ASSURING THE SAFETY, QUALITY AND EFFICACY OF VETERINARY MEDICINES

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DECENTRALISED PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

Effitix 26.8 mg/240 mg spot-on solution for very small dogs
Effitix 67 mg/600 mg spot-on solution for small dogs
Effitix 134 mg/1200 mg spot-on solution for medium dogs
Effitix 268 mg/2400 mg spot-on solution for large dogs
Effitix 402 mg/3600 mg spot-on solution for very large dogs

MODULE 1

PRODUCT SUMMARY

EU Procedure number	UK/V/0502/001/DC UK/V/0502/002/DC UK/V/0502/003/DC UK/V/0502/004/DC UK/V/0502/005/DC
Name, strength and pharmaceutical form	Effitix 26.8 mg/240 mg spot-on solution for very small dogs Effitix 67 mg/600 mg spot-on solution for small dogs Effitix 134 mg/1200 mg spot-on solution for medium dogs Effitix 268 mg/2400 mg spot-on solution for large dogs Effitix 402 mg/3600 mg spot-on solution for large dogs
Applicant	Virbac S.A. 1ère Avenue – 2065 - L.I.D. 06516 Carros France
Active substance(s)	Fipronil Permethrin
ATC Vetcode	QP53AC54
Target species	Dogs
Indication for use	In dogs, to be used against infestations with fleas and/or ticks when repellent (anti-feeding) activity is also necessary against sand-flies and/or mosquitoes. <u>Fleas:</u> Treatment and prevention of infestations by fleas *(Ctenocephalides felis).* Fleas on dogs are killed within 24 hours following treatment. One treatment provides persistent efficacy against new infestations with adult fleas for four weeks.

	The product can be used as part of a treatment strategy for the control of Flea Allergy Dermatitis (FAD) where this condition has previously been diagnosed by a veterinarian. <u>Ticks:</u> One application provides persistent acaricidal efficacy against tick infestations (*Ixodes ricinus*, *Dermacentor reticulatus* and *Rhipicephalus sanguineus*) for four weeks. If ticks are present at the time of application, not all ticks may be killed within 48 hours but they may be killed within a week. <u>Sand-flies and mosquitoes:</u> One treatment provides repellent (anti-feeding) activity against sand-flies (*Phlebotomus perniciosus*) and against mosquitoes (*Culex pipiens, Aedes aegypti*) for four weeks.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Heads of Medicines Agencies (veterinary) (HMA(v)) website (www.hma.eu).

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Fixed combination application in accordance with Article 13b of Directive 2001/82/EC as amended.
Date of completion of the original decentralised procedure	23rd April 2014
Date product first authorised in the Reference Member State (MRP only)	Not applicable
Concerned Member States for original procedure	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, The Netherlands, Norway, Poland, Portugal, Slovakia, Spain, Sweden.

I. SCIENTIFIC OVERVIEW

These products are spot-on solutions containing fipronil and permethrin with a range of strengths to treat dogs of increasing sizes. The products can be used against infestation with fleas and / or ticks when repellent (anti-feeding) activity is also necessary against sand-flies and / or mosquitoes. The products are intended for the treatment and prevention of infestation by fleas (*Ctenocephalides felis).* Most fleas are killed within 24 hours following treatment. An application provides persistent acaricidal efficacy against tick infestations *Ixodes ricinus*, *Dermacentor reticulatus* and *Rhipicephalus sanguineus*) for four weeks. If ticks are present when the product is applied, all the ticks may not be killed with 48 hours. The products provide repellent activity against sand-flies (*Phlebotomus perniciosus*) and against mosquitoes (*Culex pipiens, Aedes aegypti*) for four weeks.

The products can be used as part of a treatment strategy for the control of Flea Allergy Dermatitis (FAD) when this has been previously diagnosed by a veterinary surgeon.

The product is produced and controlled using validated methods and tests which ensure the consistency of the product released on the market. It has been shown that the product can be safely used in the target species; the slight

reactions observed are indicated in the SPC[1]. The product is safe for the user, and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC. The efficacy of the product was demonstrated according to the claims made in the SPC. The overall benefit/risk analysis is in favour of granting a marketing authorisation.

II. QUALITY ASPECTS

A. Composition

The product contains the following amounts of the active substances fipronil and permethrin respectively 26.8 mg / 240 mg; 67 mg / 600 mg; 134 mg / 1200 mg; 268 mg / 2400 mg and 402 mg / 3600 mg.

Dog weight	Fipronil (mg)	Permethrin (mg)
1.5-4 kg	26.8	240
4-10 kg	67	600
10-20 kg	134	1200
20-40 kg	268	2400
40-60 kg	402	3600

The excipients are Butylhydroxyanisole (E320), Butylhydroxytoluene (E321), benzyl alcohol (E1519) and diethylene glycol monoethyl ether.

The container/closure system consist of a transparent multi-layer plastic single dose pipette formed by thermoforming a transparent polyacrylonitrile-methacrylate/cyclic olefin copolymer/polypropylene complex closed by heat sealing with a polyacrylonitrile-methacrylate/aluminium/polyethylene-terephthalate lid. Boxes contain individual pipette(s) placed in a coloured over-blister made from polypropylene/cyclic olefin copolymer/polypropylene and closed with a lid of polyethylene-terephthalate/aluminium/polypropylene. The particulars of the containers and controls performed are provided and conform to the regulation.

The choice of the formulation is justified. The products are established pharmaceutical forms and their development is adequately described in accordance with the relevant European guidelines.

B. Method of Preparation of the Product

The products are manufactured fully in accordance with the principals of good manufacturing practice from a licenced manufacturing site. Process validation data on the products have been presented in accordance with the relevant

[1] Summary of Product Characteristics

European guidelines. The manufacturing process consists of several solubilisation and mixing steps, followed by final fill into pipettes.

C. Control of Starting Materials

The active substances fipronil and permethrin are established active substances and not described in the European Pharmacopoeia (Ph. Eur.). Data on the active substance fipronil was supplied in the form of an Active Substance Master File (ASMF) from one manufacturer and suitable data were provided from an alternative manufacturer. Data related to the active permethrin has been provided in the form of an ASMF. The active substances are manufactured in accordance with the principals of good manufacturing practice.

The active substance specifications are considered adequate to control the quality of the material. Batch analytical data demonstrating compliance with this specification have been provided. All excipients comply with their relevant Ph. Eur. monographs.

D. Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

There are no substances within the scope of the TSE Guideline present or used in the manufacture of this product.

E. Control on intermediate products

Not applicable.

F. Control Tests on the Finished Product

The finished product specification controls the relevant parameters for the pharmaceutical form. The tests in the specification, and their limits, have been justified and are considered appropriate to adequately control the quality of the product. The tests include those for appearance, density, water content, uniformity of dosage, identification of active substances and excipients, identification of impurities and microbial purity.

Satisfactory validation data for the analytical methods have been provided.
Batch analytical data from the proposed production site have been provided demonstrating compliance with the specification.

G. Stability

Stability data on the active substances have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substances when stored under the approved conditions. A retest period of 12 months has been established for one manufacturer of fipronil and 24 months for

the other manufacturer of fipronil. A retest period of 36 months has been established for the permethrin.

Stability data on the finished product have been provide in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life when stored under the approved conditions. Data available from batches were stored under real time conditions (25ºC/60% RH and 30ºC/ 60% RH) for 18 months or accelerated conditions (40ºC/75% RH) for 6 months. A small rise in water content was noted. Pipettes in their over-blister should be stored in the carton to protect the products from light. A shelf life of 24 months stored at a temperature not above 30ºC is acceptable.

H. Genetically Modified Organisms

Not applicable.

J. Other Information

Shelf life of the veterinary medicinal product as packaged for sale: 2 years
Shelf life after first opening the immediate packaging: immediate use.
Store below 30ºC. Keep the blister pack in the outer carton in order to protect from light.

III. SAFETY AND RESIDUES ASSESSMENT (PHARMACO-TOXICOLOGICAL)

III.A Safety Testing

Pharmacological Studies

Pharmacodynamics
The active substances are fipronil and permethrin. Fipronil is a broad spectrum insecticide included in sprays and spot-on veterinary medicinal products to control fleas, ticks and other ectoparasites on dogs and cats. It can be used in the treatment of Flea Allergy Dermatitis (FAD) where the condition has been previously diagnosed by a veterinary surgeon. Fipronil is a blocker of the gamma-aminobutyric (GABA)-regulated chloride ion channel, which interferes with the passage of chloride ions across the membranes. This results in uncontrolled activity of the central nervous system and death of the insect. The active substance is more toxic to insects than mammals.

Permethrin belongs to the type I synthetic pyrethroid acaricides and insecticides and also acts as a repellent. Permethrin binds to sodium channels in vertebrates and non-vertebrates, affecting the sodium channels by slowing down the activation and inactivation properties. This leads to simulation of the nerves, depolarisation, nerve block and death of the parasite.

Pharmacokinetics

Dermal application studies in rats show that levels of absorbed fipronil are very low, indicating that fipronil is poorly absorbed across rat skin *in vivo*. Another study in rats demonstrated that up to 75% of orally administered fipronil was excreted via the faeces and between 5-25% though the urine. Fipronil has several metabolites, of which the major one is a sulfone derivative, which also possesses insecticidal and acaricidial properties. The metabolite fipronil sulfone demonstrates similar toxicity to the parent compound, the photodegradation product fipronil desulfinyl has been shown to have persistent insecticidal activity on the coat. Fipronil plasma concentrations peak after 5 days and the active metabolite peaks around 14 days. Concentrations are quantifiable up to 35 days.

Permethrin displays very low levels of systemic absorption. Rats provided with a single oral dose of permethrin, followed by a repeated dose showed some retention of permethrin in fat. In rats 96% of the administered dose was recovered in the urine and faeces within 12 days. Permethrin is poorly distributed among internal organs, except in fat where residual content is higher.

Toxicological Studies

The active substances, fipronil and permethrin, are both found individually in authorised veterinary medicinal products. Data provided includes published literature in addition to specific studies using the final combination product.

- Single Dose Toxicity

Data were provided on the toxicity of fipronil. In studies the LD_{50}[2] values were cited as 97 mg/kg (oral, rats) and 95 mg/kg (oral, mice). An LC_{50}[3] of 0.42 – 0.68 mg/l/4h was noted in rats following inhalation exposure. Dermal LD_{50} exceeded 2000 mg/kg in rats and an LD_{50} of 354 mg/kg was exhibited in rabbits. Death generally occurred within 2 days, while changes to central nervous system function were noted after 7 hours. Above 50 mg/kg signs included tremors, altered gait, hunched posture, agitation and seizures.

Data were provided on the toxicity of permethrin. In studies the oral LD_{50} values were cited as 650 mg/kg in mice. Oral LD_{50} values for different species of rats ranged from 1200 mg/kg to 8900 mg/kg. Dermal LD_{50} values in rats was 1750 mg/kg, in mice and rabbits this exceeded 10,000 mg/kg and 2,000 mg/kg respectively. Permethrin toxicity varied with the vehicle used; a 10-fold increase in toxicity to rodents when used in an oily vehicle, compared to permethrin in water. Clinical signs included neurotoxicity characterised by convulsions, tremors and hypersensitivity to external stimuli.

[2] LD_{50} – dose that will destroy half of a test population.
[3] LC_{50} – concentration that will destroy half a target population.

- Repeated Dose Toxicity

A review of a series of repeat dose studies were provided. NOAEL[4] were established for some of the studies. A 13 week study in rats, administered fipronil in the diet, resulted in a NOAEL of 0.33 – 0.37 mg/kg bodyweight. Changes in the haematological parameters and clinical history were observed in addition to an increase in thyroid weight. A 52 week dietary study which combined toxicity and carcinogenicity resulted in a NOAEL of 0.019 – 0.025 mg/kg bodyweight. Neurotoxicity, seizures resulting in death were observed, in addition to enlarged kidneys and liver, adrenal and thyroid glands. Benign and malignant neoplastic changes in the thyroid gland occurred. A three week dermal study in rabbits resulted in a NOAEL of 5 mg/kg. No dermal irritation or deaths were recorded. At 10 mg/kg, bodyweight gain and food consumption were reduced. In a 13 week oral study (capsule) in dogs, a NOAEL of 0.5 – 2 mg/kg was observed. At 10 mg/kg, neurotoxological effects were observed. Two further oral studies in dogs conducted over a year, resulted in a NOAEL of 0.5 mg/kg (capsules) and 0.3 mg/kg (fipronil in diet) respectively. In the first study neurotoxicity was seen at doses higher than 2 mg/kg and in the second study, at the next highest dose with the study, which was 3 mg/kg.

A 28 day study in rats and mice, administered permethrin in the diet resulted in a NOAEL of 50 mg/kg and 140 mg/kg respectively. In rats doses above 100 mg/kg caused tremors and an increase in liver weights, in mice 280 mg/kg per day induced liver changes. A 14 day oral study in dogs established a NOAEL of 250 mg/kg per day, tremors and ataxia were observed at 500 mg/kg per day. Two further oral studies in dogs conducted over 96 days and 52 weeks resulted in a NOAEL of 50 mg/kg per day and 5 mg/kg respectively. In the first study liver changes and neurotoxic signs were observed at a dose of 500 mg/kg and in the second study reduced bodyweight and increased liver weight at a dose of 100 and 1,000 mg/kg.

- Reproductive Toxicity, including Teratogenicity:

During a two-generation reproductive study, parental toxicity of fipronil was equivalent to a NOAEL of 0.25 – 0.27 mg/kg per day, increased thyroid weights and decreased body weight were observed. A NOAEL for reproductivity was 2.5 – 2.7 mg/kg bodyweight per day. Decreased litter size bodyweight and a decrease in the percentage of animals that mated were noted in offspring. A study in which fipronil-based spot on product was applied topically noted that pregnancy rates in rats were reduced at higher doses of 280 mg/kg while hormonal changes were found at lower doses (70 mg/kg). Doses used in this study are considered higher than those that may be expected to be applied topically. A maternal and developmental toxicity study in rats administered fipronil orally demonstrated a NOAEL of 4 mg/kg bodyweight per day and 20 mg/kg bodyweight per day respectively. There were no deaths, abortions, premature deliveries or clinical signs in the adult females. Maternal effects were only observed in animals receiving high doses and included reduced body weight gain, increased water consumption and decreased food consumption. A

[4] NOAEL – No observed adverse effect limit

second study showed no treatment-related effects on pregnancy rates or litter size and weights in rabbits administered fipronil. A NOAEL for developmental toxicity of 1 mg/kg bodyweight per day was observed.

In a study permethrin was administered in the diet of rats at various concentrations for three generations. There were no effects on bodyweights of the parents, nor on their reproduction and the number of births or on the survival or growth of their offspring. A NOAEL for reproductive toxicity was 6.7 mg/kg per day. A second three generation study in rats also showed no effects that the higher dose of 6.7 mg/kg bodyweight per day and gave a NOAEL for systemic and reproductive toxicity of 180 mg/kg per day. Several teratogenicity studies have been carried out in rats to study the effects of permethrin up to 225 mg/kg per day administered orally in corn oil between days 6 and 16 of gestation. No developmental toxicity issues, effects on bodyweight, number of live foetuses, foetal bodyweight, and litter size were noted. A second rabbit study with doses of 600, 1,200, 1,800 mg/kg per day from days 6 to 18 showed maternal toxicity in all doses. Signs included tremors, death, hypothermia, salivation and body weight impairment. No malformations were reported. A NOAEL for maternal toxicity could not be established. A NOAEL for development was given as 1,2000 mg/kg per day.

The studies indicate that fipronil is a non-teratogenic substance and does not cause reproductive toxicity. Permethrin has no reproductive or embryo/foeto toxicity at doses non-toxic for mothers and is therefore not considered reprotoxic, foetotoxic, or to be a teratogen.

- Mutagenicity

Fipronil and its metabolites are considered as non-genotoxic. Permethrin is also considered as non-genotoxic on the basis of the results of a battery of genotoxicity tests.

- Carcinogenicity

Fipronil has been shown to increase the incidence of thyroid tumours in rats, although this is regarded as a species specific effect and is not considered to be carcinogenic to humans.

A study in mice was conducted with permethrin in a diet for 24 months at doses of 0, 3, 15, 75 and 200 mg/kg per day. Overall no carcinogenic effects were observed and the NOAEL was established at 15 mg/kg per day. Two other studies increasing doses beyond 200 mg/kg also showed no evidence of a significant increase in tumour incidence or carcinogenic effects. Lifetime studies in rats dosed with permethrin at oral doses up to 125 mg/kg per day for 24 months did not show spontaneous death or an increase in neoplastic / non-neoplastic lesions. Although the EPA[5] regard permethrin as likely to be a carcinogen by ingestion based on studies in mice, the risk of developing cancer through dermal or oral exposure is of minimal concern as the incidence in

[5] EPA – Environmental Protection Agency

tumours was within the historical range, as described in an MRL Summary Report (CVMP 2000) for permethrin.

Other Studies

Neurotoxicity

Clinical signs of toxicity were evaluated in rats following a single dose of fipronil administered orally at doses of 0, 0.5, 5 and 50 mg/kg bodyweight. 6 rats treated at 50 mg/kg bodyweight died during the study from diffuse brain haemorrhages. Clinical signs of toxicity were only observed at the high dose. A NOAEL of 0.5 mg/kg bodyweight was established. A 13 week study in rats treated with a fipronil diet led to the establishment of a NOAEL of 0.3 mg/kg per day. A reduction in bodyweight gain and food consumption was observed. In a neurotoxicity study, fipronil was administered in capsules to young female dogs. Animals received 20 mg/kg bodyweight per day for a maximum of 14 days. Administration of fipronil was discontinued immediately after the appearance of neurotoxic signs. All treated animals displayed neurotoxic signs which included hypoactivity, salivation, ataxia, convulsions, tremors, stiffened body, and muscle twitching. A NOAEL for neurotoxicity could not be established.

A neurotoxicity study in rats administered permethrin at a dose of 0, 100, 750, 1500, 3000, 4000 and 5000 ppm showed dose-related responses. At doses greater than 1500 ppm animals displayed tremors, splayed hind legs and staggered gait. Deaths were observed at 4000 ppm. A NOAEL of 750 ppm was established. In a second study lasting 13 weeks, rats received varying doses of permethrin. No deaths were observed, but at doses greater than 1500 ppm symptoms observed included staggered gait, splayed hind legs and reduced bodyweight. A NOAEL for neurotoxicity was established at 15 mg/kg per day.

Studies with fipronil metabolites

Studies in rats have been conducted with fipronil sulfone, fipronil desulfinyl amide, fipronil carboxylic acid, fipronil sulfonyl amide and fipronil detrifluoromethyl sulfonate. Acute oral toxicity of fipronil sulfone was investigated in rats at doses of 50, 65, 90 and 120 mg/kg. Neurotoxicity was observed at all doses, with death occurring at 65 mg/kg and higher. The acute oral LD_{50} was calculated at 69 mg/kg for males, 100 mg/kg for females and 83 mg/kg for the combined sexes. The dermal LD_{50} for fipronil sulfone was in excess of 2000 mg/kg. The acute oral LD_{50} in rats for fipronil sulfone was 15 mg/kg in male rats and 18 mg/kg in female rats. Chronic feeding of fipronil desulfinyl to rats was undertaken for 2 years. Male and female rats were give oral doses of 0, 0.025, 0.098, 0.050 mg/kg per day and 0, 0.032, 0.130 and 0.550 mg/kg per day respectively. Rats showed increased incidence of aggression and irritability at higher doses.

Studies on final formulation

Haircoat distribution

Six dogs received the recommended dose of the product. Hair samples were taken in four areas, (neck, shoulder, back and thigh), at regular time points up to

35 days post-application. Fipronil, fipronil sulfone and permethrin were measured at each time point. No treatment related clinical effects were noted during the study. Peak concentrations in hair samples were found at day 3 for permethrin and fipronil and at day 14 for fipronil sulfone with the highest concentration sampled from the back area. The concentrations of permethrin and fipronil / fipronil sulfone remained above the LLOQ[6] (5 µg/g and 100 ng/g respectively).

Wipe test

A wipe test was performed in eight dogs treated with the product at the recommended dose to determine the amount of residual fipronil (including its sulfone derivative) and permethrin that could be wiped off the application site. The dorsal cervical area was wiped prior to application. Dogs were treated with a 2.2 ml pipette of the product applied topically. Dogs were wiped at Day 0 and at regular time points up to 672 hours. A nitrile glove covered in a cotton glove was placed on the petting hand and the animal was stroked ten times from head to tail. Following wiping the cotton glove was assessed for fipronil, fipronil sulfone and permethrin. No adverse events occurred. 1 hour post application levels of residual fipronil and permethrin were approximately 4% which rapidly declined to about 1% of the applied dose 24 hours post dosing. After 28 days, 0.1% of initial dose was removed by petting. No fipronil sulfone was detected in any sample. The applicant's conclusion that potential exposure to the end user is not expected to be significant is supported.

Acute oral toxicity of the final formulation

Acute oral toxicities achieved with each active substance given alone or in combination with the formulation were investigated in rats. The final formulation combination product administered resulted in similar neurological signs as seen when each substance was administered alone. At 550 mg/kg, no mortality occurred for fipronil but the combination product and permethrin alone were lethal. The oral LD_{50} for the final formulation was estimated to be 550 mg/kg with 175 mg/kg showing no mortality.

Acute dermal toxicity

A GLP[7] study was conducted and the test formulation was applied to the skin of male and female rats at a single dose of 2000 mg/kg. The test formulation covered approximately 10% of the total body surface of the rat for a period of 24 hours. Following this period rats were washed. Rats were observed daily for 14 days. Following necropsy no treatment-related signs of toxicity were observed. Pruritus was observed in 2 of the 5 female animals, but signs of local reaction were reversible. The dermal LD_{50} was determined to be in excess of 2000 mg/kg. Neither toxicity nor mortality was reported at 2000 mg/kg.

[6] LLOQ - Lower limit of quantification
[7] GLP - Good laboratory practice

Skin irritation

A GLP skin irritation study was conducted using a reconstituted three-dimensional human skin model and the final formulation of the test product. The formulation was applied to the skin for 15 minutes. A negative control of saline with magnesium and calcium and a positive control of 5% SDS[8] in distilled water were used. The results showed that the test product may be classed as non-irritant to the skin.

Eye Irritation

An eye irritation study was performed *in vitro*. Using a closed chamber method, eye tissue was exposed to the test product, a negative control of saline and a positive control of ethanol. The change in opacity for each cornea and the mean in vitro irritation scores were calculated. The mean irritation score for the test product was calculated as 140.20 and the product is classed as a potentially severe eye irritant.

Skin Sensitisation

A skin sensitisation study was conducted in mice. The animals were administered the product, a negative control or a positive control (1% phenylenediamine in DMSO) via topical administration to the dorsal surface of the ear for 3 consecutive days. The potential of the product to induce contact hypersensitivity was determined using a local lymph node assay. On Day 6 the mice were given titrated methyl thymidine and auricular lymph node cells were excised and pooled for analysis. The proliferative response of lymph node cells was counted as radioactive disintegrations per minute per lymph node and expressed as the Stimulation Index (SI).

Animals treated at 3.125%, 6.25% and 12.5% during the test period did not show clinical signs. The measurement of ear thickness did not reveal any significant increase of the ear thickness in any dosage group. The mean stimulation index SI at a concentration of 12.5% was 3.0, therefore the test product is considered to be a dermal sensitiser.

Ames Test on Final Formulation

A GLP compliant test was conducted to investigate the potential of the final formulation for its ability to induce gene mutation. Plate incorporation test and pre-incubation tests were performed with *Salmonalla typhimurium* strains. Under the conditions of the experiment, the test item did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains. The final formulation is considered to be non-mutagenic.

Observations in Humans

Eight recorded cases of human intoxication with fipronil were referenced in published data. Two cases resulted in central nervous system toxicity with seizures, sweating, nausea, vomiting and agitation. All other patients were

[8] SDS – Sodium Dodecyl Sulphate

asymptomatic within 12 hours of ingestion. One patient died however it was not clear if this was attributed to fipronil overdosing. Dermal exposure to permethrin in humans may result in skin irritation, erythema and reversible paraesthesia, symptoms lasting no longer than 24 hours. Ocular exposure results in pain, redness or burning sensation and ingestion causes vomiting, abdominal pain and sore throat. Exposure of pregnant women to permethrin spray to treat scabies showed no adverse events.

Microbiological Studies

The proposed product is not described as having any antimicrobial activity *in vivo* in any species therefore no microbiological data have been submitted.

User Safety

The applicant has provided a user safety assessment in compliance with the relevant guideline.

Fipronil and permethrin are both well-known active substances that have been used separately in other veterinary medicines. A photostability study showed that only limited degradation of fipronil occurred, no degradation of permethrin was observed. The lowest oral NOAEL for fipronil was 0.5 mg/kg per day from a study conducted in dogs for 13 weeks. Following a 21 day study in rabbits the lowest dermal NOAEL was 5 mg/kg per day. For permethrin a NOAEL of 5 mg/kg per day from a 90 day oral rat study was derived. A dermal NOAEL of 1000 mg/kg per day was calculated from a 21 day rabbit study where skin irritation was observed without any systemic signs.

Following a study conducted in rats the highest non-lethal dose of the final formulation corresponded to 175 mg/kg bodyweight by oral ingestion. No toxicity effects were seen in a single dose acute dermal test in rats using the final formulation at a dose of 2000 mg/kg. The final formulation was found to be non genotoxic, non-irritating to the skin, a mild dermal sensitiser and a potentially severe eye irritant.

Oral exposure following hand-to-mouth transfer and accidental oral ingestion were considered. The toxicities of the individual substances of the formulation have been reviewed in the context of safety for the end user and warnings and precautions as listed on the product literature are adequate to ensure safety to users of the product.

- The product may cause neurotoxicity. The product may be harmful if swallowed. Avoid ingestion including hand-to-mouth contact. In the event of accidental ingestion, seek medical advice immediately and show the package leaflet or the label to the physician.
- This product can cause eye and mucous membrane irritation. Therefore, avoid contact between the product and the mouth or eyes including hand-to-mouth and hand-to-eye contacts. In the event of accidental contact

between the product and eyes, immediately and thoroughly flush the eyes with water. If eye irritation persists, seek medical advice and show the package leaflet or the label to the physician.

- Avoid contact with the skin. Should the product come into contact with skin, wash the contacted area immediately with soap and water.
- Wash hands thoroughly after use.
- Do not eat, drink or smoke while handling the veterinary medicinal product.
- People with a known hypersensitivity (allergy) to fipronil, permethrin or any of the other ingredients should avoid contact with the veterinary medicinal product, which, on very rare occasions, can cause respiratory irritation and dermal reactions in certain individuals.
- Treated animals should not be handled or played with until the application site is dry and for about 12 hours after treatment. It is therefore recommended to treat the animals in the early evening or late afternoon in order to minimise contact with the treated animal. On the day of treatment, treated animals should not be permitted to sleep with their owner, especially children.
- Keep the stored pipettes in the original packaging. In order to prevent children from getting access to used pipettes, dispose of used pipettes immediately in a proper way.

Ecotoxicity

The applicant provided a Phase I environmental risk assessment in compliance with the relevant guideline. It was agreed that the product will under normal circumstances only be placed within the coat of individual dogs. Warnings and precautions as listed in the product literature are adequate to ensure safety to the environment when the product is used as directed.

- Fipronil and permethrin may adversely affect organisms. Dogs should not be allowed to swim in water courses for 2 days after application.
- Any unused veterinary medicinal product or waste material derived from such veterinary medicinal product should be disposed of in accordance with local requirements.
- Do not contaminate ponds, waterways or ditches with the veterinary medicinal product or empty container as this may be dangerous for fish and other aquatic organisms.

IV CLINICAL ASSESSMENT (EFFICACY)

IV.A Pre-Clinical Studies

Pharmacology

Pharmacodynamics

The applicant has provided a literature review covering relevant pharmacodynamics information for each of the active substances and potential interactions between the active substances have been addressed separately in four GLP studies.

In veterinary medicinal medicine, fipronil is a broad spectrum insecticide used as a spray or concentrated spot-on formulation to control flea, ticks and other ectoparasites on dogs and cats and to control Flea Allergy Dermatitis. Fipronil is a member of the phenylpyrazole family. Its mode of insecticidal action is interference with the passage of chloride ions through the gamma aminobutyric (GABA)-regulated chloride ion channel, which results in uncontrolled central nervous system activity and subsequent death of the insect.

Permethrin is a synthetic pyrethroid insecticide, effective against a wide range of insect pests. The insecticidal action is due to interaction with ion channels and axons of the central nervous system of the target species. It binds to sodium channels causing a slowing of their rate resulting in repetitive firing of nerves, depolarisation and nerve block.

- Interactions - Insecticidal and acaricidal

Four *in vivo* interaction studies were conducted to investigate potential negative interactions on insecticidal and acaricidal activities of fipronil and permethrin when the active substances are used in combination.

Each of these studies were GCP-compliant, controlled efficacy studies comprising of eight animals per groups. The first group of dogs received a combination of permethrin and fipronil, the second group fipronil only and the third group received permethrin only. Each dog was infected with 100 fleas (*Ctenocephalides felis*) and 50 ticks (*Rhipicephalus sanguineus* and *Dermacentor reticulatus*). The test products were administered between the shoulder blades and in the lumbar area. Fleas and ticks were counted on various assessment days. The study designs were consistent with the CVMP[9] guideline on efficacy testing for antiparasitic substances (EMEA/CVMP/ERP/005/200-Rev.2). The first study was an interaction efficacy study of the active ingredients against fleas. The applicant demonstrated that all three treatment groups yielded data which demonstrated immediate and persistent efficacies, supporting a lack of drug/drug interaction between active substances when administered within the SPC-stated dose range. In the second study the recommended minimum dose of fipronil and permethrin was used according to body weight ranges. The

[9] CVMP – The Committee for Medicinal Products for Veterinary Use

efficacy of the proposed product was at least 95% for fleas at each counting and supported a lack of drug/drug interaction between the active substances when administered at the lowest recommended dose. The third study was an interaction efficacy study of the active ingredients against ticks (*Rhipicephalus sanguineus*). This study demonstrated that there was no interaction between the active ingredients that influenced the effectiveness of the combination product against ticks when administered at the lowest recommended dose. The final study was an interaction study against ticks (*Dermacentor reticulatus*). The study supported the conclusion that no interaction between the active substances influenced the effectiveness against ticks when the product was administered at the minimum recommended dose.

During the studies no treatment-related adverse events were observed. The majority of local tolerance observations related to expected changes following treatment with a topical spot-on and / or flea infestation.

- Interactions - repellency

The applicant justified the absence of interaction studies for repellency based on bibliographic data and five efficacy studies were completed which are discussed in the dose confirmation study section. The insecticidal / acaricidal studies presented were consistent with the CVMP guideline on efficacy testing for antiparasitic substances (EMEA/CVMP/ERP/005/200-Rev.2). Studies were conducted with relevant flea and tick populations and acceptable levels of efficacy were demonstrated for the proposed product.

Two literature studies were provided demonstrating the anti-feeding effect of permethrin in target organisms when used as a single active substance. This is discussed in more detail in the dose confirmation study section.

- Interactions – toxicological

Three GLP-compliant studies were conducted in female rats. The formulation was given orally as either fipronil, permethrin or fipronil and permethrin. At a dose rate of 175 mg/kg no mortality was observed. Mortality was observed at dose rates of 550 mg/kg with the combination product and in the presence of permethrin alone. At 2000 mg/kg mortality was seen in all groups. The LD_{50} of the combination product was not higher than recorded for the same formulation containing only permethrin. The lack of toxicological interaction was accepted.

- Dose determination – fipronil

Literature was provided to support the common dose rate of registered fipronil products (minimum 6.7 mg/kg) for the control of flea and tick infestations. Fipronil products are available as sprays and as spot-on formulations, and also in combination with other active substances.

- Dose determination – permethrin

Three randomised, blinded GCP laboratory studies on dogs were provided to confirm the repellent (anti-feeding) effect of the product on the sand fly

Phlebotomus perniciosus, and to investigate the optimum mode of administration of the combination and to determine the minimal effective dose of permethrin. These studies contributed to the final assessment and subsequent authorisation of the recommended dose.

- Justification of combination

Studies were provided which contributed to the overall establishment of the recommended dose.

Pharmacokinetics

The pharmacokinetics of the active substances were discussed in a literature review, in addition to the provision of a GLP-compliant pharmacokinetic study conducted in dogs. The study was performed using the final formulation of the proposed product at the recommended dose rate. Hair and plasma concentrations of the active substance and relevant metabolites were recorded.

Following topical application to dogs under normal conditions of use, permethrin and fipronil together with its major metabolite are well distributed in the hair of the dog within one day of application. The concentrations of fipronil, fipronil sulfone and permethrin in the hair decrease with time and are detectable for at least 35 days after application. Fipronil plasma concentrations peak after 5 days and concentrations of its active metabolite peak at around 14 days. Permethrin displays low levels of systemic adsorption.

Tolerance in the Target Species of Animals

The applicant has provided a target animal safety study and has also presented the safety data from all the preclinical and clinical studies performed with the product. A GLP-compliant study was performed in 36 clinically eligible dogs, using a product containing 1x, 3x or 5x the expected therapeutic dose of the final product. The animals were dosed at 3 weekly intervals. Placebo was used as a negative control. Dogs receiving the placebo and 1 x dose received a total of 6 doses at 3 weekly intervals, and dogs receiving the 3x and 5x dose received a total of 3 doses at 3 weekly intervals.

Appropriate observations and clinical measurements were made at suitable time points. Results show that final formulation was well tolerated locally and systemically at all dose levels. These data, along with pooled data from submitted studies contributed to the safety warnings as described in the SPC.

The product literature accurately reflects the type and incidence of adverse effects which might be expected.

Resistance

The bibliography references were provided in relation to the possible resistance of target organisms to fipronil and permethrin. The studies provide no evidence of established resistance to fipronil or permethrin in the target parasites. Due to

the different modes of action of the two active substances, cross-resistance is unlikely.

IV.B Clinical Studies

Laboratory Trials

The applicant conducted dose confirmation studies for the selected doses of fipronil and permethrin against fleas, ticks, sandflies and mosquitoes. The studies were performed with the final formulation using the recommended dose.

Dose confirmation studies:

Study title	Interaction/efficacy study of a proposed product containing fipronil and permethrin against fleas (*Ctenocephalides felis*) on dogs.
Objectives	To assess the interaction and confirm efficacy of the proposed product, and fipronil and permethrin separately against fleas (*Ctenocephalides felis*) on dogs.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	A topical spot-on – 6.1% fipronil 54.5% permethrin administered once at day 0 based on bodyweight range: 1.1 ml/dog >4-10 kg 2.2 ml/dog >10-20 kg
Control product	6.1% fipronil 54.5% permethrin
Animals	Three groups, 8 dogs in each group (each group was administered either combination product, or fipronil or permethrin)
Outcomes/endpoints	Flea counts occurred on various days of assessment. This was performed by calculating the mean number of live fleas on each of the dogs in the treated groups.
Randomisation	Randomised
Blinding	Blinded
Method	The dose of the relevant product was drawn into a 1ml syringe, divided into equal volumes and applied directly onto the skin between the shoulder blades and in the lumbar area. At various time points according to the dosing schedule animals were infested as appropriate (approximately 100 fleas per dog), flea counts were performed on various days of assessment.
Statistical method	Statistical analysis was performed using appropriate software. Efficacy against fleas was calculated using Abbott's formula. Criteria for adulticidal efficacy was

	assessed as being the termination of ≥ 95% of fleas.
RESULTS	
Outcomes for endpoints	No treatment-related adverse events were observed. The three products had clinically comparable immediate persistent efficacy against *C. felis* infestation. No interaction between the two active ingredients was evident.
DISCUSSION	The study demonstrated that all three treaded groups had clinically comparable immediate and persistent efficacies with no statistical difference in arithmetic means. Efficacy of the proposed product at the recommended dose against the target parasite was at least 95% for fleas at each counting+, and persistence of efficacy was >95% for four weeks. No interaction between test materials was seen

Study title	Interaction/efficacy study of a proposed product containing fipronil and permethrin against fleas (*Ctenocephalides felis*) on dogs.
Objectives	To assess the interaction and confirm efficacy of the proposed product, and fipronil and permethrin separately against fleas (*Ctenocephalides felis*) on dogs.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	A topical spot-on – 6.1% Fipronil 54.5% permethrin administered once on Day 0 at the minimum recommended dose of 0.11 ml/kg bodyweight
Control product	6.1% fipronil 54.5% permethrin
Animals	Three groups, 8 dogs in each group (each group was administered either combination product, fipronil or permethrin)
Outcomes/endpoints	Flea counts occurred on various days of assessment by calculating the mean number of live fleas on each of the dogs in the treated groups.
Randomisation	Randomised
Blinding	Blinded
Method	The dose of the relevant product was drawn into a 1ml syringe, divided into equal volumes and applied directly onto the skin between the shoulder blades and in the lumbar area. At various time points according to the dosing schedule animals were infested as appropriate (approximately 100 fleas per dog), flea counts were performed on various days of assessment.

Statistical method	Statistical analysis was performed using appropriate software. Efficacy of the proposed product against fleas was calculated using Abbott's formula. Criteria for the measurement of adulticidal efficacy was set at ≥ 95% for fleas.
RESULTS	
Outcomes for endpoints	No treatment-related adverse events were observed. The efficacy of the proposed product against the target parasite was at least 95% for fleas at each counting.
DISCUSSION	The applicant has adequately demonstrated that data from all three treated groups demonstrated that there was clinically comparable immediate and persistent efficacy against the target parasite (>95%). No interaction between test materials was seen.

Study title	Efficacy of a proposed product containing fipronil and permethrin against ticks (*Dermacentor reticulatus*) against fleas (*Ctenocephalides felis*) on dogs water showered and shampooed.
Objectives	To confirm the efficacy of the proposed product against ticks (*Dermacentor reticulatus*) and fleas (*Ctenocephalides felis*) on dogs water showered and shampooed
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	A topical spot-on – 6.1% Fipronil 54.5% permethrin Group 2: shampooed 2 hours prior to treatment Group 3: shampooed 16 days post-treatment Group 4: water showered 16 days post treatment Group 5: not shampooed / showered
Control product	Untreated control untreated water showered and shampooed.
Animals	Five groups of 7 dogs.
Outcomes/endpoints	Following treatment, tick and flea infestations were performed. Tick and flea counts were performed post-application / infestation.
Randomisation	Randomised
Method	The test formulation was administered at the recommended dose rate, topically, in two spots on day 0. At various time points according to the dosing schedule, tick infestations were performed using 50 adult *D. reticulatus* on each dog, and flea infestations were performed using 100 adult *C. felis* on each dog. Flea and tick counts were performed on various days of the assessment.

Statistical method	Statistical analysis was performed using appropriate software. Efficacy against fleas was calculated using Abbott's formula. Criteria for adulticidal efficacy was set at ≥ 90% for ticks and ≥ 95% for fleas.
RESULTS	
Outcomes for endpoints	The data contributed to the environmental warnings as displayed on the SPC
DISCUSSION	Efficacy against *C. felis* was demonstrated to be >95% at all-time points for all studies. Efficacy against *D. reticulatus* was shown to have a delayed effect, and this is reflected in the SPC

Study title	Interaction/efficacy study of a proposed product containing fipronil and permethrin against ticks (*Rhipicephalus sanguineus*) on dogs.
Objectives	To confirm the efficacy of the proposed product, and fipronil and permethrin alone against ticks (*Rhipicephalus sanguineus*) on dogs.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	A topical spot-on – 6.1% Fipronil 54.5% permethrin administered once on Day 0 at the minimum recommended dose of 0.11 ml/kg bodyweight
Control product	6.1% fipronil 54.5% permethrin
Animals	Three groups, 8 dogs in each group (each group was administered either the combination product, or fipronil or permethrin alone)
Outcomes/endpoints	Tick counts occurred on various days of assessment by calculating the mean number of live ticks and the mean number of live and dead engorged ticks within each of the treated groups.
Randomisation	Randomised
Blinding	Blinded
Method	The dose of the relevant product was drawn into a 1ml syringe, divided into equal volumes and applied directly onto the skin between the shoulder blades and in the lumbar area. At various time points, according to the dosing schedule, animals were infested as appropriate (approximately 50 ticks per dog), tick counts were performed on various days of assessment.
Statistical method	Statistical analysis was performed using appropriate software. Efficacy against fleas was calculated using Abbott's formula. Criteria for adulticidal efficacy was set at ≥ 90% for ticks.

RESULTS	
Outcomes for endpoints	No treatment related adverse events were observed. The efficacy of the proposed product was at least 90% at all-time points
DISCUSSION	The applicant has adequately demonstrated that there was >90% efficacy of the product for up to 30 days. None of the test materials demonstrated immediate efficacy. No interaction between the test materials was seen.

Study title	Interaction/efficacy study of a proposed product containing fipronil and permethrin against ticks (*Dermacentor reticulatus*) on dogs.
Objectives	To assess the interaction and confirm efficacy of the proposed products and fipronil and permethrin alone against ticks (*Dermacentor reticulatus*) on dogs.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	A topical spot-on – 6.1% Fipronil 54.5% permethrin administered once on Day 0 at the minimum recommended dose of 0.11 ml/kg bodyweight
Control product	6.1% fipronil 54.5% permethrin
Animals	Three groups, 8 dogs in each group (each group was administered either combination product, fipronil or permethrin)
Outcomes/endpoints	Tick counts occurred on various days of assessment by calculating the mean number of live ticks and the mean number of live and dead engorged ticks on each of the treated groups.
Randomisation	Randomised
Blinding	Blinded
Method	The dose of the relevant product was drawn into a 1ml syringe, divided into equal volumes and applied directly onto the skin between the shoulder blades and in the lumbar area. At various time points according to the dosing schedule animals were infested as appropriate (approximately 50 ticks per dog), tick counts were performed on various days of assessment.
Statistical method	Statistical analysis was performed using appropriate software. Efficacy against fleas was calculated using Abbott's formula. Criteria for adulticidal efficacy was set at ≥ 90% for ticks.
RESULTS	
Outcomes for	No treatment-related adverse events were observed.

endpoints	The efficacy of the proposed product was at least 90% for all time points
DISCUSSION	The applicant has adequately demonstrated that there is no interaction between the active substances that influences product effectiveness against *D. reticulatus* ticks when it is administered at the lowest recommended dose. Immediate efficacy was not seen but all other time points efficacy was adequately demonstrated.

Study title	Dose confirmation study to determine the efficacy after a single application of a proposed spot-on product when compared with an untreated control group against artificially induced infestations of ticks (*Ixodes ricinus*) on dogs.
Objectives	To confirm the efficacy after a single application of a proposed spot-on product compared with an untreated control group against artificially induced infestations of ticks (*Ixodes ricinus*) on dogs.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	A topical spot-on administered at the recommended dose in two spots on target animals
Control product	No treatment
Animals	Two groups of six dogs
Outcomes/endpoints	Tick counts occurred on various days of assessment. Calculations were based on female ticks only, as male *I. ricinus* do not attach to a host.
Randomisation	Randomised
Blinding	Partially blinded
Method	Dogs were infected with 50 unfed adult *I. ricinus* at various time points according to the dosing schedule. Tick counts were conducted 48±2 post application / tick infestations.
Statistical method	Statistical analysis was performed using appropriate software. Efficacy against fleas was calculated using Abbott's formula.
RESULTS	
Outcomes for endpoints	Based on the appropriate arithmetic means, the test product was effective against *I. ricinus*. The efficacy of the proposed product was at least 90% at all-time points.
DISCUSSION	It was demonstrated that the proposed product provided persistent acaricidal efficacy against *I. ricinus* for four weeks

Study title	A study to confirm the adulticidal and the repellency effect on the sand-fly *Phlebotomus perniciosus* of a proposed product containing fipronil and permethrin, when applied to dogs at 2 points on the body. Comparison was made with a commercial product, Advantix Spot-on Solution for Dogs.
Objectives	To confirm the adulticidal and the repellency effect on *Phlebotomus perniciosus* of a proposed spot-on applied on dogs at 2 points and compared with a commercial product, Advantix.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	A topical spot-on administered at the recommended dose Advantix administered at the recommended dose
Control product	No treatment
Animals	Three groups each included 6 dogs
Outcomes/endpoints	Following treatment and infestation, anti-feeding and mortality effects were calculated according to measured engorgement and mortality rates.
Randomisation	Randomised
Blinding	Blinded
Method	Six groups were included per group. Each dog was infested with 80±2 female *P. perniciosus* on various days of assessment. Anti-feeding and mortality effects were calculated according to engorgement and mortality counts. Criteria for efficacy were not established "*a priori*".
Statistical method	Suitable statistical measurements were made in order to evaluate statistical differences in anti-feeding and adulticidal effects.
RESULTS	
Outcomes for endpoints	Results demonstrated repellent and insecticidal effects following administration of the proposed spot-on at two points on the body.
DISCUSSION	Based on the studies, one treatment of the proposed product protects dogs from sand-flies for four weeks, providing immediate and persistent repellent (anti-feeding) and insecticidal effects. These effects were demonstrated to persist for four weeks.

Study title	A study to confirm adulticidal and the repellency effects of a proposed product containing fipronil and permethrin on the mosquito *Culex pipiens* as applied to dogs at two points.
Objectives	To confirm the adulticidal and repellency effects on *Culex pipiens* of a proposed spot on product, applied to dogs at two points.
Test site(s)	Single centre
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	A topical spot-on administered at the recommended dose
Control product	No treatment
Animals	Six dogs were included per group – one group received no treatment, the other the recommended dose of proposed product.
Outcomes/endpoints	Following treatment anti-feeding and mortality effects were calculated.
Randomisation	Randomised
Blinding	Blinded
Method	Each dog was infested with 80±2 female *Culex pipiens* on various days of assessment. 104.14 spot on was administered on day 0. Counts were performed according to the timelines. Anti-feeding and mortality effects were calculated.
Statistical method	Anti-feeding and mortality effects were calculated using Abbott's formula according to engorgement and mortality counts.
RESULTS	
Outcomes for endpoints	The proposed product had an anti-feeding efficacy of more than 96% (based on arithmetic means), against artificially induced infestations of *Culex pipiens* on dogs for 28 days after treatment.
DISCUSSION	The proposed product demonstrated immediate and persistent efficacy >90% for four weeks against the *Culex pipiens* species of mosquito.

Study title	A dose confirmation study to determine the anti-feeding and insecticidal efficacy of a proposed spot-on product containing fipronil and permethrin, against the mosquito *Aedes aegypti* in dogs.
Objectives	A study to determine the anti-feeding and insecticidal efficacy of the proposed spot-on product against *Aedes aegypti* in dogs.
Test site(s)	Single centre
Compliance with	Good Clinical Practice (GCP)

Regulatory guidelines	
Test Product	A proposed spot-on administered at the recommended dose
Control product	No treatment
Animals	Six dogs were included per group – one group received no treatment, the other the recommended dose of product.
Outcomes/endpoints	Following treatment anti-feeding and mortality effects were calculated.
Randomisation	Randomised
Blinding	Blinded
Method	Each dog was exposed to 75(±5) females and 10-26 male viable non-blood fed adult *Aedes aegypti* on various days of assessment. The proposed product was administered on Day 0. Counts were performed according to the timelines. Anti-feeding (primary efficacy), and mortality, (secondary efficacy) effects were calculated.
Statistical method	Anti-feeding and mortality effects were calculated using Abbott's formula according, to engorgement and mortality counts.
RESULTS	
Outcomes for endpoints	The proposed spot-on had an anti-feeding efficacy of >96% (based on arithmetic means), against artificially induced infestations of *Aedes aegypti* on dogs, for up to 28 days after treatment.
DISCUSSION	The proposed product demonstrated immediate and persistent efficacy at >90% for four weeks against the *Aedes aegypti* species of mosquito.

Field Trials

Study title	Field clinical study to confirm the efficacy and safety of a proposed product containing fipronil and permethrin in dogs naturally infested by ticks.
Objectives	To confirm the efficacy and the safety of a product containing fipronil and permethrin, after spot-on application on dogs naturally infested by ticks, within normal conditions of use of the product in the field, and when compared to a positive reference product, Advantix Spot on Solution for Dogs.
Test site(s)	Multi-centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	A proposed spot-on solution containing 61 mg/ml fipronil and 545 mg/ml permethrin.

Control product/placebo	Control product: Advantix – a spot-on solution containing 100 mg/ml imidacloprid and 500 mg/ml permethrin
Animals	182 dogs were included in the study. A large diversity of both pure breeds and mixed breeds was included in the study. A total of 123 dogs were treated with the proposed product and 59 with Advantix. Inclusion criteria: • at least 3 live and attached ticks • at least 1.5 kg body weight and 12 weeks of age (i.e. 84 days) • a maximum of 4 dogs per household • privately (client) owned dog • healthy or well-controlled concurrent disease • healthy application site(s), dry fur • signed owner consent and owner's agreement to attend all protocol stated visits • one dog cannot be included more than once in the study Exclusion criteria: • pregnant, lactating or females intended for breeding during the study • treatment of the dog or environment with a parasiticide product with ongoing tick efficacy as per label • major surgery within 7 days prior to or planned during the study period • severe life-threatening chronic disease • requirement for a forbidden concomitant treatment • requirement for a shampoo before visit V3 (day 14±2 days), • known hypersensitivity and/or an adverse event to a spot-on or to one of the ingredients of the products tested
Outcomes/endpoints	Efficacy: • primary outcome parameter: live tick count (D0, D7, D14, D21, D28 and other date) • duration of the tick count process (end time minus start time), dead tick count, ticks species: number of dead and of live (adults, larvae, nymphs) Safety:

	• product exposition: quantity of product administered (ml) and dose completely administered (yes/no), premature withdrawals (completion yes/no, reason for premature withdrawal) • number of adverse events (AE) and number of serious adverse event (SAE) • number of concomitant treatments related or not related to an AE Tolerance: • rectal temperature, physical examination, abnormal signs, application site abnormality, physical examination of co-habiting cats Other: • time between visits, water immersion
Randomisation	Randomised.
Blinding	Blind
Method	At the first visit each dog received a single treatment on Day 0 according to the randomisation plan. Each dog was examined weekly for four weeks. At each examination attached ticks were counted and identified. Clinical examination included application site examination for possible adverse reactions. Any abnormal clinical signs on cohabitating cats were considered as related to the administration of permethrin to the dogs living with the cats.
Statistical method	In order to obtain a statistically significant result, 180 dogs were required to be enrolled into the study. A primary efficacy criterion 'percent reduction of live tick count was assessed using 95% confidence intervals between the test product and the positive control. The non-inferiority margin was set at 10%.
RESULTS	
Outcomes for endpoints	Acaricidal activity was statistically calculated to be within defined parameters throughout the 28 day study.
Adverse events	Some adverse effects were noted. The most widespread adverse effects were skin and appendages disorder, with 12 cases of pruritus exhibited. The SPC carries suitable warnings.
DISCUSSION	The data confirmed that the proposed product is safe to use when used as directed in the SPC.

Study title	Field clinical study to confirm the efficacy and safety of a proposed product containing fipronil and permethrin, in dogs naturally infested by fleas
Objectives	To confirm the efficacy and safety of a proposed product, a combination of fipronil and permethrin, after spot-on application on dogs naturally infested by fleas, within normal conditions of use in the field, and in comparison with a reference product, Advantix Spot on Solution for Dogs.
Test site(s)	Multi-centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	Test product: A spot-on solution containing 61 mg fipronil and 545 mg/ml permethrin.
Control product/placebo	Control product: Advantix – a spot-on solution containing 100 mg/ml imidacloprid and 500 mg/ml permethrin.
Animals	190 dogs were included in the study and 15 supplementary dogs were included. Inclusion criteria: • single dog household: the dog had at least 7 live fleas • multi-dog household: at least one dog had at least 7 live fleas • at least 1.5 kg body weight and12 weeks of age (i.e. 84 days) • a maximum of 4 dogs and 4 cats per household • privately (client) owned dog • healthy or well-controlled concurrent disease • healthy application site(s), dry fur • signed owner consent and owner's agreement to attend all protocol stated visits Exclusion criteria: • no treatment of primary or supplementary dog with the IVP or CVP or any cat (e.g. not possible due to pregnancy, known hypersensitivity) • treatment of dog or environment with a parasiticide product with ongoing flea efficacy as per label • major surgery within 7 days prior to or during the study period • expected introduction of a new dog/cat during the study period • requirement for a forbidden concomitant treatment requirement for a shampoo before visit V3 (day 14 +/- 2 days)

	Post inclusion removal criteria: • upon request of the animal owner • within a multi-dog household, if a primary or supplementary dog was not treated with the IVP or CVP • if a cat in the same household was not treated with the appropriate commercial veterinary spot-on product on day 0 or day 1 at the latest • major surgery has to be carried out during the study period • an adverse event occurs and interferes with the study objectives • the dog receives any forbidden concomitant treatment including alternative flea treatment • an ectoparasiticide environmental product was applied in the household • any other event occurs that could invalidate the data • shampoo within 14 days after treatment
Outcomes/endpoints	Efficacy: • Primary outcome parameter: live flea count (D0, D14, D28) • Other parameters: o duration of the flea count (end time - start time) Safety: • product exposition: quantity of product administered (ml) and dose completely administered (yes/no), premature withdrawals (completion yes/no, reason of premature withdrawal) • number of adverse events (AE) and number of serious adverse event (SAE) • number of concomitant treatments related or not related to an AE Tolerance: • rectal temperature, physical examination, abnormal signs, application site abnormality, physical examination of co-habiting cats Other: • time between visits, water immersion
Randomisation	Randomised.
Blinding	Blinded
Method	Each dog was assigned to one of the treatment groups according to the randomisation plan. At the first visit each dog (primary and supplementary) received a single treatment. Each dog was the examined every two weeks

	for four weeks. At each visit live flea counts (primary dogs only) were completed. Clinical examination included application sure examination and potential adverse reactions were investigated. Any abnormal clinical signs on cohabitating cats were considered as related to the administration of permethrin to the dogs living with the cats.
Statistical method	The primary efficacy variable was the percent reduction of the live flea counts.' This was assessed using 95% confidence intervals of the difference between the test product and the positive control. The non-inferiority margin was defined as 5%.
RESULTS	
Participant flow	Total of 5 dogs were not included in the ITT population. Four dogs were withdrawn from the study. Two cases were lost to follow up and were excluded from the ITT populations. One case received a forbidden concomitant treatment. One was withdrawn as other dogs in the same household were not included in the study. From the PP population, as additional 22 dogs were excluded due to major deviations, post inclusion withdrawal due to eligibility criteria.
Outcomes for endpoints	Data were considered sufficient to support the claims within the SPC.
Adverse events	Some adverse effects were noted. The most widespread AE was skin and appendages disorder characterised by pruritis and alopecia. The second common AE was application site pruritus and inflammation.
DISCUSSION	Data were considered sufficient to support the claims within the SPC.

V OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the benefit/risk profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.

MODULE 4

POST-AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the Heads of Medicines Agencies (veterinary) (HMA(v)) website (www.hma.eu).

This section contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

•	19 September 2014	Change in the quantitative composition, with respect to excipients, of the finished product.