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.

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

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

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

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

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

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

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

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.

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.

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.

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".

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.

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.

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

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

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.

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

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

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.

References:

Bourguinat C, Keller K, Bhan A, Peregrine A, Geary T, Prichard R. Macrocyclic lactone resistance in *Dirofilaria immitis*. Vet Parasitol. 2011 Sep 27;181(2-4):388-392

Snyder DE, Wiseman S, Cruthers LR, Slone RL. Ivermectin and milbemycin oxime in experimental adult heartworm (*Dirofilaria immitis*) infection of dogs. J Vet Intern Med. 2011 Jan-Feb;25(1):61-64

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

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

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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