 and GL19 in 4 to 9 month old male and female Beagle dogs which were free from heartworms at study initiation, as confirmed by the absence of microfilaria and specific antibodies in blood. Dogs were infected subcutaneously on study D-30 with sufficient numbers of 3rd stage larvae of *D. immitis* strains “Georgia” or “Michigan”, which had been isolated 2006 and 2007 in the related US states from dogs not given heartworm preventive treatments before. With this scheme successful infection was demonstrated in vehicle treated control groups.

Dogs from the treatment groups received the final test product orally on the scheduled treatment days 30 minutes after they had been given some of their daily food ration. The actual treatment doses administered to dogs were those approved in the US and were lower than the minimum doses indicated in 4.9 of the SPC (45 mg spinosad and 0.75 mg milbemycin oxime per kg bw) but were, regarding milbemycin oxime, still slightly higher than the dose of 0.5 mg/kg bw approved for the heartworm claim in EU countries. Spinosad was administered in a dose range of 29.7–44.8 mg/kg bw, milbemycin was administered in a range from 0.49–0.74 mg/kg bw. To examine the effect of treatment at different intervals after infection as well as of repeated administrations, dogs were treated 30 or 45, 30 and 60, 45 and 75 or 30 and 60 and 90 days after artificial inoculation with infective third stage larvae. With these treatment regimens the period of infectious third stage larvae moulting to fourth stage larvae in the skin, which takes place around 7 to 14 days after infection, has not been covered, whereas the period of migrating fourth stages and their moulting to pre-adult fifth stage larvae approximately 45 to 60 days (6–8 weeks) after infection has been covered.

In contrast to the treatment regimens employed in these studies, the treatment of dogs in practice should be initiated prior to their potential exposure to heartworm infection, according to section 4.9 of the SPC. Taking into account that fact that repeated treatments at monthly intervals are recommended, all larval stages of *D. immitis* would be met by such regular monthly treatments.

Dogs were euthanized 6 months after artificial infection. From the cardio-pulmonary tract of all vehicle-treated control dogs adult male and female heartworms were collected in sufficient numbers. This allowed for the clear discrimination of the product efficacy between the different treatment groups.

Because of the severity of heartworm disease in dogs, VICH guideline GL19 requests a 100% efficacy regarding heartworm counts (reduction efficacy) and numbers of dogs carrying heartworms (prevention rate). However, for the claim “prevention of heartworm disease” proposed for Trifexis, “a

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few residual worms could be accepted" according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines.

Actually, 100% efficacy in terms of percentage reduction of heartworms and in terms of prevention rate was achieved in some but not all treatment groups. Study results demonstrate that under US conditions there may be a strain-dependent difference in the susceptibility of *D. immitis* to preventive treatment with the spinosad/milbemycin oxime combination. The treatment length needed to provide 100% prevention was one treatment 30 days after inoculation for the "Michigan" strain and three consecutive monthly treatments (30, 60 and 90 days after inoculation) for the less susceptible "Georgia" strain.

Efficacy studies with European *D. immitis* strains have not been provided. However, according to the literature, the susceptibility of European *D. immitis* strains to milbemycin oxime is not lower than in the US. Furthermore the continuation of treatment for one month after the last exposure of dogs to mosquitoes is sufficient for Europe (SPC, section 4.9) and this is in line with the European Scientific Counsel Companion Animal Parasites (ESCCAP) Guideline No. 5, 2012. In dogs travelling to endemic heartworm regions, treatment should be started within 30 days after arrival according to ESCCAP guidelines.

Administration of the test product provoked vomiting, mostly within 1–2 hours after administration, in a total of 6 of the 70 dogs (8.6%) in the test groups, and was graded as not serious by the study authors. As a result, the administered tablet, or part of it, could be lost so there is an increased risk of treatment failure due to insufficient absorption of the active substance. Failure to prevent heartworm disease would have far more serious consequences for the dog than failure to prevent, for example, flea infestation. However, sufficient advice is included in the SPC (and other product literature) concerning the need to monitor the dog closely after administration of the tablet, and re-dose the dog with another full dose if vomiting occurs within an hour of administration and the tablet is visible. This should ensure efficacy of the product in the prevention of heartworm disease.

Target animal tolerance

Several studies on target animal safety, including the safety in subgroups, have been provided. These comprise of a six month pivotal target animal safety study and its three months pilot, a single dose emesis study in 7 week old puppies, a reproductive toxicity study, studies in avermectin sensitive dogs, a study in dogs with patent heartworm infection, and a six months field safety study conducted in the US. All pivotal studies were conducted according to GCP or GLP requirements and used the intended final formulation of the product. All these studies were conducted using target doses of 45–60 mg spinosad/kg bw plus 0.75–1 mg milbemycin oxime/kg bw, which is below the maximum label dose of 70 mg spinosad/kg bw plus 1.17 mg milbemycin oxime/kg bw. Target animal safety information can also be derived from studies that were primarily conducted for other purposes, including pharmacokinetic and dose finding studies, and from the EU clinical field studies. Additionally, the report of a GLP study on the effects of acute single overdoses up to 3X the maximum label dose (which is 70 mg spinosad/kg bw plus 1.17 mg milbemycin oxime/kg bw) in dogs aged 10.9–15.7 months has been provided.

The doses of the single substances in the combination do not exceed the doses authorised for both active substances. However, potential effects arise from the combination of both substances.

Regarding both active substances separately, the following adverse reactions in dogs are known:

For spinosad, the most frequently observed adverse event is vomiting, which most commonly occurs in the first 48 hours after dosing and is most likely caused by a local effect on the small intestine. This

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effect is dose dependent and within the authorised dose band of 45–70 mg/kg bw a higher incidence of vomiting is seen in dogs dosed at the upper end of the dose band (8% in studies with the monovalent product). Other adverse reactions are uncommon or rare, and include lethargy, anorexia, diarrhoea, ataxia and seizures. Overdose leads to a further increase in vomitus, affecting the vast majority of dogs following dosing with approximately 2.5 times the recommended dose of 70 mg/kg bw. Long term overdose led to mild elevations in alanine aminotransferase and phospholipidosis in lymphoid tissues.

For milbemycin oxime, lethargy, neurological and gastrointestinal symptoms occur in very rare cases following treatment with the label dose. Massive overdose may lead to signs of avermectin toxicosis.

For the combination, abundant target animal safety data on the effects of up to the medium label dose (up to 60 mg spinosad/kg bw plus 1.0 mg milbemycin oxime/kg bw) are available. Regarding the effects of acute overdoses in dogs, a report of a study using placebo, 1x, 1.5x and 3x the intended maximum label dose in 12 dogs each was presented. Some additional information on acute overdose was achieved in some dogs in the single dose emesis study (maximum actual dose: 1.4x the intended maximum label dose) and in dogs of two pharmacokinetic studies (with doses corresponding to 1.3x and 1.7x the intended maximum label dose). However, since these studies were not designed for the detection of adverse events despite emesis, other reactions, especially those of neurological nature, could have been missed. Field safety data is not robust regarding the full dose range. In the 6 months US field safety study, dogs received doses of 30–60 mg spinosad/kg bw plus 0.5–1.0 mg milbemycin oxime/kg bw. In the EU studies, only five dogs (less than 2%) received a dose >65 mg spinosad/kg bw plus 1.1 mg milbemycin oxime/kg bw, and only a single treatment was applied.

The following acute adverse events were noted after treatment with Trifexis:

Emesis occurred in many studies, with a variable frequency, and most commonly within 48 hours. The highest rate could be observed in dogs younger than the minimum age of 14 weeks: in the single dose emesis study (maximum actual dose: 1.4x the intended maximum label dose), 19/36 of the 7 week old puppies vomited following treatment. In older dogs, vomitus could be observed as a dose dependent effect. In the acute single overdose study, vomitus in dogs aged 10.9–15.7 months occurred in 0/12 dogs in the placebo group, 1/12 in the 1x group, 2/12 in the 1.5x group and 6/12 in the 3x group (in this group, 3 dogs vomited more than once). In two pharmacokinetic studies, using a single overdose corresponding to 1.3x to 1.7x the intended maximum label dose respectively, 4/6 and 4/8 dogs vomited. This can be considered an unacceptable high rate of emesis, but the number of dogs is too low to draw final conclusions. In other studies, including the pivotal 6 month target animal safety study (mean dose corresponding to 0.7x the maximum label dose), the emesis rate is considerably lower. During the US field safety study vomitus was a commonly observed adverse event. In the 2 European clinical field efficacy/safety studies, only a few adverse events were observed. Diarrhoea occurred in many studies, with a lower frequency than emesis. During the US field safety study diarrhoea was observed in 21/176 of the dogs.

Effects of potentially neurotoxic origin were also observed following treatment with Trifexis. Milbemycin oxime is a macrocyclic lactone. Depending on the substance and dose, macrocyclic lactones have the potential to exert neurological activity in vertebrates in case they reach the central nervous system, where they can lead to severe or fatal effects (so-called avermectin toxicosis). This is normally prevented by the blood-brain-barrier, namely the efflux pump P-glycoprotein, which is encoded by the multidrug resistance gene MDR1. Spinosad has been shown to be a substrate of P-glycoprotein. There is no evidence for a specific neurological toxicity of spinosad, despite the report of uncommon/rare effects of ataxia and seizures. Based on this situation it seems possible that spinosad “blocks” P-glycoprotein, leading to higher concentrations of milbemycin oxime in the CNS in dogs with the usual expression of P-glycoprotein (so-called MDR1 +/+ or wild type dogs; dogs with mutations of the MDR1

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gene are addressed separately, below). Salivation and tremors could be signs of neurological effects and were seen in the reproduction toxicity study and one of the EU clinical field efficacy/safety studies, and in the pivotal target animal safety study, respectively. In the acute single overdose study, (hyper-)salivation was noted in 1/12 dogs of the 1.5x group and 2/12 dogs in the 3x group; in one case of the latter, this was associated with decreased activity and partially closed eyelids. Another dog of this group stumbled, which occurred in the same time frame as vomitus. It is noteworthy that in the chronic overdose studies high plasma levels were achieved by consecutive dosing of an approximate medium label dose, which did not lead to such concerns. Lethargy occurred in the US field safety study in 22/176 (12.5%) dogs, as well as one case of seizures.

Determining the frequency rates of adverse reactions is hampered by the fact that data in laboratory studies were usually achieved using doses which did not include the upper part of the intended dose band. The US field study used a lower dose band than the one intended for the EU, and in the EU field studies performed for only 2 and 4 weeks, respectively, less than 2% of the animals received a dose above 65 mg spinosad/1.1 mg milbemycin oxime.

In order to adequately reflect the adverse reactions observed, the following wording is used in section 4.6 of the SPC (followed by the standard QRD table of frequency grouping):

“A commonly observed adverse reaction is vomiting, which occurs in the first 48 hours after dosing. In the majority of cases, vomiting was transient and mild and did not require symptomatic treatment.

At doses of 30 to 60 mg spinosad and 0.5 to 1 mg milbemycin oxime per kg bodyweight, lethargy, anorexia/decreased appetite, diarrhoea, pruritus, dermatitis and reddening of the skin and pinna were commonly seen. Hypersalivation, muscle tremors, ataxia and seizures were uncommon. Post-marketing reports for spinosad indicate that in very rare cases, blindness, impaired vision and other eye disorders were observed.”

Data on monthly treatment and chronic overdose were generated using doses considerably lower than the maximum label dose for each application. The pivotal target animal safety study used a mean dose of 51 mg/kg bw spinosad plus 0.84 mg/kg bw milbemycin oxime in the 1x group. This corresponds to 0.7x the maximum intended label dose. Additionally 5/8 dogs of the 1x group were treated with a dose below the minimum label dose on one occasion. Furthermore, overdose was achieved by application of a single low to medium label dose on one day for the 1x group or on 3 or 5 consecutive days for the 3x and 5x groups, respectively, at the beginning of each treatment round. These treatment rounds were conducted every 28 days for three months in one study, or for six months in another study. Plasma levels of spinosad were determined in the pivotal target animal safety study for all treatment groups and it could be demonstrated that an adequately high exposure to spinosyns A and D was reached in the overdose groups. However, due to the low single dose applied, the resulting cumulative dose administered per treatment round in the 3x and 5x groups thus corresponded to 2.2x and 3.6x the maximum label dose, respectively. This possibly explains the unexpectedly low rate of observed events recorded, even in the highest dose group. No other data is available from other studies which would compensate this deficiency.

Pharmacokinetic data suggest that dosing with the medium label dose leads to an accumulation of spinosyns and milbemycin oxime in the body without reaching a plateau during the tested period of six months. In juvenile dogs, trough concentrations of spinosad doubled monthly up to month 5. The increase in plasma concentrations of both substances was strongly correlated with an increase in their terminal elimination half-lives. Since a similar increase in half-lives following repeated administration could be observed in adult dogs, this effect cannot solely be attributed to growing physiology – to the contrary, one might assume that an increase in trough concentrations might also occur in adult dogs. Regarding the pharmacokinetic parameters AUC and C_{max}, data were not sufficient to conclude on their

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behaviour following repeated administration. Presented bioequivalence analyses for adult dogs considered only a three-month period and resulted in upper confidence limits for the ratio of C_{max} of 119% and 149% for spinosad A and milbemycin A_4 5-oxime, respectively, thus showing that one possibly could expect even higher increases in longer time-spans. Six-month data on juvenile dogs showed a numerical increase of C_{max} and AUC, however they were based on three dogs only and have thus to be considered with caution. The pivotal EU clinical field studies using the intended label dose were conducted with a single treatment and the observation period was only 2 and 4 weeks, respectively. Therefore, the European field studies do not allow conclusions on long-term safety of Trifexis. The US field study was conducted for six months, but the maximum tested dose was 60 mg spinosad/kg bw plus 1.0 mg milbemycin oxime/kg bw. Thus, field safety has not been adequately characterised with respect to accumulation of the active substances. However, during the 6 month US field safety study reddening of the skin or the pinna belonged to the adverse events with the highest frequency (in almost 7% of the dogs), which is adequately addressed in section 4.6 of the SPC.

In consequence, the following conclusions on long term use of Trifexis are restricted to maximum duration of treatment of six months at a maximum cumulative overdose of 3.6x. Only very few potential effects of chronic use could be identified in the pivotal target animal safety study. Laboratory findings comprised of an increase in alanine aminotransferase, total protein and calcium. All these effects lacked clinical correlation. Apart from that, sporadic haematology and clinical chemistry parameters were statistically different from controls, but these deviations were minor and not considered biologically relevant. In 2/4 male dogs of the high dose group, histology revealed hypospermatogenesis, in contrast to 0/4 male control dogs; however, no histopathology was not performed on dogs of the other groups. This had been considered as a spontaneous finding in peripubertal dogs, and supported by literature. However, animal numbers examined in this study are too low to rule out toxic effects of the product on spermatogenesis. In accordance to the wording used in the product information for the spinosad monopreparation, Comfortis, information is included in section 4.7 of the SPC that the safety of the product in male dogs used for breeding has not been determined.

Taken together, no reliable margin of safety can be derived beyond a treatment duration of six months. Based on the available data, the safety of repeated use of Trifexis has been demonstrated for a maximum of six consecutive months within one year. Related information is included in section 4.9 of the SPC: “This combination product (Trifexis) must, however, not be given for more than 6 consecutive months in any one year”.

The findings of the margin of safety study are reflected in section 4.10 of the SPC.

In contrast to the results of studies with spinosad as single substance, no signs for phospholipidosis were detected which may be a result of the comparatively low doses used in the respective Trifexis target animal safety study.

Data on pharmacokinetics indicate that the dog’s gender may have an impact on its plasma level. Female dogs showed a higher drug exposure compared to males. During the pivotal target animal safety study with only few exceptions, in each treatment period and each group (0.7x, 2.2x, 3.6x of the recommended maximum treatment dose, overdoses achieved by cumulative dosing) the plasma levels of each substance were higher in females than in males; for milbemycin A_4 5-oxime, this difference was statistically significant in the highest dose group. However, the increase from month to month was similar for both sexes, that is, no difference in the accumulation between the sexes could be observed.

Concerning dogs with mutations of the MDR1 gene and/or ivermectin sensitive dogs, one pilot and one pivotal study were provided. Such dogs do not express P-glycoprotein, or do so to a lower extent.

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Thus, substances which cannot reach the brain in MDR1 +/+ (wild type) dogs, can enter the CNS (see above). This effect is dose dependent. In both studies, no signs of avermectin toxicosis were observed following treatment with Trifexis using the medium label dose once or as consecutive overdose over three or five days. However, the pilot study shows flaws and doubts remain on the degree of avermectin sensitivity of the dogs in the pivotal study. Taken together, although an increase in the risk of neurological toxicity by combination of the two substances at the medium intended dose and following consecutive dosing was not observed, the value of the data is considered too limited to allow for firm conclusions on the safety of the proposed product, especially following administration of the maximum label dose or accidental overdoses in dogs with an MDR1 mutation/ ivermectin sensitivity. Therefore, information is included in section 4.5 of the SPC that avermectin sensitive dogs and/or dogs with an MDR1 mutation should be treated with special caution.

It was demonstrated that the use of spinosad plus milbemycin oxime in dogs suffering from a patent heartworm infection does not lead to additional concerns. This study was also conducted with a dose below the intended label dose, but it is considered unlikely that special concerns regarding this subgroup would arise when using the high end of the label dose.

Since no studies have been performed in sick or convalescent dogs, information is given in section 4.5 of the SPC that in such dogs, the product should only be used based on a benefit-risk-assessment of the responsible veterinarian.

Additionally, section 4.5 of the SPC contains the following information: “It is recommended to observe the treated dog up to 24 hours post-administration of the product for possible adverse reactions (see SPC section 4.6). In case of adverse reactions consult your veterinarian.”

A study on reproductive toxicity was also conducted, using 10 bitches per group (control, 1x group using the lower half of the intended treatment dose, so-called 3x group receiving the 1x dose once weekly over three weeks). Treatment was applied monthly from at least 43 days prior to mating until weaning at 42 days post-partum. Most reproductive parameters were equal in placebo and treatment groups. Due to the small sample size final conclusions cannot be drawn, but a treatment of bitches with the lower half of the intended label dose does not appear to influence fertility or puppy viability. Therefore, in section 4.7 of the SPC, information is provided that the safety of the product in pregnant and lactating bitches has not been sufficiently established, and that spinosad is excreted with the milk. It is stated that during pregnancy and lactation the product should be used according to the benefit/risk assessment by the veterinarian.

The proposed minimum age for Trifexis is 14 weeks. This is in line with the minimum age of the authorised spinosad only product, Comfortis, which was introduced because of observations in puppies of reduced weight gain and a higher frequency of vomiting (related to the spinosad). This is consistent with the observation made for Trifexis. In the single dose emesis study, with puppies of 7 weeks of age, the emesis rate was approximately 50% (19/36 dogs; 5/12 dogs considering those treated with a formulation comparable to the final formulation). Additionally, 10/12 pups gained weight comparing the day before treatment and the day of treatment, whereas 11/12 pups lost weight comparing the day of treatment and the following day. In the pharmacokinetic study, 2/8 dogs (2/4 female dogs), treated for 6 months with 60 mg spinosad/kg bw plus 1.0 mg milbemycin oxime/kg bw were thin and gained less weight than the others. The CVMP noted their disagreement with some of the fundamental issues of the design of 2 of the studies regarding management of the animals.

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Summary on target animal tolerance

Several studies on target animal safety have been provided, including, but not limited to, a study on repeated use over six months, a six months field safety study conducted in the US and studies on the safety in subgroups. All pivotal studies were conducted according to GCP or GLP requirements and used the intended final formulation of the product at a target doses of 45–60 mg spinosad/kg bw plus 0.75–1 mg milbemycin oxime/kg bw (which is lower than the maximum label dose of 70 mg spinosad/kg bw plus 1.17 mg milbemycin oxime/kg bw). Target animal safety information can also be derived from studies that were primarily conducted for other purposes, including pharmacokinetic and dose finding studies, and from the single treatment EU clinical field studies. Additionally, the report of a study on the effects of acute single overdoses up to 3-fold the maximum label dose in dogs aged 10.9–15.7 months has been provided.

The following acute adverse events were noted within 48 hours after treatment with Trifexis:

Emesis occurred in many studies, with a variable frequency but overall suggesting a dose-dependency especially following doses exceeding the label dose. Data from studies using up to the medium label dose of Trifexis showed low emesis rates in the preclinical studies and emesis as a common event in the six month US clinical field study. In studies using overdoses of 1.3x to 3x the maximum label dose, about half of the dogs vomited, some of dogs dosed at 3x vomited repeatedly. Effects of potentially neurotoxic origin were noted in some cases following treatment with the medium label dose in preclinical studies (tremors, salivation), and the US field study (lethargy as common adverse reaction, and one case of seizures). One case of ataxia was recorded in the EU field study. Adverse reactions following overdoses indicate a dose dependency of this type of adverse events, with one case of hypersalivation following a 1.5x dose and two cases of (hyper-)salivation following a single 3x dose, one of which was associated with decreased activity. One case of stumbling was observed at a 3x overdose. Diarrhoea was also observed in many studies, including the US field study where it occurred as a common event. Further common adverse events following use of up to the medium dose of Trifexis include anorexia/decreased appetite, dermatitis and reddening of the skin and pinna.

None of these adverse reactions required medical treatment.

All adverse reactions are adequately addressed in section 4.6 of the SPC. In section 4.5 of the SPC, a recommendation is included to observe the treated dog up to 24 hours post-administration of the product for possible adverse reactions (see SPC section 4.6), and to consult the veterinarian in case of adverse reactions. Additionally, information is included that no studies have been performed in sick and convalescent dogs, and that therefore the product should only be used based on a benefit-risk assessment of the responsible veterinarian.

Following repeated monthly treatment over six months with mean doses of 0.7x of the maximum label dose, given on one day/month or three or five days/month (corresponding to a cumulative overdose of 2.2x and 3.6x, respectively), only few adverse events were noted. Field data of repeated use of the full range of the label dose are not available. Only very few potential effects of repeated use up to six months could be identified, and the mild laboratory findings lacked clinical correlation. Pharmacokinetic data suggest that dosing with the medium label dose leads to an accumulation of spinosyns and milbemycin oxime in the body without reaching a plateau during the tested period of six months, thus conclusions on potential effects of repeated use beyond this time cannot be drawn.

Based on the available data, the safety of repeated use of Trifexis has been demonstrated for a period of up to 6 months, and the maximum treatment duration has been limited to no more than six consecutive months in any one year.

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Some data on the use of Trifexis in MDR1 mutated/avermectin sensitive dogs has been generated. No signs of increased neurotoxicity in these dogs following single or consecutive treatment with 0.7x of the maximum label dose of Trifexis could be observed. However, since the maximum label dose has not been tested and doubts remain on the degree of avermectin sensitivity of the tested dogs, a precautionary warning is included in section 4.5 of the SPC to treat such dogs with caution.

It was demonstrated that the use of spinosad plus milbemycin oxime in dogs suffering from a patent heartworm infection does not lead to additional concerns.

No final conclusion could be drawn on the reproductive toxicity of Trifexis due to small sample sizes in the respective studies. Related information is included in section 4.7 of the SPC for female and male dogs. A recommendation is included that the product should be used according to the benefit/risk assessment by the veterinarian.

The minimum age for use of Trifexis is 14 weeks. This is in line with the authorised minimum dog age for the spinosad only product (Comfortis), introduced because of observations of reduced weight gain in puppies and a higher frequency of vomiting, attributed to spinosad. This is consistent with observations made for Trifexis.

Field trials

Treatment and prevention of flea infestations (*Ctenocephalides felis*)

One GCP-compliant multi-site randomised blinded controlled clinical field study was conducted in dogs from April to July 2011 in France. Flavoured tablets were orally administered at a single dose of 45–70 mg spinosad/kg bw and 0.75–0.17 mg milbemycin oxime/kg bw. A spot-on solution containing selamectin was used for comparison and dosed according to the labelling. A total of 266 dogs living in single- or multi dog households suffering from flea infestations were included of which 178 dogs received the test product and 88 dogs the control product. Both treatment groups were comparable with respect to breed (purebred/mixed breeds), age and weight range. No cases of flea allergic dermatitis were included into this field study. Under the conditions of this study, both the test and positive control group proved to be very effective against flea infestations. Success rates on D14 and D30, defined by a ≥90% individual flea count reduction, were 96.7% and 88.7% in the test group and 85.9% and 73.2% in the control group, respectively; although the success rates in the control group were high, those in the test group were even significantly superior. Since large numbers of dogs were even flea free (89.3% and 80% of the dogs treated with the tablet formulation, and 77.5% and 70.4% treated with selamectin on D14 and D30), success rates would have been comparably high if success had been defined by a more appropriate 95% threshold. Efficacy against flea infestations based on Abbott's formula showed reductions in flea counts (based on arithmetic means) of 98.42% and 94.74% in the group treated with the final formulation, and 95.88% and 84.86% in the control group on D14 and D30. Thus the test product missed the threshold of 95% only on D30 narrowly.

Given the low overall rate of adverse events (5.6%), flavoured spinosad/milbemycin tablets appeared to be well tolerated. Adverse events included diarrhoea, hypersalivation, ataxia, renal insufficiency, erythema and lethargy with an incidence of 0.6% for each of these events. The rate of emesis, a well-known common adverse effect of spinosad, occurring on the day of dosing or the day after dosing, was low (3 animals/1.7%). Only one dog vomiting 1 h after dosing needed to be re-dosed. However, only 3 dogs received doses above 65 mg spinosad/1.0 mg milbemycin/kg bw, and one of these dogs vomited on the day of dosing.

The tablet acceptability was 65.2% by free choice (by hand, on the floor or with food) and 34.8% "pilled".

Medicinal product no longer authorised

Treatment of nematode infections (*Toxocara canis, Toxascaris leonina, Trichuris vulpis, Ancylostoma caninum* and *Uncinaria stenocephala*).

A GCP-compliant multi-centre randomised blinded controlled clinical field study was conducted in dogs in France and Ireland following a single oral dose of 45–70 mg/kg bw spinosad and 0.75–1.17 mg/kg bw milbemycin oxime.

Milbemycin oxime containing tablets were used for control at the recommended single dose of 0.5 mg/kg bw in Europe. A total of 229 dogs living in single- or multi-dog households were enrolled into the study including a variety of different breeds, mixed breeds, ages, and weights representative of the target population.

As part of the justification of the combination product data were collected on the presence/absence of fleas prior to treatment to demonstrate concurrent infestations. About 37% of dogs in the safety population showed flea infestations on day 0 (38.4% in the test and 35.1% in the control group).

Eggs of *T. vulpis* were found most frequently, followed by *T. canis, A. caninum* and *U. stenocephala*. Eggs of *T. leonina* were only found in a total of 7 dogs. The nematode species present prior to treatment were well balanced between the two treatment groups. 56% of dogs in the test group and 56.2% of dogs in the control groups had single nematode infections, while the rest had mixed infections (2–4 nematodes).

The range of pre-treatment egg counts in all nematode species was wide. Box plots to illustrate the individual faecal egg count data by nematode species clearly showed that the distribution of data was skewed, particularly at the pre-treatment time point. In the post treatment samples (day 8 (7–10) post-treatment), the range of egg counts for all nematode species was much narrower, with the exception of *U. stenocephala*. The efficacy for each type of nematode was based on geometric means.

For spinosad/milbemycin oxime tablets, the success rate, based on the proportion of animals with ≥90% faecal egg count reduction was 94.3% for *T. canis*, 100% for *T. leonina*, 90% for *T. vulpis*, 100% for *A. caninum*, and 65.7% for *U. stenocephala*. Success rates for the control product were 88.5% for *T. canis*, 100% for *T. leonina*, 89.7% for *T. vulpis*, 90.9% for *A. caninum*, and 47.8% for *U. stenocephala*. Based on these success rates, spinosad/milbemycin oxime tablets proved to be non-inferior to the control product (milbemycin oxime); superiority could not be demonstrated as the lower bound of the 95% confidence intervals was <0.

The results confirm that flavoured spinosad/ milbemycin oxime tablets at the single oral recommended dose are effective in the treatment of mixed and single infections of *T. canis, A. caninum*, and *T. vulpis*. The data basis for *T. leonina* (5 test and 2 control animals, only) is too limited in order to allow reliable conclusions. However, taking into account the favourable results of the laboratory dose confirmation studies, the treatment claim for this type of nematode is accepted.

The data for *U. stenocephala* are not convincing. The proved non-inferiority of spinosad/milbemycin oxime tablets compared to an EU authorised product containing milbemycin oxime and praziquantel is not meaningful, since the control product is not authorised for the treatment of *U. stenocephala* infections. The percentage reduction in faecal egg counts following spinosad/milbemycin oxime tablets was 92.5% (AM 84%) compared to 79% (AM 57%) for the control product (milbemycin). Success rates, i.e., the proportion of dogs with individual ≥90% reductions of their faecal egg counts, was too low. This data, together with the inadequate results obtained in the laboratory dose confirmation studies, do not support a claim for *U. stenocephala*.

Under the conditions of this field study spinosad/milbemycin tablets proved to be well tolerated. The rate of adverse events was low (2%). Adverse events included diarrhoea and dermatitis/eczema.

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Surprisingly emesis was not observed at all. However, only 2 dogs received doses above 65 mg spinosad/1.0 mg milbemycin per kg bw.

The duration of this study (2 weeks) is furthermore too short in order to allow conclusions on the field safety of the product when used at monthly intervals as foreseen in the product literature.

The tablet acceptability by the dogs was 73.5% by free choice (that is, giving the tablet by hand, on the floor or with food) and 25.6% “pilled”. Based on these data the proposed text in section 4.9 of the SPC regarding palatability was not accepted by the CVMP.

Trifexis was launched on the US market at the beginning of 2011. The US PSURs for Trifexis, covering 22 months, have been provided as additional supporting data. It is worth noting that these pharmacovigilance data are derived from use of the product at a lower dose compared to the intended EU dose. The incidence of all adverse events for Trifexis is low The data do not allow any correlation between the occurrence of adverse events with the duration of administration of Trifexis. Based on the data provided, no conclusions on the safety of Trifexis in certain sensitive subpopulations (MDR1 mutated dogs, young animals) can be drawn.

Furthermore, PSUR data for the monovalent spinosad product, Comfortis, marketed in the US for 5 years and in the EU for almost 2 years, have also been provided as additional supporting data. As with Trifexis, the incidence of all adverse events is low, both in countries outside the EU (label dose 30–60 mg/kg bw) and also in countries inside the EU (label dose 45–70 mg/kg bw).

Pharmacovigilance data for the monocomponent spinosad product, Comfortis, in the target species (dogs), showed very rare (‘very rare’ is less than 1 animal in 10,000 animals, including isolated reports) cases of eye disorders.

Recent information from the US, as reported by the applicant, indicate that such very rare cases of eye disorders have also been reported after use of Trifexis, including blindness, impaired vision and other eye disorders.

Some inconsistencies were noted regarding the pharmacovigilance data provided.

Based on the PSUR data provided to date, an in-depth causality assessment in relation to the dose and duration of use of Trifexis is not possible, in particular for neurological and ophthalmic adverse events.

The applicant is therefore requested to conduct a post-authorisation safety study with the objective of a targeted monitoring of treated animals to give an idea of the incidence of those adverse events in a non-interventional study and to evaluate the nature and risk of neurological and ophthalmic adverse reactions induced by Trifexis.

Other studies

To assess the palatability of Trifexis tablets, two pilot laboratory studies have been conducted. Both studies evaluated the palatability of tablets containing spinosad and milbemycin oxime and varying amounts of a flavouring agent (beef flavour) with a commercially authorised product containing only milbemycin oxime.

These studies were conducted under specially controlled conditions which do not reflect the conditions in the field.

In addition, the acceptance of the final formulation was assessed in the US field safety study and in the two EU clinical field studies.

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During the field safety study, dogs that did not receive their dose before a meal were excluded from palatability population, which is not in line with the administration recommended in the SPC.

In both clinical field studies free choice was not differentiated from ingestion with food but included by hand, on floor, or in food. The number of dogs which had to be pilled was high in the clinical field studies (a fourth and one third, respectively).

Taking into account all the data presented on tablet acceptability, the proposed SPC text regarding tablet palatability was not supported by the Committee.

Overall conclusion on efficacy

Trifexis is a new fixed combination for oral use containing the insecticidal active spinosad which is already licensed at a minimum recommended dose of 45 mg/kg bw orally in fed dogs for the treatment and prevention of flea infestation for up to 4 weeks and the nematocidal macrocyclic lactone milbemycin oxime at a minimum recommended dose of 0.75 mg/kg bw orally against several nematode species incl. immature L4, L5 and adults of the zoonotic *A. caninum* and L5 and adults of the zoonotic *T. canis* but also as an preventative against heartworm infections (*D. immitis).* Veterinary medicinal products containing milbemycin oxime are on the EU market since more than a decade but at a lower minimum recommended oral dose of 0.5 mg/kg bw (range 0.5–2 mg/kg bw orally) against adult of lumen dwelling nematodes and for heartworm prevention.

Zoonotic hookworms are regarded as the dose limiting species for milbemycin oxime, thus, *A. caninum* was used for both studying dose determination and non-interference between the actives. Efficacy against adults of *A. caninum* was >99% at oral point doses of 0.5 and 0.75 mg/kg bw milbemycin oxime in combination with 30 mg spinosad/kg bw, confirming both efficacy and non-interference with the insecticidal spinosad at an oral dose of 0.5 mg milbemycin oxime/kg bw in dogs. However, insufficient efficacy against L4 (32.4%) and L5 (76.8%) of *A. caninum* was shown at an oral point doses of 0.5 mg/kg bw milbemycin oxime when combined with 30 mg spinosad/kg bw. In two additional studies adequate efficacy above 95% against L4 and L5 stages of *A. caninum* could be demonstrated at the increased dose rate of 0.75–1.0 mg milbemycin oxime/kg bw orally in fixed combination with 45–60 mg spinosad/kg bw. Thus, the minimum dose of 0.75 mg milbemycin oxime/kg bw in the combination is justified according to the anthelmintic guidelines. With regard to adults of the zoonotic roundworm *T. canis* both point doses of 0.5 mg and 0.75 mg milbemycin oxime/kg bw in fixed combination with 45 mg spinosad/kg bw orally resulted in 100% efficacy. Against immature L5 of *T. canis* efficacies of 96.9% and 99% were found but the results are statistically not different.

Dose confirmation studies were conducted for treatment and prevention of flea infestations for up to 4 weeks using the final formulation either at the lower US dose range of 30–45 mg spinosad in combination with 0.5–0.75 mg milbemycin oxime/kg bw, or at 45–60 mg spinosad in combination with 0.75–1 mg milbemycin oxime/kg bw. No interference between spinosad and milbemycin oxime was found.

Dose confirmation studies were carried out using the final formulation in dogs artificially or naturally infected with adult roundworms of *T. canis, T. leonina* and *T. vulpis* at the lower oral dose range of 0.5–0.75 mg milbemycin oxime/kg bw in combination with the lower dose range of 30–45 mg spinosad per kg bw resulting in efficacy of 93.3–100%. No interference between spinosad and milbemycin oxime was detected.

A dose confirmation study was also conducted in L5 immature adults of *Toxocara canis.* Efficacy of 96.2% after oral treatment with the final formulation at the dose range of 0.75–1 mg milbemycin

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oxime in fixed combination with 45–60 mg spinosad per kg bw was calculated. Study results justify the addition of the claim for immature adults (L5) of *Toxocara canis*.

With regard to heartworm disease caused by *Dirofilaria immitis* three GCP-compliant dose confirmation studies after artificial infection were conducted in the US with the final formulation at the lower oral US dose range of 30–45 mg spinosad and 0.5–0.75 mg milbemycin oxime/kg bw. Since heartworm characteristics are comparable in the US and Europe, the US study results were considered appropriate to demonstrate the preventive efficacy of Trifexis against heartworm disease in European countries.

In recent years, reduced efficacy of macrocyclic lactones in the prevention of canine heartworm disease has been reported from southern areas of North America (ESCCAP-Guideline no. 5, 2nd edition, 2012). Therefore, measures to reduce the risk of resistance development in European heartworm strains are included in the product literature of Trifexis.

Two GCP-compliant European multi-centre controlled clinical field studies confirmed the efficacy of the proposed product at the proposed dose range of 45–70 mg spinosad and 0.75–1.17 mg milbemycin oxime/kg bw in the treatment of single and multi-nematode infections (*T. canis, T. leonina, T. vulpis* and *A. caninum*) and the treatment and prevention of flea infestations for 4 weeks. In one of the field studies it was shown that 37% of dogs suffered from nematode infections and concurrent flea infestations prior to treatment, hence, justifying the proposed combination product from a clinical point of view. The claim for the reduction of the level of *U. stenocephala* infections is not accepted due to the low percentage of worm reduction achieved in the controlled laboratory studies. The rate of adverse events including emesis associated with the use of the product was generally low, however, due to the short duration of the field studies (2 and 4 weeks), no final conclusions on field safety could be drawn from this study. The palatability of the product could not be confirmed based on the studies provided.

Adverse events of potentially neurotoxic origin were noted in single cases following treatment with an average (mid-range) label dose in preclinical and clinical studies. Adverse reactions following overdoses indicate a dose dependency of this type of adverse events. However, the entire target animal safety data package did not show an unacceptable risk in relation to target animal safety after treatment at the recommended label dose, since these adverse reactions were transient and did not require symptomatic treatment.

Pharmacovigilance data from the US for Trifexis, and from the US and EU for Comfortis (spinosad only), indicate a low incidence of all adverse events for both products. The data do not allow any correlation between the occurrence of adverse events with the duration of administration of Trifexis. Based on the data provided, no conclusions on the safety of Trifexis in certain sensitive subpopulations (MDR1 mutated dogs, young animals) can be drawn.

Pharmacovigilance data for the monocomponent spinosad product, Comfortis, in the target species (dogs), showed very rare ('very rare' is less than 1 animal in 10,000 animals, including isolated reports) cases of eye disorders.

As confirmed by the applicant, recent information from the US indicate that these very rare cases of eye disorders have also been reported after use of Trifexis, including blindness, impaired vision and other eye disorders.

Some inconsistencies were noted regarding the pharmacovigilance data provided.

Based on the pharmacovigilance data provided to date, an in-depth causality assessment of neurological and eye disorders in relation to the dose and duration of use of Trifexis is not possible.

The Committee agreed a condition of the marketing authorisation for a post-authorisation non-interventional safety study to be performed to provide targeted monitoring of treated animals in order

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to further characterise the safety profile with respect to potential neurological and ophthalmic adverse events.

Part 5 – Benefit-risk assessment

Introduction

The application is for Trifexis chewable tablets for use in dogs, a fixed combination product containing spinosad and milbemycin oxime as active ingredients. Spinosad is a new substance authorised for use in dogs in the European Union in 2011. Milbemycin oxime is a well-established endectocide in the EU. The product is presented in five tablet strengths. The application is supported by a full dossier.

Benefit assessment

Direct therapeutic benefit

Trifexis is a fixed combination, the justification for which is based on the broadening of the spectrum of activity by combination of the insecticide spinosad with the endectocide milbemycin oxime. This is considered acceptable and supported by the pivotal European field study on nematode infections where 37% of the study population suffered from concurrent flea infestations.

Trifexis chewable tablets are intended for the treatment and prevention of flea infestations (*Ctenocephalides felis*) in dogs, and for the concurrent prevention of heartworm disease (*Dirofilaria immitis*) and/or treatment of gastrointestinal nematode infections caused by hookworm (L4, immature adult L5 and adult *Ancylostoma caninum*), roundworms (immature adult L5 and adult *Toxocara canis* and adult *Toxascaris leonina*) and whipworm (adult *Trichuris vulpis*). The product can be used as part of a treatment strategy for the control of flea allergy dermatitis. The recommended treatment dose is 45–70 mg spinosad and 0.75–1.17 mg milbemycin oxime/kg bw. The treatment can be repeated at monthly intervals up to six months depending on the indication and epidemiological situation.

The minimum recommended treatment dose of 0.75 mg milbemycin oxime/kg bw which is higher than the established milbemycin oxime dose in Europe and also in the US, is justified. L4 and L5 stages of the zoonotic *A. caninum* is proven to be the dose limiting species requiring the higher dose of 0.75 mg milbemycin oxime/kg bw, as confirmed on artificially infected dogs in two US dose confirmation studies.

In well-conducted GCP-compliant dose confirmation studies at the lower dose range of 0.5–0.75 mg milbemycin oxime/kg bw in combination with 30–45 mg spinosad/kg bw proved to be highly effective in the treatment of adult nematode infections of *T. canis* incl. immature adult L5, *T. leonina*, *A. caninum*, and *T. vulpis*. These favourable results were confirmed in a GCP-compliant controlled European field study using the recommended dose regimen.

The efficacy of Trifexis in the prevention of heartworm disease was confirmed in GCP-compliant controlled clinical studies in the US in artificially infected dogs at single or monthly oral doses of 0.5–0.75 mg milbemycin oxime/kg bw and 30–45 mg spinosad/kg bw. The close morphological and genetic similarity of heartworm strains from the US and the EU have been demonstrated by recent literature. The data basis is considered sufficient to justify the claim for prevention of heartworm disease in principle.

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Well-conducted GCP-compliant EU clinical field studies proved that Trifexis at the recommended dose is effective in the treatment and prevention of flea infestations for 4 weeks. The claimed indication that the product can be used as part of a treatment strategy for the control of flea allergy dermatitis, and also the claimed rapid killing effect leading to a reduction in egg production are supported by reference to the studies submitted with the monocomponent, Comfortis (spinosad) application.

Additional benefits

Trifexis increases the range of available treatment possibilities for the treatment of concurrent flea infestations and single/ mixed nematode infections in dogs and can also be used for the prevention of heartworm disease in dogs at risk.

Risk assessment

- *For the target animal*

Preclinical and clinical data reveal that Trifexis was generally well tolerated in dogs at dose levels corresponding to the lower half of the label dose. Emesis was the most frequently observed effect. Less frequently observed adverse reactions were diarrhoea, lethargy, anorexia/decreased appetite, pruritus, dermatitis, reddening of the skin and the pinna, tremors, salivation, ataxia and seizures.

At acute overdoses corresponding to 1.5 times the maximum recommended dose, vomiting occurred in 17% of the dogs, and hypersalivation occurred in 8% of the dogs.

At acute overdoses corresponding to 3 times the maximum recommended dose, vomiting occurred in half of the animals, sometimes repeatedly. Adverse events of potentially neurological origin (decreased activity, hypersalivation or stumbling) were observed in 25% of the animals. Additionally, cases of tremor were observed in dogs treated with doses below the maximum treatment dose. These data were obtained in healthy young dogs.

Regarding pharmacokinetics, both spinosad and milbemycin oxime may interfere at the blood-brain-barrier as both are substrates of P-glycoprotein. There is an interaction of the actives which leads to increased exposure to milbemycin oxime. Furthermore, both substances accumulated in the blood plasma of juvenile dogs and possibly also of adult dogs without reaching steady state within six months.

All adverse events observed in the preclinical and clinical trials were mild and transient in nature and did not require symptomatic treatment.

Trifexis is intended for repeated use. A six months target animal safety study using monthly cumulative overdoses corresponding to 0.7x, 2.2x and 3.6x of the maximum label dose, showed a low rate of adverse reactions. Repeated use lead to a dose proportional increase in systemic exposure to spinosyns and milbemycin oxime. No plateau in plasma levels of either spinosyns or milbemycin oxime could be detected even after 6 months in the juvenile dogs tested.

Field safety after repeated use of the product at the intended EU dose could not be fully assessed because the data provided was produced using the US dose range.

Based on the data presented, the safety of the product following repeated use can only be considered demonstrated for up to six consecutive months within any one year.

Pharmacovigilance data from the US for Trifexis, and from the US and EU for Comfortis (spinosad only), indicate a low incidence of all adverse events for both products. In terms of reporting rates, no

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relationship between the dose and emesis (the most frequently reported adverse event) or any other adverse reactions, including neurological adverse events, could be established. The data also do not allow any correlation between the occurrence of adverse events with the duration of administration of Trifexis. Based on the data no conclusions on the safety of Trifexis in certain sensitive subpopulations (MDR1 mutated dogs, young animals) can be drawn.

Pharmacovigilance data for the monocomponent spinosad product, Comfortis, in the target species (dogs), showed very rare ('very rare' is less than 1 animal in 10,000 animals, including isolated reports) cases of eye disorders.

As confirmed by the applicant, recent information from the US indicate that these very rare cases of eye disorders have also been reported after use of Trifexis, including blindness, impaired vision and other eye disorders.

Some inconsistencies were noted regarding the pharmacovigilance data provided.

Based on the safety data provided to date, an in-depth causality assessment of the above events in relation to the dose and duration of use of Trifexis is not possible, and therefore a targeted monitoring of treated animals is necessary in order to further characterise the safety profile with respect to potential neurological and ophthalmic adverse events.

Information on adverse reactions following recommended use of the product and following acute and chronic subsequent overdoses are reflected in the SPC, but may be subject to changes in the future after marketing of the product, depending also on the outcome of the post-authorisation study referred to above. Additionally, precautions are included that treated dogs should be observed up to 24 hours post administration for possible adverse reactions and to contact the veterinarian if such reactions are observed, and that the product should only be used based on a risk-benefit assessment of the responsible veterinarian in sick or convalescent dogs, in the absence of related studies.

The minimum age for Trifexis is set at 14 weeks, which is in line with the authorised spinosad only product (Comfortis). This minimum age takes into account the higher emesis rate in younger puppies and the possibility that reductions in weight gain may occur.

- *for the user*

There is no health concern for adults, including pregnant and nursing women, administering this product (to dogs) in accordance with the SPC. Child-resistant packaging and all the proposed warning phrases are considered satisfactorily to minimise the risk for children.

- *for the environment*

Trifexis 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.

- *for the consumer*

Not applicable.

Risk management or mitigation measures

Regarding the target animal, provisions to ensure a safe and efficacious use are included in the SPC.

The Committee agreed to a condition of the marketing authorisation for a post-authorisation non-interventional safety study to be performed to provide targeted monitoring of treated animals in order

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to further characterise the safety profile with respect to potential neurological and ophthalmic adverse events.

The CVMP also considered the inconsistencies noted during the assessment procedure regarding the pharmacovigilance data provided, and agreed that an early pharmacovigilance system inspection should be performed.

Evaluation of the benefit-risk balance

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

The product has been shown to be efficacious at the recommended dosing regimen in the treatment and prevention of flea infestations for up to 4 weeks, the prevention of heartworm disease in dogs (monthly treatment interval) and the treatment of adult nematode infections (including immature adults of *T. canis*) in dogs.

The formulation and manufacture of Trifexis are well-described and the specifications set will ensure that a product of consistent quality will be produced.

Data from pre-clinical studies performed in healthy young dogs indicate potential neurological adverse reactions following the use of Trifexis. Events of potential neurotoxic nature were also observed in the clinical field study, in a dose range lower than the EU label dose. Both active substances (milbemycin oxime and spinosad) are substrates of P-glycoprotein. Following repeated treatments, both substances accumulate in juvenile dogs and possibly also in adult dogs. Despite the fact that all adverse reactions reported in these studies were mild and transient in nature and did not require symptomatic treatment, additional details on the safety profile in a broader target animal population will be necessary to confirm the safe use of the product in the field. Therefore, a post-authorisation safety study is needed with the objective of a targeted monitoring of treated animals for further characterisation of the safety profile including the incidence of those adverse events.

Additionally, an early pharmacovigilance inspection should be performed.

Trifexis tablets present a low risk for users and the environment and appropriate warnings have been included in the SPC and other product information.

Conclusion

The overall benefit-risk evaluation is deemed positive with a sufficiently clear and complete SPC and product literature and taking into account the defined risk management measures. Based on the original and complementary data presented, it is concluded that the quality, safety and efficacy of Trifexis were considered to be in accordance with the requirements of Directive 2001/82/EC.

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References:

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Divergent position on the CVMP opinion for Trifexis (EMEA/V/C/002635)

Pursuant to Article 31 of Regulation (EC) No 726/2004, Eli Lilly and Company Limited submitted to the European Medicines Agency on 23 January 2012 an application for a marketing authorisation for Trifexis, containing spinosad/milbemycin oxime.

Having considered all the information presented, it is the opinion of the undersigned that a real risk to target animal safety is present with this product, due to the combination of active substances spinosad/milbemycin oxime.

The following points, in particular are noted:

- Regarding both active substances separately, the following adverse reactions in dogs are known. For spinosad, the most frequently observed adverse event is vomiting, which most commonly occurs in the first 48 hours after dosing. This effect is dose dependent and within the authorised dose band of 45–70 mg/kg bw a higher incidence of vomiting is seen in dogs dosed at the upper end of the dose band (8% in studies with the monovalent product). Other adverse reactions are uncommon or rare, and include lethargy, anorexia, diarrhoea, ataxia and seizures. For milbemycin oxime, lethargy, neurological and gastrointestinal symptoms occur in very rare cases following treatment with the label dose. Massive overdose may lead to signs of avermectin toxicosis.

- The CVMP assessment brought out the fact that another currently authorised product, Comfortis,

is on the market, containing the single active substance, spinosad. Pharmacovigilance has identified serious adverse events in dogs (*e.g.* neurologic signs) from the use of this product. There is no reason to conclude that allowing Trifexis on the market, with the same active substance would not result in that same or a higher rate of serious adverse events. In fact, Trifexis has two active substances (spinosad/milbemycin oxime) compared to Comfortis, both of which can lead to potential neurotoxic effects. Currently, the known adverse effects of Trifexis include vomiting as most common, which usually occurs in the first 48 hours after dosing. Although such vomiting post-treatment is relatively common, in the majority of cases the vomiting is transient, mild and does not require symptomatic treatment. Lethargy, anorexia/decreased appetite, diarrhoea, pruritis, dermatitis and reddening of the skin and pinna were also commonly seen.

- Spinosad is a mixture of spinosyns, a group of systemically acting insecticides. Corresponding findings with spinosad and milbemycin oxime demonstrated that the kinetics of milbemycin oxime were significantly changed in terms of an increased exposure, when administered together with spinosad to Beagle dogs. Based on the findings with ivermectin and spinosad, a potential interaction of milbemycin oxime and spinosad on the level of transport glycoproteins P-gp was discussed, which may reduce the efflux of milbemycin oxime from cells and, by this, increase its concentration in different organs (e.g. brain). Despite these observations no dose adjustment of milbemycin oxime was considered for the combination product (Trifexis), although the $AUC_{0-\infty}$ of the compound was increased by more than the 3-fold in the presence of spinosad. It remains unknown whether the metabolism of milbemycin oxime was affected at least quantitatively in the presence of spinosad, because no control group treated with milbemycin oxime alone had been included in the studies. It has been speculated that milbemycin oxime and spinosad may interfere at the CYP450-enzymes which trigger the conversion of many lipophilic drugs to hydrophilic degradation products. The reverse case, *i.e*. the potential influence of milbemycin oxime on the metabolic degradation of spinosad was not specifically investigated because the applicant had not identified a pharmacokinetic interaction between milbemycin oxime and spinosad.

- Effects of potentially neurotoxic origin were also observed following treatment with Trifexis, representing the combination of spinosad/milbemycin oxime. Milbemycin oxime is a macrocyclic lactone. Depending on the substance and dose, macrocyclic lactones have the potential to exert neurological activity in vertebrates in case they reach the central nervous system, where they can lead to severe or fatal effects (so-called avermectin toxicosis). This is normally prevented by the blood-brain-barrier, namely the efflux pump p-glycoprotein (P-gp), which is encoded by the multidrug resistance gene MDR-1. Spinosad is probably also a substrate of P-gp. Based on this situation it seems possible that spinosad “blocks” P-gp, leading to higher concentrations of milbemycin oxime in the CNS in dogs with the usual expression of P-gp (so-called MDR-1 +/+ or wild type dogs; dogs with mutations of the MDR-1 gene are addressed separately, below). Determining the frequency rates of adverse reactions was further hampered by the fact that data in laboratory studies were based on doses which did not include the upper part of the intended dose band. The US field study used a lower dose band than the one intended for the EU, and in the EU field studies performed for only 2 and 4 weeks, respectively, less than 2% of the animals received a dose above 65 mg spinosad/1.1 mg milbemycin oxime.

- These neurologic adverse events related to spinosad are serious, and regardless of whether or not dogs recovered from these adverse reactions, the events create distress and problems for dogs, owners, and veterinarians. It is difficult to understand as to how this is an acceptable risk

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for a product intended as an elective treatment for common ectoparasites, when other efficacious and safer products are currently authorised.

- The CVMP opinion is heavily contingent on a post-authorisation safety study. The undersigned are of the opinion that there are concerns of this additional clinical tolerance study, and not considered appropriate, for the following reasons:

  - In the Summary of Product Characteristics, there is a specific provision for stopping treatment after 6 months of repeated treatment. This is in line with the fact that the target animal study (TAS) study was conducted at the lower range of the dose range for spinosad and that animals have not be treated for a period longer than 6 months. This requirement is considered to be stringent, but its practicability is questioned. It remains to be determined if this risk mitigation measure is adequate to prevent or reduce serious adverse events.
  - The robustness of such a future study is not fully assured. Participating owners will be informed by veterinarians, but it is unclear how they shall undertake this. It may well be either that full blinding is not feasible, or that recruitment will be low because alternatives for flea control exist.
  - Furthermore, the study is not to be conducted according to Good Clinical Practice (GCP). It may well be that scientifically a study can be considered robust despite the lack of GCP status; however, in the present case, robustness is questioned. The lack of GCP, in this case, is understood to contribute potentially to the bias inherently present in the study and hence the results will be more questionable. Other sources of potential bias could include those of veterinarians (*e.g.* excluding dog breeds as carriers of the MDR-1 gene mutations) and owners. The study will likely include strict diagnostic criteria (*e.g.* combined nematode and flea infestations) that further restrict the dog population to be monitored and different from pharmacovigilance identified for Comfortis. Issues related to a control treatment group for comparison to Trifexis have not been fully addressed.
  - The currently proposed condition does not indicate the number of animals to be used, but it is considered unreasonable to expect that the study, even under the best conditions, has sufficient statistical power to show an effect if it is really present because such a study would require a high number of animals (hundreds or thousands depending on the expected incidence rates of the side-effect in question).
  - For Comfortis, a mono-product containing spinosad, belonging to the same applicant/marketing authorization holder, the available pharmacovigilance data indicate that there is a risk for neurological signs in treated animals including blindness that may be imputated to the use of the product. This is considered a serious, although very rare event at present and will be further investigated separately.
  - The justification for an additional study comes primarily from the seriousness of the blindness, and not foremost from any of the other side-effects, even though they may be linked to eventual blindness. Identifying such a link, though it may be theoretically supported, is not considered possible in the proposed study.
  - The protocol was unavailable at the time of positive CVMP opinion. Also, there is uncertainty as to how the results will be interpreted of such a post-authorisation study; particularly, it is uncertain as to how the endpoints of this study could be interpreted if the product could remain as a positive CVMP opinion on benefit-risk assessment. Thus, the condition of this post-authorisation study is disproportionately important in the overall conclusions on the benefit:risk balance.

Without a robust, agreed-upon post-authorisation study, then it is uncertain if the current CVMP

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opinion would remain positive. The signing CVMP members, under these provisions, cannot support a positive benefit:risk balance. Although the current Trifexis dossier appears to meet the strict legal requirements (in terms of target animal safety, based primarily on young healthy dogs) - the experience with Comfortis (spinosad only) has demonstrated that in the general dog population there are serious adverse events. The same situation is expected for this combination product (spinosad/milbemycin oxime) which is unacceptable.

London, 17 July 2013

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