ASSURING THE SAFETY, QUALITY AND EFFICACY OF VETERINARY MEDICINES

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DECENTRALISED PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

Duoflect spot-on solution for cats 1-5 kg
Duoflect spot-on solution for dogs 2-10 kg and cats > 5kg
Duoflect spot-on solution for dogs 10-20 kg
Duoflect Duo spot-on solution for dogs 20-40 kg
Duoflect Duo spot-on solution for dogs 40-60 kg

Date created: July 2014

PuAR correct as of 28/03/2018 when RMS was transferred to FR. Please contact the RMS for future updates.

MODULE 1

PRODUCT SUMMARY

EU Procedure number	UK/V/0489/001/DC UK/V/0489/002/DC UK/V/0489/003/DC UK/V0489/004/DC UK/V0489/005/DC
Name, strength and pharmaceutical form	Duoflect spot-on solution for cats 1-5 kg Duoflect spot-on solution for dogs 2-10 kg and cats > 5kg Duoflect spot-on solution for dogs 10-20 kg Duoflect spot-on solution for dogs 20-40 kg Duoflect spot-on solution for dogs 40-60 kg
Applicant	Ceva Animal Health Ltd Unit 3, Anglo Office Park White Lion Road Amersham Buckinghamshire HP7 9FB
Active substance	Fipronil (S)-methoprene
ATC Vetcode	QP53AX65
Target species	Dogs, Cats
Indication for use	<u>Dogs</u> Treatment and prevention of flea and/or tick infestations. - Treatment and prevention of flea infestations (*Ctenocephalides spp*). Immediate insecticidal efficacy against new infestations with adult fleas persists for 9 weeks. Prevention of the multiplication of fleas by inhibiting the hatching of flea eggs (ovicidal activity) and the

	development of flea eggs into adult fleas for 8 weeks after application. - Treatment and prevention of tick infestation (*Dermacentor reticulatus*, *Rhipicephalus sanguineus*). The product has immediate and persistent acaricidal efficacy for 6 weeks after application. The product can be used as part of a treatment strategy for the control of Flea Allergy Dermatitis (FAD) in dogs. <u>Cats</u> Treatment and prevention of flea and/or tick infestations. - Treatment and prevention of flea infestations (*Ctenocephalides spp*). Immediate insecticidal efficacy against new infestations with adult fleas persists for 8 weeks. Prevention of the multiplication of fleas by inhibiting the hatching of flea eggs (ovicidal activity) and the development of flea eggs into adult fleas persists for 6 weeks after application - Treatment and prevention of tick infestation (*Rhipicephalus turanicus*). The product has immediate and persistent acaricidal efficacy for 5 weeks after application. The product can be used as part of a treatment strategy for the control of Flea Allergy Dermatitis (FAD).

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	Full application in accordance with Article 12 (3) of Directive 2001/82/EC as amended.
Date of completion of the original decentralised procedure	29th January 2014.
Date product first authorised in the Reference Member State (MRP only)	Not applicable.
Concerned Member States for original procedure	Belgium, France, Germany, Italy, Luxembourg, Spain.

I. SCIENTIFIC OVERVIEW

These products are intended for the treatment of flea and/or tick infestations. In dogs, there is immediate insecticidal activity against new infestations of adult fleas (*Ctenocephalides* spp) persisting for 9 weeks, and prevention of the multiplication of fleas and development of adults from flea eggs for 8 weeks after application. The products for dogs also treat and prevent tick infestation (*Dermacentor reticulatus*, *Rhipicephalus sanguineus*) for 6 weeks post-application.

In cats, there is immediate insecticidal activity against new infestations of adult fleas (*Ctenocephalides* spp) persisting for 8 weeks and prevention of the multiplication of fleas and development of adults from flea eggs for 6 weeks after application. The products for cats also treat and prevent tick infestation (*Rhipicephalus turanicus*) for 5 weeks post-application. The products may be used to treat Flea Allergy Dermatitis (FAD) in both dogs and cats.

The products are 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 products can be safely used in the target species, the slight reactions observed are indicated in the SPC. The product is safe for the user, and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC.[1] 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.

[1] Summary of Product Characteristics.

II. QUALITY ASPECTS

A. Composition

The products contain fipronil and (S)-methoprene.

Duoflect solution for cats 1-5 kg: one 0.4 ml pipette contains 68 mg fipronil and 34 mg (S)-methoprene.
Duoflect spot-on solution for dogs 2-10 kg and cats > 5kg: One 0.7 ml pipette contains 121 mg fipronil and 60 mg (S)-methoprene.
Duoflect spot-on solution for dogs 10-20 kg: One 1.4 ml pipette contains 240 mg fipronil and 120 mg (S)-methoprene
Duoflect spot-on solution for dogs 20-40 kg: One 2.8 ml pipette contains 480 mg fipronil and 240 mg (S)-methoprene.
Duoflect spot-on solution for dogs 40-60 kg: One 4.2 ml pipette contains 720 mg fipronil and 360 mg (S)-methoprene.

The container/closure system consists of:-

Front foil:	Polypropylene / polyethylene terephthalate
Lidding foil:	Polyester /aluminium / polyester / polyethylene terephthalate

Pipettes are packed in child resistant blisters. Packs containing 1, 3, 6, 12, 24, 60 or 120 pipettes. The particulars of the containers and controls performed are provided and conform to the regulation. The choice of the formulation and the presence of preservative are justified. The product is an established pharmaceutical form and its 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 principles of good manufacturing practice from a licensed manufacturing site. Process validation data on the product have been presented in accordance with the relevant European guidelines. The manufacturing process is a mixing and filtering procedure, followed by shipping of bulk product for filling into pipettes.

C. Control of Starting Materials

The active substances are fipronil and (S)-methoprene active substances not described in the European Pharmacopoeia (Ph. Eur). These established active substances are manufactured in accordance with the principles of good manufacturing practice.

Each active substance specification is considered adequate to control the quality of the material. Batch analytical data demonstrating compliance with this specification have been provided. Relevant ASMF were submitted.

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

The tests performed during production are described and the results of 3 consecutive runs, conforming to the specifications, are provided.

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. 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. Tests include those for appearance, identity and content of active substances, impurities, water content, preservative content, uniformity of dosage units and microbiological purity.

G. Stability

Stability data on the active substances have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions.

Fipronil

Three production batches were stored in commercial packaging at 25°C/60% RH and 40°C/75% RH. Data for 36 months at 25°C/60% RH and 6 months at 40°C/75% RH demonstrated that the active substance is stable under these conditions. A retest period of 3 years is acceptable.

(S)-methoprene

Commercially prepared batches of the active substance were stored under nitrogen in light-resistant containers at 25°C/60% RH and at 40°C/75% RH. Active substance stored at 25°C/60% RH was tested at 0, 3, 6, 12, 18 and 24 months. Active substance stored at 40°C/75% RH was tested at 0, 1, 3 and 6 months. Retest time was established as being 2 years when stored at 2-8°C.

Stability tests on the finished product were provided for product stored in commercial packaging for up to 24 months at 25°C/60% RH and at up to 6 months at 40°C/75% RH. A shelf-life of 3 years was established.

H. Genetically Modified Organisms

Not applicable.

J. Other Information

Shelf-life of the veterinary medicinal product as packaged for sale: 3 years.

III. SAFETY AND RESIDUES ASSESSMENT (PHARMACO-TOXICOLOGICAL)

The applicant provided bibliographical data in support of the toxicological aspects of the two active substances, and also provided studies on the irritation and dermal sensitisation of the products. A user risk assessment (URA and environmental risk assessment (ERA) were also provided.

III.A Safety Testing

Pharmacological Studies

Pharmacodynamics

Fipronil

In the target organisms, fipronil antagonises the gamma aminobutyric acid (GABA) channels resulting in the blocking of the pre-synaptic transfer of chloride ions across the cell membrane. This results in uncontrolled activity of the nervous system, a manifestation of the insecticidal and acaricidal activity of fipronil. Fipronil additionally binds glutamate activated chloride channels not found in mammals.

(S)-methoprene

This active substance is an analogue of the juvenile hormone of insects. Addition of (S)-methoprene therefore causes abnormalities to occur during the developmental stages of the target organisms, it additionally, has negative effects on the reproductive capacity of adult insects.

Pharmacokinetics

Fipronil

Dermal absorption studies were provided for humans, rats and rabbits. At 200 mg/ml, dermal penetration was seen to a greater extent in rabbits and rats, but at 0.2 mg/ml dermal penetration was similar between human, rabbits and rats. In a rat dermal study using ^{14}C-fipronil, the quantity of active substance absorbed was less than 1% of the dose applied. In a single oral dose study in rats using radio-labelled fipronil, the active substance was found to be widely distributed. Faeces appeared to be the main route of excretion.

Sulfone, amide and reduction products are common metabolites of fipronil metabolism, and fipronil is commonly found within the hair and hair follicles of treated animals.

(S)-methoprene

A single radio-labelled dose of 25 mg/kg was administered orally to rats in one study. Peak concentration occurred after approximately 6 hours, which was followed by a slow decline with a 48 hour half-life. Distribution in rats was found to be in the liver, kidney and lungs, with peak concentration seen at 6-12 hours after dosing. Less of the active substance was seen in fat and muscle. A further study on the metabolism of (S)-methoprene mammals, showed that much is oxidised. The active substance is in general excreted biphasically, with a rapid first phase, followed by a slower second phase.

Suitable data were submitted on the use of the combination of the active substances.

Toxicological Studies

The applicant provided bibliographical data.

- Single Dose Toxicity

Fipronil was found to be acutely toxic to rats and mice following oral administration, and was slightly toxic to rabbits via the dermal route. In general, (s)-methoprene is considered less toxic than fipronil.

- Repeated Dose Toxicity

Fipronil

Studies were provided for repeat dose studies. In rats, dietary doses were given at 0, 0.07, 0.33, 1.93, or 19.87 mg/kg/day to males and 0, 0.07, 0.37, 2.28 and

24.03 mg/kg/day to females. In this study, the NOEL[2] was established at 0.33 mg/kg/day for males and 0.37 mg/kg/day for females. An oral study in dogs given doses at 0, 0.5, 2.0 and 10 mg/kg/day provided NOEL of 2 mg/kg/day for males and 0.5 mg/kg/day for females. In a further study, gelatine capsules given to dogs at 0, 0.2, 2 or 5 mg/kg/day product a NOEL of 0.2 mg/kg/day in both males and females. Neurotoxic effects were noted.

(S)-methoprene

Racemic methoprene was administered to male and female dogs at doses of 6.2, 12 and 120 mg/kg/day for 90 days. No deaths occurred, but an increase in liver weight was noted. A NOEL of 8.6 mg/kg/day was confirmed.

- Reproductive Toxicity, including Teratogenicity

Fipronil

In one study, rates were administered fipronil in the diet at 0, 0.25, 2.5 and 26 mg/kg/day (male rats), and 0, 0.27, 2.7 and 28 mg/kg/day (female rats). At amounts greater than 2.5 mg/kg/day, systemic effects were seen in the parental animals. Litters of treated parental animals showed adverse effects when given the fipronil-containing diet, and a reduction in fertility was also noted The NOAEL for parental toxicity was 0.25 mg/kg/day while for reproductive toxicity, it was observed to be 2.5 mg/kg/day.

A study in rats in which fipronil was administered to the cervical region of female rats at doses of 70, 140 and 280 mg/kg day showed that progesterone levels were increased and oestrodial levels were reduced. This altered the ovulating cycle of the rats. Fipronil reduced the pregnancy index in the highest dose group during mating studies, although other factors such as body weight, weaning weight, implantation and the number of resorptions were not affected.

In addition to further data provided for embryotoxic studies, it was established that fipronil has an effect on reproduction in laboratory animals. Suitable warnings appear in the SPC for use of the product in dogs and cats, i.e. use of the product during pregnancy and lactation is recommended only after the benefit/risk assessment by the responsible veterinarian. A further study in

rabbits, administered fipronil at 0, 0.1, 0.2, 0.5 and 1 mg/kg/day demonstrated no treatment-related effect, and established the NOAEL[3] at 1 mg/kg/day.

[2] NOEL – No observed effect level.
[3] NOAEL – No observed adverse effect level.

(S)-methoprene

In one study, rats were fed 0, 25 and 75 mg/kg/day racemic methoprene prior to mating. Pups from ensuing litters were utilised in follow-on mating studies. Evidence from the studies suggested that the NOAEL was 33 mg/kg/day, (29mg/kg/day when corrected for purity). A further study suggested a NOEAL for embryotoxicity of 570 mg/kg/day, (the highest dose tested).

In rabbits, a development study using racemic methoprene showed reduced body weight and increase frequency of abortions and the highest dose used, 2000 mg/kg/day. The NOAEL for both maternal and foetal toxicity was established as being 200 mg/kg/day.

- Mutagenicity

Published data were provided showing that the two active substances showed negative for genotoxic tests.

- Carcinogenicity (if necessary)

Fipronil

Fipronil was added to the diet of mice for 78 weeks at doses 0, 0.1, 0.5, 10, 30 or 60 parts per million. An adverse effect possibly due to an increase in liver weights was seen in the highest dose group only. A NOAEL was established at the equivalent of 0.055 mg/kg/day.

In a further combined carcinogenicity/toxicity study, rats were dosed in the diet with fipronil at 0, 0.019, 0.059, 1.27 or 12.68 mg/kg/day and at 0, 0.025, 0.078, 1.61, or 16.75 mg/kg/day for females. High mortality rates prevented the conclusion of the study with chemical changes and organ alterations noted at the two highest dose rates. Benign and malignant changes occurred in the thyroid glands of both sexes. The NOEL was established for males at 0.019 mg/kg/day and for females at 0.025 mg/kg/day.

(S)-methoprene

Racemic methoprene was given in the diet to mice at concentrations of 38, 150 and 380 mg/kg/day. The notable adverse effect was on changes to the liver which led to the establishment of a NOAEL equivalent to 130 mg/kg/day when corrected for purity.

Other Studies

Fipronil

Fipronil was administered orally to rats at doses of 0, 0.5, 5 and 50 mg/kg. Clinical toxicity was only observed at the highest dose, with neurotoxic signs seen only in male mice and convulsions seen in both species. A NOAEL of 0.5 mg/kg was established. A further study using a spot-on solution was performed in rats, with the active substance applied at 1, 2 and 4 times recommended dose; equivalent to 70, 140 and 280 mg/kg. Some behavioural abnormalities were observed, related particularly to the highest dose.

In a developmental neurotoxicity study in rats, fipronil was administered at 0.05, 0.90 and 15 mg/kg/day. The maternal NOEL was 0.90 mg/kg/day. The NOEL for developmental toxicity was established as 0.05 mg/kg/day, based on a reduction in pup weights and an increase in preputial separation in males.

Studies on thyroid function, which may be altered by fipronil, indicated that biliary clearance of the hormone was affected. This may cause an increase in thyroid-stimulating hormone.

(S-methoprene)

(S)-methoprene was shown to have no androgenic, oestrogenic, glucocorticoid or anabolic activity.

Combined product

In a variety of studies, the combined product was shown not to be an irritant or sensitiser to skin. Fipronil desulfinyl, the significant metabolite of fipronil seen after photodegradation, was also not observed to cause irritation or sensitisation problems. An investigation to determine the level of active substances on gloves after petting dogs showed that levels were highest 12 hours after administration for all compounds and declined over the course of the treatment to 0.5% by 7 days.

Observations in Humans

Published data on the adverse effects of fipronil in humans suggests that acute exposure causes no long-term harm and is self-limiting. Adverse reactions include vomiting, drowsiness headache, vertigo and sweating. There were no data relating to the absorption of (S)-methoprene by humans.

User Safety

The applicant has provided a user risk assessment in compliance with the relevant guideline, which discussed possible routes of exposure.

Warnings and precautions as listed on the product literature are adequate to ensure safety to users of the product.

- This product can cause eye irritation.
- Avoid contact of the product with skin, eyes or mouth. People with known hypersensitivity to any of the ingredients should not treat their animal with this product.
- Treated animals should not be handled or played with for at least 12-hours after treatment. Animals should be treated in the evening 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.
- Do not eat, drink or smoke while handling the product.
- Wash hands thoroughly after use.
- In case of accidental spillage on skin, wash off immediately with soap and water.
- If the product accidentally gets into the eyes, they should be thoroughly flushed with water.
- If the product is accidentally swallowed, seek medical advice immediately and show the package leaflet to the physician.

Ecotoxicity

The applicant provided a Phase I environmental risk assessment in compliance with the relevant guideline. The product is not to be used in food-producing animals, and the product is not considered to cause a threat to the environment when used as recommended. Warnings and precautions as listed on the product literature are adequate to ensure safety to the environment when the product is used as directed, with a direction that recently treated dogs should not enter watercourses.

IV CLINICAL ASSESSMENT (EFFICACY)

IV.A Pre-Clinical Studies

Pharmacology

Refer to Section III, Safety Testing, Pharmacological Studies. Suitable data were provided.

Fipronil - Mode of action
The mode of action has been supported by bibliographical references. The principal drug target for fipronil is the GABA gated chloride channel. Binding results in GABA antagonism leading to neurotoxicity and death in invertebrates. Fipronil can result in neurotoxicity in mammals at high doses however; insects are significantly more sensitive to the toxic effects. Fipronil has been demonstrated to have an adulticidal effect in fleas and ticks.

S-methoprene - Mode of action
The mode of action of S-methoprene has been supported by bibliographical references. S-methoprene is an insect growth regulator (IGR) with ovicidal and larvicidal activity. Specifically it is defined as a juvenile hormone analogue and its principal target is the insect neuroendocrine system where it exerts a regulatory effect at the level of gene expression. Studies have demonstrated the inhibitory effects of S-methoprene on insect development, with the key periods of sensitivity being early embryonic development and metamorphosis. S-methoprene has both an ovicidal and larvicidal effect on fleas when used topically to treat cats and dogs.

Fixed combination of fipronil/(s)-methoprene
The insecticidal and acaricidal effects of fipronil can be demonstrated alongside the IGR effects of S-methoprene in the same animal after topical application.

Tolerance in the Target Species of Animals

The applicant has conducted target animal tolerance studies using multiples of the recommended dose in the target species. A GLP[4]-compliant study was performed in a suitable number of clinically eligible young cats, (32 kittens: 18 male and 14 female; age: 8 weeks ± 2 days on day 0; weight range: 0.27 kg to 0.97 kg on day 0), using a spot-on product containing 1x, 3x or 5x the maximum recommended dose of the final product, over a period of 7 fortnightly applications. Placebo was used as a negative control. This was a five phase, parallel group, randomised, blinded, controlled study. Appropriate observations and clinical measurements were made at suitable time points. Analysis showed

[4] GLP – Good Laboratory Practice.

that the product was tolerated at x 5 the highest maximum recommended dose level (1.75 ml/kg), for a 12 week period.

A second study evaluated the oral safety of the combined product in adult cats. A 0.35 ml/kg dose was administered to a suitable number of clinically eligible animals, along with a placebo administered at the same dose to additional animals. This was a GLP-compliant study. The study design was parallel grouped, randomised, blinded and controlled. Placebo was used as a negative control. Appropriate observations and clinical measurements were made at suitable time points. No adverse reactions were seen.

A further study was conducted in young dogs. In this study, a suitable number of clinically eligible young dogs, (32 puppies: 12 male and 20 female; age: 8 weeks ± 2 days on day 0; weight range: 1.38 kg to 3.82 kg on day 0), were treated using a spot-on product containing 1x, 3x or 5x the maximum recommended dose of the final product, over a period of 7 fortnightly applications. Placebo was used as a negative control. This was an eight phase, parallel group, randomised, blinded, controlled study. Appropriate observations and clinical measurements were made at suitable time points. Analysis showed that the product was safe for use, no adverse reactions were seen.

A fourth study evaluated the oral safety of the combined product in adult dogs. A 0.36 ml/kg dose was administered to a suitable number of clinically eligible animals, along with a placebo administered at the same dose to additional animals. This was a GLP-compliant study. The study design was parallel group, randomised, blinded and controlled. Placebo was used as a negative control.

Appropriate observations and clinical measurements were made at suitable time points. Salivation was observed in all animals and vomiting was observed in 50% of the animals. The SPC carries suitable warnings demanding the avoidance of oral ingestion of the product. The adverse reactions observed did not have a systemic affect.

Resistance

Bibliographical references were provided in relation to the possible resistance of ticks and fleas to fipronil, not thought to be a major issue in Europe at the current time. No data is available for (S)-methoprene. Adequate warnings and precautions appear on the product literature and in the SPC.

IV.B Clinical Studies

Laboratory Trials

The applicant conducted dose titration and dose confirmation studies, and field clinical trials to demonstrate the efficacy in cats and dogs. Efficacy summary for cats:

IGR efficacy against the further development of flea eggs and larvae:

- During a dose titration study, ovicidal and larvicidal efficacy was demonstrated at 6 mg/kg S-methoprene.
- Persistence of IGR (ovicidal activity and prevention of the development of flea eggs to adult fleas) efficacy was shown during two dose confirmation studies until 47 days and 52 days post-treatment respectively.

Adulticidal efficacy against fleas:

- Minimum effective dose 12 mg/kg of fipronil against *Ctenocephalides felis* was shown in a dose titration study.
- Immediate efficacy above 95% was demonstrated during dose titration and dose confirmation studies
- Persistence of adulticidal efficacy until 44 days and 58 days post-treatment was shown during two dose confirmation studies respectively.
- A field study in representative cats across two geographic regions demonstrated non-inferiority to the reference product Frontline Combo Cat, Merial. These studies were randomised controlled studies over 28 days.

Adulticidal efficacy of fipronil against ticks:

- Minimum effective dose 12 mg/kg of fipronil against *Rhipicephalus turanicus*. was shown in a dose titration study.
- Immediate efficacy against *R. turanicus* was demonstrated during two dose dose confirmation studies. Persistence of efficacy against *R. turanicus* for 30 days and 37 days post-treatment was demonstrated during two dose confirmation studies respectively.

The following indications in cats are supported for the proposed fixed combination:

”Treatment and prevention of flea and/or tick infestations.
The product can be used as part of a treatment strategy for the control of Flea Allergy Dermatitis (FAD).
Treatment and prevention of flea infestations (*Ctenocephalides spp*). Immediate insecticidal efficacy against new infestations with adult fleas is seen persisting for 8 weeks. Prevention of the multiplication of fleas by inhibiting the hatching of flea eggs (ovicidal activity) and the development of flea eggs into adult fleas for 6 weeks after application.

Treatment and prevention of tick infestation (*Rhipicephalus turanicus*). The product has immediate and persistent acaricidal efficacy for 5 weeks after application."

Efficacy Summary for Dogs:

IGR efficacy against the further development of flea eggs and larvae:

- During a dose titration study, ovicidal and larvicidal efficacy was demonstrated at 6 mg/kg S-methoprene.
- Persistence of IGR (ovicidal activity and prevention of the development of flea eggs to adult fleas) efficacy was shown during one dose confirmation study until 61 days and until 8 weeks during a water challenge study.
- Weekly water immersion or a single pre-treatment shampoo in emollient shampoo was not shown to influence the persistence of IGR efficacy until day +62. Weekly shampooing with emollient shampoo or chlorhexidine containing shampoo was shown to reduce the persistence of IGR efficacy to 6 weeks.

Adulticidal efficacy of fipronil against fleas:

- Minimum effective dose 12 mg/kg of fipronil against *Ctenocephalides felis* was shown in the dose titration study.
- Immediate efficacy above 95% was demonstrated during dose titration and dose confirmation studies.
- Persistence of this effect for 65 days post-treatment was demonstrated during the dose titration and one dose confirmation studies.
- Water emersion weekly or a single pre-treatment shampoo in emollient shampoo product was not shown to influence immediate adulticidal efficacy. Weekly shampooing with emollient shampoo or chlorhexidine containing shampoo was shown to reduce the persistence of adulticidal efficacy to three weeks.
- A field study in representative dogs across two geographic regions indicated non-inferiority to the reference product Frontline Combo Dog, Merial. These studies were randomised controlled studies over 28 days.

Acaricidal efficacy of fipronil against ticks:

- Minimum effective dose 12 mg/kg of fipronil against *Rhipicephalus sanguineus* was shown in a dose titration study.
- Immediate efficacy against against *Dermacentor reticulatus* and *Rhipicephalus sanguineus* ticks was demonstrated during two dose confirmation studies.
- Persistence of acaricidal efficacy was demonstrated for 37 days post-treatment against *Rhipicephalus sanguineus* during one dose confirmation study and for 44 days post-treatment during two dose confirmation studies against *Dermacentor reticulatus* and *Rhipicephalus sanguineus* ticks respectively.

- A field study in representative dogs across two geographic regions demonstrated non-inferiority to the reference product Frontline Combo Dog, Merial. These studies were randomised controlled studies over 28 days.

The following indications are supported in dogs for the proposed fixed combination:

”Treatment and prevention of flea and/or tick infestations. The product can be used as part of a treatment strategy for the control of Flea Allergy Dermatitis (FAD). Treatment and prevention of flea infestations (*Ctenocephalides spp*). Immediate insecticidal efficacy against new infestations with adult fleas persists for 9 weeks. Prevention of the multiplication of fleas by inhibiting the hatching of flea eggs (ovicidal activity) and the development of flea eggs into adult fleas for 8 weeks after application. Treatment and prevention of tick infestation (*Dermacentor reticulatus, Rhipicephalus sanguineus*). The product has immediate and persistent acaricidal efficacy for 6 weeks after application.”

The followining warning is also added in the SPC:
“The effect of bathing dogs on the duration of product efficacy against fleas has been studied.
Weekly water immersion of dogs following treatment had no effect on the duration of efficacy. Shampooing of dogs with an emollient shampoo 48 hours prior to treatment had no effect on duration of efficacy. Weekly shampooing with an emollient shampoo in dogs may reduce the duration of efficacy to 3 weeks against adult fleas and to 6 weeks against immature stages of fleas. Weekly bathing with a chlorhexidine shampoo may reduce effectiveness against adult fleas to 3 weeks. No data on the effect of bathing/shampooing on the efficacy of the product in cats is available.”

Further Laboratory Trials

The applicant provided appropriate information on dose determination studies.

A number of dose confirmation studies were presented:

Study 1

Study title	Dose confirmation efficacy study of a fipronil/(S)-methoprene spot-on formulation against fleas (*Ctenocephalides felis*) on cats
Objectives	• To confirm the adulticidal efficacy of a fipronil/S-methoprene spot-on formulation against fleas (*Ctenocephalides felis*) on cats. • To confirm the efficacy of a fipronil/S-methoprene spot-on formulation against the further development of flea (*Ctenocephalides felis*) eggs. • To observe any possible adverse events related to the administration of the investigational veterinary product.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoprene Dose administered dependent on day 0 weight: • 0.35 ml/cat weighing 1 - 5 kg • 0.71 ml/cat weighing >5 - 10 kg
Control product/placebo	Negative control.
Animals	16 healthy cats, 8 animals in each group
Outcomes/endpoints	Primary endpoints: The number of adult fleas, the proportion of hatched eggs, and the proportion of adults emerging at each time point in both control and treatment groups. Safety endpoints: No specific adverse events were defined in the protocol, however during the study the health of animals was monitored closely to identify potential adverse events.
Randomisation	Randomised.
Blinding	Blinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment depending of their respective group. At various time points according to study schedule, animals were infested as appropriate (approximately 100 fleas per cat), flea counts were performed, flea eggs were collected, egg hatch and adult flea emergence were assessed to establish adulticidal, ovicidal and larvicidal efficacy.
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Comparisons

	were made by ANOVA. Efficacy against adult fleas, flea egg and larval development (inhibition of adult emergence) was calculated for the various assessment days using the arithmetic mean. The IVP was regarded effective if the efficacy against egg hatch and adult flea emergence was >90% and the adulticidal efficacy against fleas was >95%.
RESULTS	
Outcomes for endpoints	Adulticidal efficacy of the IVP remained above 95% at all time points and until day +44. Ovicidal efficacy of the IVP remained above 90% at all time points and until day +47. Larvicidal efficacy remained above 90% until day +33; on day +40 the efficacy was 89.3% and on day +47 the efficacy was 97.4%. No treatment related adverse events were observed.
DISCUSSION	A fixed combination of 17% fipronil and 8.5% S-methoprene, administered at the recommended dose, demonstrated immediate adulticidal efficacy against existing flea (*Ctenocephalides felis*) infestations on cats and was persistently effective for 6 weeks. The product was effective in preventing the further development of flea eggs (ovicidal activity) for at least 6 weeks post-treatment (47 days).

Study 2

Study title	Dose confirmation study of a fipronil/(S)-methoprene spot-on combination formulation against the further development of flea (*Ctenocephalides felis*) eggs (ovicidal and larvicidal activity) collected from treated cats.
Objectives	To confirm the efficacy of the target dose of a fipronil / (s)-methoprene spot-on combination formulation against the further development of flea (*Ctenocephalides felis*) eggs (ovicidal and larvicidal activity) collected from treated cats.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoprene Dose: 0.071 ml/kg (minimum product dose to be received, based on proposed product posology).
Control product/placebo	Negative control.
Animals	16 healthy cats, 8 animals in each group.
Outcomes/endpoints	Primary endpoint: Inhibition of adult emergence. Proportion of emerged adult fleas per incubated egg. Secondary endpoint: Ovicidal product effect. Proportion of hatched larvae per incubated egg. Safety endpoints: None specifically defined, however health of enrolled animals

	was monitored closely to identify potential adverse events.
Randomisation	Randomised.
Blinding	Blinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment depending of their respective group. At various time points according to study schedule, animals were infested as appropriate (approximately 100 fleas per cat), flea eggs were collected, egg hatch and adult flea emergence were assessed to establish ovicidal and larvicidal efficacy.
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Comparisons were made by ANOVA. Inhibition of adult emergence and ovicidal product effect were calculated for the various assessment days using the arithmetic mean. The IVP was regarded effective when inhibition of adult flea emergence was >90% compared to controls.
RESULTS	
Outcomes for endpoints	Reported ovicidal efficacy was >90% at all time points and for up to +45 days post treatment. Adult flea emergence efficacy was >90% at all time points and for up to +52 days post treatment. None of the adverse events observed during the study were in the opinion of the examining veterinarian related to administration of the IVP.
DISCUSSION	The IVP was safe in cats and >90% effective in preventing the further development of flea eggs to adult fleas for up seven weeks (52 days) post treatment.

Study 3

Study title	A dose confirmation study to determine the efficacy of a combined fipronil/(S)-methoprene product against fleas (*Ctenocephalides felis*) on cats under laboratory conditions.
Objectives	The objective of this dose confirmation study was to determine the efficacy after a single application, of a combined product (17% fipronil / 8.5% S-methoprene) when compared with an untreated control group against artificially induced infestations of fleas (*Ctenocephalides felis*) on cats.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoproene Dose: 0.071 ml/kg (minimum product dose to be received,

	based on proposed product posology).
Control product/placebo	Negative control.
Animals	16 healthy cats, 8 animals each group.
Outcomes/endpoints	Primary endpoint: flea adulticidal activity. Flea count compared to baseline (control) count. Safety endpoints: None specifically defined, however health of enrolled animals was monitored closely to identify potential adverse events.
Randomisation	Randomised.
Blinding	Blinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment depending of their respective group. At various time points according to study schedule, animals were infested as appropriate (approximately 100 fleas per cat) and flea counts were performed to establish adulticidal efficacy.
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Comparisons were made by ANOVA. Efficacy against adult fleas was calculated for the various assessment days using the arithmetic mean. The IVP was regarded effective when >95% reduction in flea count for treated animals compared to controls was achieved.
RESULTS	
Outcomes for endpoints	Adulticidal efficacy of the IVP was 100% at all time points and until day +58. There were no adverse reactions.
DISCUSSION	The results of this study demonstrate that a single topical application of a flea treatment (containing 17% fipronil and 8.5% (S)-methoprene solution) administered at 0.071 ml/kg was 100% effective (based on arithmetic means) against artificially induced infestations of Ctenocephalides felis fleas on cats when compared to the untreated control cats. There were no adverse reactions.

Study 4

Study title	Dose confirmation efficacy study of a fipronil/S-methoprene spot-on formulation against ticks (*Rhipicephalus turanicus)* on cats.
Objectives	To confirm the efficacy of the target dose of a fipronil/(S)-methoprene combination formulation against ticks (*Rhipicephalus turanicus*) on cats. To observe any possible adverse events related to the

	administration of the investigational veterinary product.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoprene Dose administered dependent on day 0 weight: • 0.35 ml/cat weighing 1 - 5 kg • 0.71 ml/cat weighing >5 - 10 kg
Control product/placebo	Negative control.
Animals	14 healthy cats, 7 animals each group.
Outcomes/endpoints	Primary endpoint: Adulticidal efficacy against ticks. Safety endpoints: None specifically defined, however health of enrolled animals was monitored closely to identify potential adverse events.
Randomisation	Randomised.
Blinding	Blinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment depending of their respective group. At various time points according to study schedule, animals were infested as appropriate (50 ticks per cat), tick counts were performed to establish acaricidal efficacy. (Tick counts were conducted 48 ± 2 hours post infestation or treatment).
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Comparisons were made by ANOVA. Efficacy against adult ticks was calculated for the various assessment days using the arithmetic mean. The IVP was regarded as effective when the efficacy was greater than or equal to 90%.
RESULTS	
Outcomes for endpoints	Adulticidal efficacy of the IVP remained above 90% at all time points (until at least day +28). No treatment related adverse events were recorded in any cats.
DISCUSSION	17% fipronil and 8.5% S-methoprene, administered at the target dose had a greater than 90% immediate (day +2) efficacy and persistent efficacies greater than 90% against *Rhipicephalus turanicus* for four weeks (day +28).

Study 5

Study title	Dose confirmation efficacy study of a fipronil / (S)-methoprene spot-on formulation against ticks (*Rhipicephalus turanicus*) on cats.
Objectives	To confirm the efficacy of the target dose of a fipronil / (S)-methoprene combination formulation against ticks (*Rhipicephalus turanicus*) on cats. To observe any possible adverse events related to the administration of the investigational veterinary products.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoprene Dose: 0.071 ml/kg (minimum product dose to be received, based on proposed product posology.
Control product/placebo	Negative control.
Animals	16 healthy cats, 8 animals each group.
Outcomes/endpoints	Primary endpoint: Adulticidal efficacy against ticks. Safety endpoints: None specifically defined, however health of enrolled animals was monitored closely to identify potential adverse events.
Randomisation	Randomised.
Blinding	Blinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment depending of their respective group. At various time points according to study schedule, animals were infested as appropriate (50 ticks per cat), tick counts were performed to establish acaricidal efficacy. Elizabethan collars were used to prevent ticks being groomed off. Tick counts were performed 48 hours post-treatment or infestation according to a systematic protocol.
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Comparisons were made by ANOVA. Efficacy against ticks was calculated for the various assessment days using the arithmetic mean. The IVP was regarded effective when adulticidal efficacy was >90% compared to untreated controls.
RESULTS	
Outcomes for endpoints	Reported adulticidal efficacy: 96.3% on day +2, and between 99.2% and 100% at all other assessment points up to +35days. For both day +2 and +30 the mean tick count from control animals was below 12.5 ticks (25%) and therefore below the CVMP specified minimum. On day +2 this was

	considered to be due to the four day period between infestation and assessment; on day +30 the lower count was not considered of impact on the conclusions due to the zero tick recovery from IVP treated cats. Efficacy was therefore >90% from +2 to +37 days post treatment. Adverse events observed were not due to treatment.
DISCUSSION	The IVP (17% fipronil and 8.5% (S)-methoprene solution) was immediately and persistently effective (≥90%) against *Rhipicephalus turanicus* infestations on cats for up to five weeks post treatment when administered at a dosage of 0.071 ml/kg.

Study 6

Study title	Dose confirmation efficacy study of a fipronil / (S)-methoprene spot-on formulation against fleas (*Ctenocephalides felis*) on dogs.
Objectives	To confirm the adulticidal efficacy of a fipronil/S-methoprene spot-on formulation against fleas (*Ctenocephalides felis*) on dogs. To confirm the efficacy of a fipronil/S-methoprene spot-on formulation against the further development of flea (*Ctenocephalides felis*) eggs. To observe any possible adverse events related to the administration of the investigational veterinary product.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoprene Dose of IVP: 0.71 ml/dog weighing >2 - 10 kg 1.41 ml/dog weighing >10 - 20 kg 2.82 ml/dog weighing >20 - 40 kg 4.24 ml/dog weighing >40 kg
Control product/placebo	Negative control.
Animals	16 healthy dogs, 8 animals in each group.
Outcomes/endpoints	Primary endpoints: adult flea count, flea egg hatch, and adult flea emergence. Safety endpoints: No specific adverse events were defined in the protocol, however during the study the health of animals was monitored closely to identify potential adverse events.
Randomisation	Randomised.
Blinding	Blinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment depending of their respective group. At various time points according to study schedule, animals were infested as appropriate (approximately 100

	fleas per dog), flea counts were performed, flea eggs were collected, egg hatch and adult flea emergence were assessed to establish adulticidal, ovicidal and larvicidal efficacy.
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Comparisons were made by ANOVA. Efficacy against adult fleas, flea egg and larval development (inhibition of adult emergence) was calculated for the various assessment days using the arithmetic mean. The IVP was regarded effective if the adulticidal efficacy against fleas was >95% and the efficacy against egg hatch and adult flea emergence was ≥90%.
RESULTS	
Outcomes for endpoints	Adulticidal efficacy remained above 97% at all time points and until day +65. Ovicidal efficacy remained 100% at all time points and until day +61. Larvicidal efficacy was 100% at all time points; however, the proportion of emerged adults per hatched egg in the control group was low during the study. No treatment related adverse events were observed.
DISCUSSION	A fixed combination of 17% fipronil and 8.5% S-methoprene, administered at the recommended dose, demonstrated immediate adulticidal efficacy against existing flea (*Ctenocephalides felis*) infestations on dogs and was persistently effective for 9 weeks. The product was effective in preventing the further development of flea eggs for 61 days post-treatment.

Study 7

Study title	Water immersion and shampoo impact study on the efficacy of a fipronil/(S)-methoprene spot-on formulation against fleas (*Ctenocephalides felis*) on dogs.
Objectives	To determine the effect of weekly post treatment water immersions on the adulticidal, ovicidal and larvicidal efficacy of a fipronil/(S)-methoprene spot-on formulation against fleas (*Ctenocephalides felis*) on dogs. To determine the effect of weekly post treatment shampooing with an emollient shampoo on the adulticidal, ovicidal and larvicidal efficacy of a fipronil/(S)-methoprene spot-on formulation against fleas (*Ctenocephalides felis*) on dogs. To determine the effect of a single shampooing with an emollient shampoo 48 hours prior to treatment on the adulticidal, ovicidal and larvicidal efficacy of a fipronil/(S)-methoprene spot-on formulation against fleas (*Ctenocephalides felis*) on dogs. To determine the effect of weekly post treatment shampooing with a chlorhexidine shampoo on the adulticidal, ovicidal and larvicidal efficacy of a fipronil/(S)-methoprene spot-on

	formulation against fleas (*Ctenocephalides felis*) on dogs. To observe any possible adverse events related to the administration of the investigational veterinary product.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoprene Dose of IVP: 0.71 ml/dog weighing >2 - 10 kg 1.41 ml/dog weighing >10 - 20 kg 2.82 ml/dog weighing >20 - 40 kg 4.24 ml/dog weighing >40 kg
Control product/placebo	Negative control.
Animals	35 healthy dogs, 7 animals each group.
Outcomes/endpoints	Primary endpoints: adult flea count, flea egg hatch, and adult flea emergence. Safety endpoints: No specific adverse events were defined in the protocol, however during the study the health of animals was monitored closely to identify potential adverse events.
Randomisation	Randomised.
Blinding	Unblinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment, depending of their respective group: - Group 1: Negative untreated control, water immersed and shampooed with emollient shampoo and chlorhexidine shampoo . - Group 2: Dogs treated with the IVP and shampooed weekly with chlorhexidine shampoo. - Group 3: Dogs treated with the IVP and shampooed weekly with emollient shampoo. - Group 4: Dogs shampooed with emollient shampoo on Day -2 and treated with the IVP. - Group 5: Dogs treated with the IVP and water immersed weekly. At various time points according to study schedule, animals were infested as appropriate (approximately 100 fleas per dog), flea counts were performed, flea eggs were collected, egg hatch and adult flea emergence were assessed to establish adulticidal, ovicidal and larvicidal efficacy.
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Comparisons were made by ANOVA. Efficacy against adult fleas, flea egg and larval development (inhibition of adult emergence) was calculated for the various assessment days using the arithmetic mean. The IVP was

	regarded effective if the adulticidal efficacy against fleas was >95% and the efficacy against egg hatch and adult flea emergence was ≥90%.
RESULTS	
Outcomes for endpoints	Summary: Immediate adulticidal efficacy was shown in groups 3 - 5, however group 2 was marginally below the threshold for efficacy on day +2. Persistent adulticidal efficacy was demonstrated as follows: group 2 for 3 weeks; group 3 for 3 weeks; group 4 for 8 weeks; and group 5 for 8 weeks. Ovicidal and larvicidal efficacy was shown to persist as follows (days rounded down to nearest week): group 2 for 3 weeks and 7 weeks; group 3 for 4 weeks and 6 weeks; group 4 for 8 weeks (62 days); and group 5 for 8 weeks (62 days). No treatment related adverse events were observed.
DISCUSSION	The persistence of efficacy demonstrated by the IVP was shown to be reduced by weekly shampooing with either an emollient shampoo or a chlorhexidine based shampoo. However shampooing 48 hours pre-treatment with an emollient shampoo (group 4) and weekly immersion in water (group 5) did not affect persistence of efficacy.

Study 8

Study title	A dose confirmation study to determine the efficacy of a combined fipronil/(S)-methoprene product against ticks (*Rhipicephalus sanguineus*) on dogs under laboratory conditions.
Objectives	To determine the efficacy after a single application, of a combined fipronil/(S)-methoprene product when compared with an untreated control group against artificially induced infestations of ticks (*Rhipicephalus sanguineus*) on dogs.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoprene Dose: 0.071 ml/kg (equivalent to minimum product dose to be received, based on proposed product posology).
Control product/placebo	Negative control.
Animals	16 healthy dogs, 8 animals in each group.
Outcomes/endpoints	Primary endpoint: tick adulticidal activity. Tick count compared to baseline (control) count Safety endpoints: Health of enrolled animals was monitored closely to identify potential adverse events.
Randomisation	Randomised.
Blinding	Partially blinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment depending of their respective

	group. At various time points according to study schedule, animals were infested as appropriate (50 ticks per dog), tick counts were performed to establish acaricidal efficacy
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Efficacy against ticks was calculated for the various assessment days using the arithmetic mean. Comparisons were made by ANOVA for baseline (day -1) bodyweight; ANOVA for tick count day +2 and all other between group tick count comparisons were made using a non-parametric Kruskal-Wallis test. The IVP was regarded effective when adulticidal efficacy was >90% compared to untreated controls using Abbot's formula.
RESULTS	
Outcomes for endpoints	Tick counts: Control: On day +2, +9 and +30 three, two and one dog respectively had tick attachment rates below 25%; however, on all occasions the mean tick attachment rate of the eight dogs was significantly above 25%. Adulticidal efficacy of the IVP remained above 90% at all time points (until at least day +44). No adverse reactions were seen.
DISCUSSION	The results of this study demonstrate that a single topical application of the IVP (containing 17% fipronil and 8.5% s-methoprene solution) administered at 0.071 ml/kg was effective (> 90% efficacy based on arithmetic means) against artificially induced infestations of *Rhipicephalus sanguineus* ticks on Beagle dogs for up to 44 days (against existing infestations and subsequent weekly tick challenge post-treatment) when compared to the untreated control animals.

Study 9

Study title	Dose confirmation efficacy study of a fipronil / (S)-methoprene spot-on formulation against ticks (*Dermacentor reticulatus*) on dogs.
Objectives	To confirm the efficacy of the target dose of a fipronil / (S)-methoprene combination formulation against ticks (*Dermacentor reticulatus*) on dogs. To compare the efficacy of the target dose of a fipronil / (S)-methoprene combination formulation with that of Frontline® Plus against ticks (*Dermacentor reticulatus*) on dogs. To observe any possible adverse events related to the administration of the investigational and control veterinary products.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoprene Dose: 0.071 ml/kg (equivalent to minimum product dose to be received, based on proposed product posology).
Control product/placebo	Negative control. Positive control (Frontline® Plus).
Animals	24 healthy dogs, 8 animals in each group.

Outcomes/endpoints	Primary endpoint: Adulticidal efficacy against ticks at 48 hours post-infestation (or treatment). Secondary endpoint: Adulticidal efficacy against ticks at 24 hours post-infestation (or treatment) Safety endpoints: Health of enrolled animals was monitored closely to identify potential adverse events.
Randomisation	Randomised.
Blinding	Partially blinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment depending of their respective group. At various time points according to study schedule, animals were infested as appropriate (50 ticks per dog), tick counts were performed to establish acaricidal efficacy. Tick counts were performed 24 hours (in situ count) or 48 hours (removal and tick sexing) post-treatment or post-infestation according to a systematic protocol.
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Efficacy against ticks was calculated for the various assessment days using the arithmetic mean. A between group comparison was made comparing the results for the negative control group with the IVP and CVP groups using an ANOVA. Detachment rate of tick (%) from 24 to 48 hours post-treatment or post-infestation was also assessed. Effectiveness claim: The IVP was regarded effective when adulticidal efficacy was >90% compared to untreated controls.
RESULTS	
Outcomes for endpoints	Tick counts: Control group arithmetic mean tick counts 24 hours post treatment or infestation range from 19.1 - 29.0 ticks (assessment day +1 to +43). Control group arithmetic mean tick counts 48 hours post treatment or infestation range from 19.9 - 29.6 ticks (assessment day +2 to +44). This indicates a vigorous challenge. ANOVA identified a significant difference ($p<0.05$) between the IVP and negative control groups at all post treatment assessments. A significant difference between the CVP and negative control groups was identified at all post treatment assessments except on day +1. For the comparison of the IVP and CVP groups ANOVA identified a significant difference ($p<0.05$) between groups as follows: for the 24 hour assessments from day +22 onwards the IVP treated group had significantly less ticks; for the 48 hour assessments from day +37 onwards the IVP treated group had significantly fewer ticks.

	Adulticidal efficacy: IVP 24 hour assessment efficacy above 90% from day +8 (100%) until day +36 (94%); efficacy on day +1 and +43 was 46.2% and 70.6% respectively. IVP 48 hour assessment efficacy above 90% from day +2 (90.7%) until day +44 (96.2%) CVP 24 hour assessment efficacy above 90% from day +8 (100%) until day +15 (99.3%); efficacy on day +1, +22, +29, +36 and +43 was 24.0%, 72.4%, 61.2%, 41.8% and 40.3% respectively. CVP 48 hour assessment efficacy above 90% from day +9 (100.0%) until day +30 (92.8%); efficacy on day +1, +37 and +44 was 64.2%, 73.8% and 60.8% respectively. Detachment rates: Detachment rates (%) for the IVP treated Group ranged from 30.8 to 100%, and from 23.7 to 77.6% for the CVP treated Group. No treatment related adverse events (other than cosmetic changes that were expected following treatment with a topical product) were observed during this study.
DISCUSSION	In this study the IVP (17% fipronil and 8.5% (S)-methoprene solution) was immediately effective (>90%) against pre-existing *D. reticulatus* tick infestations on dogs treated with the minimum recommended dose and persistently effective against new infestations for six weeks post treatment when assessed at 48 hours post infestation.

Study 10

Study title	Dose confirmation efficacy study of a fipronil/(S)-methoprene spot-on formulation against ticks (*Rhipicephalus sanguineus*) on dogs.
Objectives	To confirm the efficacy of the target dose of a fipronil/(s)-methoprene combination formulation against ticks (*Rhipicephalus sanguineus*) on dogs. To observe any possible adverse events related to the administration of the investigational veterinary product.
Test site(s)	Single centre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP).
Test Product	17% fipronil and 8.5% (S)-methoprene Dose of IVP: 0.71 ml/dog weighing >2 - 10 kg 1.41 ml/dog weighing >10 - 20 kg 2.82 ml/dog weighing >20 - 40 kg 4.24 ml/dog weighing >40 kg
Control product/placebo	Negative control.
Animals	16 healthy dogs, 8 animals each group
Outcomes/endpoints	Primary endpoint: tick adulticidal activity. Safety endpoints:

	Health of enrolled animals was monitored closely to identify potential adverse events
Randomisation	Randomised.
Blinding	Blinded.
Method	This was a parallel-grouped study. After acclimatisation, animals were given treatment depending of their respective group. At various time points according to study schedule, animals were infested as appropriate (50 ticks per dog), tick counts were performed to establish acaricidal efficacy.
Statistical method	Statistical analysis was performed using appropriate software. Level of significance was set at 5% ($p<0.05$). Efficacy against ticks was calculated for the various assessment days using the arithmetic mean. Comparisons were made by ANOVA. The IVP was regarded effective when adulticidal efficacy was >90% compared to untreated controls.
RESULTS	
Outcomes for endpoints	Efficacy against ticks: From day +9, the efficacy of the IVP remained above 90% up to and including day +37.
DISCUSSION	17% fipronil and 8.5% S-methoprene, administered at the target dose showed persistent efficacies greater than 90% against *Rhipicephalus sanguineus* from 9 days post-treatment until five weeks (day +37) post-treatment. No treatment related adverse events were recorded in any dogs.

Field Trials

Study 11 (Pivotal Field Study)

Study title	Field evaluation of the efficacy and safety of a topical formulation of Fipronil + (S)-methoprene product in the treatment and prevention of natural infestation of fleas on cats presented as veterinary patients in Europe.
Objectives	Evaluation of the efficacy and safety (over 28 days) of a spot-on solution for Cats (17% fipronil / 8.5% methoprene) in the treatment and prevention of naturally acquired infestations of fleas on cat. The study evaluated the product at a minimum recommended dose of 12 mg/kg of fipronil and 6 mg/kg of S-methoprene spot-on solution (IVP) administered once topically to the skin. The study also evaluated (over 28 days) the effectiveness of the IVP as part of a treatment strategy for the control of flea allergy dermatitis (FAD).
Test site(s)	Multicentre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)/ Good Laboratory Practice (GLP).
Test Product	17% fipronil and 8.5% (S)-methoprene (Final formulation) Pipette volume / dose:

	IVP1: Small cats (≥1.0 kg - ≤5 kg) – 0.35 ml IVP2: Large cats (> 5 kg) – 0.71 ml
Control product/placebo	Positive controls: The Control Veterinary Product: Frontline Combo Spot-on Cat (fipronil and S-methoprene, Merial) Pipette volume / dose: CVP1: Cats (≥1.0 kg) – 0.5 ml (50 mg fipronil and 60 mg S-methoprene).
Animals	203 cats, 95 female, 108 male, aged 3 months to 16 years.
Outcomes/endpoints	Primary endpoints: 1. Efficacy against fleas: presence or absence of fleas on cats. 2. Safety: clinical observations. Flea counts and clinical observations were performed by a veterinarian on day 0 (baseline) and day +14 (± 2 days) and +28 (± 2 days). All treated cats with 2 or more fleas on day 0 were included in the efficacy analysis. All treated cats and dogs were included in the safety analysis. Secondary endpoints: 1. Efficacy against fleas: analysis of arithmetic and geometric mean flea counts, and the reduction in geometric mean flea counts in each treatment group compared with day 0. 2. Efficacy against flea allergic dermatitis (FAD): assessed based on recorded dermatological findings in cats.
Randomisation	Randomised.
Blinding	Parallel group, blinded.
Statistical method	Statistical protocol was defined a priori. The statistical unit was the individual cat. The per protocol population (PP) were defined as all treated cats with two or more fleas on day zero that had met all the eligibility criteria and followed the protocol rigidly. The intention to treat population (ITT) were defined as all cat treated with the product regardless of the presence of fleas on day 0. Data analysis was conducted using appropriate software. A significance level of 5% ($P<0.05$) was applied to all statistical tests. No adjustment for multiple tests was performed. Application conditions for each treatment group were tested for homogeneity of variance and for normality before applying appropriate tests. Efficacy: Primary efficacy endpoint (presence or absence of fleas on 'per-protocol' (PP) cats on days +14 to +28 was tested by comparing the proportion of cats with no fleas on day +14 to the end of the trial in the IVP and CVP groups. Data was analysed with a one-sided non-inferiority test and delta -15%.

	Secondary endpoints were also identified and assessed. Flea count data were Log transformed prior to analysis. Percent efficacy for each treatment group and time point was calculated as the percent change from Day 0 in geometric mean flea count. The differences between study days in each treatment group were tested for significance, and the percentage reduction in treatment groups was compared at each post-treatment time point. Clinical signs of FAD (flea allergy dermatitis), examination of PP cats for signs of FAD on Day 0, were summarised. For PP cats with signs of FAD on day 0, a summary of the evolution of clinical signs of FAD over time in each treatment group (T01 and T02) was performed. Safety assessment: Adverse and suspected adverse drug reactions were reported and summarised in frequency tables.
RESULTS	
Outcomes for endpoints	Baseline measurements were appropriately assessed. Efficacy: The result of the analyses showed a non-significant difference between the IVP and CVP groups at both efficacy assessment time points. Statistical analysis for non-inferiority was established for both the primary efficacy criterion (the percentage of cats with no (zero) fleas from Days 14 to 28), and the secondary efficacy criteria (analysis of arithmetic and geometric mean flea counts on Days 14 and 28, and the percentage reductions in geometric mean flea counts on Days 14 and 28 compared with Day 0). Safety: The field safety of the IVP was demonstrated. The potential risk of local adverse reactions at the site of application should be considered. No serious adverse events occurred in the ITT population and no significant safety concerns were raised. Concomitant treatments were administered and none interfered with the interpretation or integrity of the study and there was no evidence of interactions.
DISCUSSION	The IVP, applied as a single topical administration at a recommended minimum dosage of fipronil 12 mg/kg and S-methoprene 6 mg/kg, was well tolerated and clinically efficacious in cats presented as veterinary patients in Europe. Statistical analysis demonstrated that it was non-inferior to the CVP (an approved product in the treatment and prevention of fleas for 28 (± 2) days on cats). Administration to a wide range of cats during the trial and with a variety of concomitant medications under typical field conditions in Europe resulted in no drug interactions and only 3 adverse events occurred during the trial from which all

	cats completely recovered. Clinical signs of FAD in cats improved throughout the study (for 28 (± 2) days), compared with Day 0, in both treatment groups. The alleviation of a range of clinical signs of FAD over time, in cats treated with IVP, indicates that the product may be beneficial as part of a treatment strategy for control of FAD in cats.

Study 12 (Pivotal Field Study)

Study title	Field evaluation of the efficacy and safety of a topical formulation of fipronil + (S)-methoprene in the treatment and prevention of natural infestation of fleas on dogs presented as veterinary patients in Europe.
Objectives	Evaluate the efficacy and safety (over 8 weeks) of a spot-on solution for dogs (17% fipronil / 8.5% methoprene) in the treatment and prevention of naturally acquired infestations of fleas on dogs presented as veterinary patients in Europe. The study evaluated the product at a minimum recommended dose of 12 mg/kg of fipronil and 6 mg/kg of S-methoprene spot-on solution (IVP) administered once topically to the skin. The study also evaluated (over 8 weeks) the effectiveness of the IVP as part of a treatment strategy for the control of Flea Allergy Dermatitis (FAD).
Test site(s)	Multicentre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP). Good Laboratory Practice (GLP).
Test Product	17% fipronil and 8.5% (S)-methoprene as proposed product. Pipette volume / dose: IVP: Small dogs (2.1 – 10.0 kg) – 0.71 ml IVP: Medium dogs (10.1 – 20.0 kg) – 1.41 ml IVP: Large dogs (20.1 – 40.0 kg) – 2.82 ml IVP: Extra large dogs (≥40 kg) – 4.24 ml
Control product/placebo	Positive controls: Frontline Combo Spot-on Dog. CVP: Small dogs – 0.67 ml (67 mg fipronil and 60.3 mg S-methoprene) CVP: Medium dogs - 1.34 ml (134 mg fipronil and 120.6 mg S-methoprene) CVP: Large dogs - 2.68 ml (268 mg fipronil and 241.2 mg S-methoprene) CVP: Extra large dogs - 4.02 ml (402 mg fipronil and 361.8 mg S-methoprene)
Animals	208 dogs, 96 female and 112 male; aged between 0.25 and 17.00 years; weight range 2.10 - 50.60 kg.
Outcomes/endpoints	Primary endpoints: 1. Efficacy against fleas: presence or absence of fleas on dogs. 2. Safety: clinical observations.

	Flea counts and clinical observations for primary endpoints were performed by a veterinarian on day 0 (baseline) and day +14 (± 2 days) and +28 (± 2 days). All treated dogs with 2 or more fleas on day 0 were included in the efficacy analysis. All treated cats and dogs were included in the safety analysis. Secondary endpoints: 1. Efficacy against fleas: analysis of arithmetic and geometric mean flea counts, and the reduction in geometric mean flea counts in each treatment group compared with day 0. 2. Efficacy against flea allergic dermatitis (FAD): assessed based on recorded dermatological findings in dogs.
Randomisation	Randomised.
Blinding	Parallel group, blinded.
Statistical method	Statistical protocol was defined a priori. The statistical unit was the individual dog. The per protocol population (PP) were defined as all treated dogs with two or more fleas on day zero that had met all the eligibility criteria and followed the protocol rigidly. The intention to treat population (ITT) were defined as all dogs treated with the product regardless of the presence of fleas on day 0. Data analysis was conducted using appropriate software. A significance level of 5% ($P<0.05$) was applied to all statistical tests. No adjustment for multiple tests was performed. Application conditions for each treatment group were tested for homogeneity of variance and for normality before applying appropriate tests. Efficacy: Primary efficacy endpoint (presence or absence of fleas on 'per-protocol' (PP) dogs on days +14 to +28 was tested by comparing the proportion of dogs with no fleas on day +14 to the end of the trial in the IVP and CVP groups. Data was analysed with a one-sided non-inferiority test and delta -15%. Secondary endpoints were also identified and assessed. Flea count data was Logn (x+1) transformed prior to analysis. Percent efficacy for each treatment group and time point was calculated as the percent change from Day 0 in geometric mean flea count. The differences between study days in each treatment group were tested for significance, and the percentage reduction in treatment groups was compared at each post-treatment time point. Clinical signs of FAD (flea allergy dermatitis), recorded by dermatological examination of PP dogs for signs of FAD on

	Day 0, were summarised. For PP dogs with signs of FAD on day 0, a summary of the evolution of clinical signs of FAD over time in each treatment group (T01 and T02) was performed. Safety assessment: Adverse and suspected adverse drug reactions were reported and summarised in frequency tables.
RESULTS	
Outcomes for endpoints	Baseline measurements were appropriately assessed. Efficacy: The result of the analyses showed a non-significant difference between the IVP and CVP groups at both efficacy assessment time points. Statistical analysis for non-inferiority was established for both the primary efficacy criterion (the percentage of dogs with no (zero) fleas from Days 14 to 28), and the secondary efficacy criteria (analysis of arithmetic and geometric mean flea counts on Days 14 and 28, and the percentage reductions in geometric mean flea counts on Days 14 and 28 compared with Day 0). Safety : The field safety of the IVP was demonstrated. The potential risk of local adverse reactions at the site of application should be considered. No serious adverse events likely to have been associated with the IVP occurred in the ITT population and no significant safety concerns were raised. Concomitant treatments were administered and none interfered with the interpretation or integrity of the study and there was no evidence of interactions.
DISCUSSION	The IVP applied as a single topical administration at a recommended minimum dosage of fipronil 12 mg/kg and S-methoprene 6 mg/kg, was well tolerated and clinically efficacious in dogs presented as veterinary patients in Europe. Statistical analysis demonstrated that it was non-inferior to the positive control, Frontline Combo Spot-on Dog, an approved product in the treatment and prevention of fleas on dogs. Administration to a wide range of dogs during the trial and with a variety of concomitant medications under typical field conditions in Europe resulted in no drug interactions and only two adverse events occurred in the IVP treated dogs during the trial from which all dogs completely recovered and no association with the product was made. Clinical signs of FAD in dogs improved throughout the study (for 28 (± 2) days), compared with Day 0, in both treatment groups. The alleviation of a range of clinical signs of FAD over time, in dogs treated with IVP, indicates that the product may be beneficial as part of a treatment strategy for control of FAD in dogs.

Study 13 (Pivotal Field Study)

Study title	Field evaluation of the efficacy and safety of a topical formulation of fipronil + (S)-methoprene in the treatment and prevention of natural infestation of ticks in dogs presented as veterinary patients in Europe.
Objectives	Evaluation of the efficacy and safety (over 28 days) of a spot-on solution for dogs (17% fipronil / 8.5% methoprene), used at a minimum recommended dose of 12 mg/kg of fipronil and 6 mg/kg of S-methoprene administered once topically to the skin for the treatment and prevention of naturally acquired infestations of ticks on dogs presented as veterinary patients in Europe.
Test site(s)	Multicentre.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP). Good Laboratory Practice (GLP).
Test Product	17% fipronil and 8.5% (S)-methoproene Pipette volume / dose: IVP: Small dogs (2.1 – 10.0 kg) – 0.71 ml IVP: Medium dogs (10.1 – 20.0 kg) – 1.41 ml IVP: Large dogs (20.1 – 40.0 kg) – 2.82 ml IVP: Extra large dogs (≥40 kg) – 4.24 ml
Control product/placebo	Positive control: Frontline Combo Spot-on Dog. CVP: Small dogs – 0.67 ml (67 mg fipronil and 60.3 mg S-methoprene) CVP: Medium dogs - 1.34 ml (134 mg fipronil and 120.6 mg S-methoprene) CVP: Large dogs - 2.68 ml (268 mg fipronil and 241.2 mg S-methoprene) CVP: Extra large dogs - 4.02 ml (402 mg fipronil and 361.8 mg S-methoprene)
Animals	168 dogs 82 female and 86 male; aged between 8 weeks and 13 years; weight range 2.10 – 75.40 kg.
Outcomes/endpoints	Primary endpoints: 1. Efficacy: absence of ticks on dogs. 2. Safety: clinical observations. Tick counts and clinical observations were performed by a veterinarian on day 0 (baseline) and day +7 (± 2 days), day +14 (± 2 days), day +21 (± 2 days), and +28 (± 2 days). All treated dogs with 1 or more ticks on day 0 were included in the efficacy analysis. All treated dogs were included in the safety analysis. Clinical examinations were performed on day 0 and day +28, and abnormal health, adverse events, and serious adverse events recorded throughout the study. Secondary endpoints: • Efficacy: analysis of arithmetic and geometric mean tick counts and reduction in the number of ticks compared with day 0.

Randomisation	Randomised.
Blinding	Parallel group, blinded.
Statistical method	Statistical protocol was defined a priori. The statistical unit was the individual dog. The per protocol population (PP) were defined as all treated dogs with one or more ticks on day zero that had met all the eligibility criteria and followed the protocol rigidly, returning for all follow-up examinations. The intension to treat population (ITT) was defined as all eligible dogs treated with the product regardless of the presence of fleas on day 0. Data analysis was conducted using appropriate software A significance level of 5% ($P<0.05$) was applied to all statistical tests. No adjustment for multiple tests was performed. Application conditions for each treatment group were tested for homogeneity of variance and for normality before applying appropriate tests. Efficacy: Primary efficacy endpoint (presence or absence of ticks on 'per-protocol' (PP) dogs on days +7 to +28 was tested by comparing the proportion of dogs with no ticks on day +7 to the end of the trial in the IVP and CVP groups. Data was analysed with a one-sided non-inferiority test and delta -15%. Non-inferiority was declared if the lower limit of the 95% confidence interval (one tail) for the difference in proportion was higher than -15%. Secondary endpoints were also identified and assessed. Safety assessment: Adverse and suspected adverse drug reactions were reported and summarised in frequency tables.
RESULTS	
Outcomes for endpoints	Baseline measurements were appropriately assessed. Efficacy: The result of the analyses showed a non-significant difference between the IVP and CVP groups at all efficacy assessment time points. Statistical analysis for non-inferiority was established for both the primary efficacy criterion (the percentage of dogs with no (zero) ticks from Days 7 to 28), and the secondary efficacy criteria (analysis of arithmetic and geometric mean tick counts all efficacy assessment time points, and the percentage reductions in geometric mean tick counts on Days 7, 14, 21 and 28 compared with Day 0). Safety: The field safety of the IVP was demonstrated. Safety assessment identified one dog with AEs possibly associated

	with the administration of the IVP (eosinophilic furunculosis). The association could not be further clarified due to a lack of information on the temporal relationship of onset with the time/day of product administration. Concomitant treatments were administered and none interfered with the interpretation or integrity of the study and there was no evidence of interactions
DISCUSSION	The IVP, applied as a single topical administration at a recommended minimum dosage of fipronil 12 mg/kg and S-methoprene 6 mg/kg, was well tolerated and clinically efficacious in dogs presented as veterinary patients in Europe against ticks. Statistical analysis demonstrated it to be non-inferior to the positive control, Frontline Combo Spot-on Dog, an approved product in the treatment and prevention of ticks on dogs. Administration to a wide range of dogs during the trial and with a variety of concomitant medications under typical field conditions in Europe resulted in no drug interactions and only one adverse event that was possibly related to the product was seen in the 84 dogs treated.

All data contributed to the formulation being assessed as acceptable for the indications as specified in 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 Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)

The post-authorisation assessment (PAA) 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.

The PAA for this product is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)