SCIENTIFIC DISCUSSION

Quality

ProMeris Duo Spot-On for Dogs is a product containing amitraz (14.34% w/w) and metaflumizone (14.34% w/w) in a non-aqueous solution, designed to be topically applied, for the treatment and prevention of flea (*Ctenocephalides canis and C.felis*) and tick (*Ixodes ricinus, Ixodes hexagonus, Rhipicephalus sanguineus, Dermacentor reticulatus* and *Dermacentor variabilis*) infestations and for the treatment of demodicosis (caused by *Demodex* spp.) and lice (*Trichodectes canis*) infestations in dogs. The product is prepared as a ready to use liquid in single use pipettes in five different filling volumes (0.67, 1.33, 3.33, 5.33 and 6.66 ml) to cover the recommended minimum dose of 20 mg/kg of body weight of both amitraz and metaflumizone to dogs. The objective of the formulation is to provide a cosmetically attractive, topical application of the active substances to the dog. The presentation in five different size pipettes allows control of dose administered according to the size of the animal.

Choice of active substances

***Metaflumizone*:**
Semicarbazone insecticide, acting as a neuronal sodium channel antagonist. It has shown excellent against fleas on dogs.

***Amitraz*:**
Formamide insecticide acting by inhibiting mono amine oxidase enzyme and as an octopamine agonist. It is effective against ticks.

The lack of flea activity by amitraz and the lack of action against ticks by metaflumizone demonstrates that the combination of these two substances is required to control both fleas and ticks.

The excipients are considered quite common for use in a spot-on formulation.

Choice of packaging

A two component package consisting of:

***Primary package*:**
A thermoformed pipette made from a laminated film.
***Secondary package*:**
The secondary package is a blister made of plasticised aluminium film.
The primary and secondary package combination was found to be acceptable in terms of compatibility (no solvents migration) and water permeation.

Mode of manufacture

The major steps in the manufacture of the present product have been discussed. Flow charts of the manufacturing process and the filling packaging operation have been included.

The bulk manufacturing process is a simple dissolving operation of the active substances in the solvent mixture, followed by a filtration. Manufacture as well as filling and packaging are standard processes.

Scale-up experiments and Stability

Scale-up experiments were performed from laboratory scale of 0.1 L, via preliminary scale-up of 7.5 L (in both cases using glass containers) to a pilot scale of 45 L (using various packaging concepts).

The product stability is directly related to storage temperature. The product shows acceptable stability at refrigeration temperature. Based on the scale-up experiments and stability results, key recommendations for packaging improvement have been made. According to the Applicant, upon developing a moisture-resistant packaging the stability of the product is expected to be satisfactory.

Production site (including batch release)

Wyeth Lederle Italia S.p.A.
Via Franco Gorgone
95030 Catania
Italy

Manufacturing process

The major steps in the manufacture of this product involve:

1. Addition of the liquid raw materials to the mixing tank.
2. Addition of active substances and mixing to dissolve the active substances in the solution.
3. The product is then treated with activated molecular sieves of 4Å grade by recirculating the product through a bed of molecular sieves.
4. In-process analytical tests are then performed on the product.
5. The product is then filtered by passing it through a 5 micron depth filter.
6. The filtered product is held in a holding tank with a nitrogen overlay until needed for the filling/packaging operation.

In-process controls

Regarding the manufacturing process, the following in-process controls are performed: water content, appearance, metaflumizone content, amitraz content and density.

The bulk solution manufacturing process and the filling packaging operation were adequately described.

Primary package: A thermoformed pipette made from a laminated film to form the primary containers. Each pipette constitutes a unit dose volume of 0.67, 1.33, 3.33, 5.33 or 6.66 ml.

Secondary package: the secondary package is a blister made of plasticised aluminium film.

The dual packaging seems necessary given the hydrolytic instability of amitraz.

Results of analysis are provided, showing compliance with the specifications set. Representative IR-spectra of the plastic components of the primary packaging by IR spectroscopy are also included.

From the Development Pharmaceutical section of the dossier, it is clear that the primary packaging is chosen in order to prevent migration of solvent and provide a barrier to oxygen and moisture.

Unfortunately, from the stability studies with the finished product it appears that due to moisture uptake at least amitraz showed rapid degradation. The packaging was consequently modified to make it impervious to moisture ingress and a second stability study was initiated with the improved package configuration.

The active substances are synthetic and free of any animal material. The excipients are also of non-animal origin. The product complies with the Note for Guidance EMEA/410/01 Rev.2.

Validation

Validation of the HPLC method for assay of metaflumizone and amitraz.

The E- and Z-isomers of metaflumizone and amitraz are determined using gradient high performance liquid chromatography with ultraviolet detection at 254 nm.

The validation report is about HPLC method M-3472.01. The current version of the method is M-3472.02 (re-issued to add structures for the active substances, to change the designation R-28153 to metaflumizone, to add the CL number for metaflumizone, and to add a recommendation on room temperature exposure of auto sampler vials). No new validations were required.

The HPLC method has been validated with respect to specificity, linearity, accuracy, repeatability, intermediate precision, stability of the solutions and robustness.

The validations presented for assay of metaflumizone and amitraz are complete and perfectly in agreement with the present requirements.

Validation of the HPLC method for analysis of impurities of metaflumizone and amitraz.

Minor components and degradation products related to metaflumizone and amitraz are determined using gradient HPLC with UV detection at 254 nm. The level of each minor component and degradation product is determined as a relative percentage by calculating the ratio of area counts of each minor component or degradation product to the area counts of either the E-isomer or amitraz peak.

The validation report is about HPLC method M-3479.01. The current version of the method is M-3479.02 (re-issued to add corrected structures for some of the degradation products, to add structures for the active substances, to change the designation R-28153 to metaflumizone, to correct response factors for some of the degradation products, to amend the CL number for one analyte, to remove one analyte from the analyte table, and to change the precision requirement for peaks < 0.3% relative amount). No new validations were required.

The HPLC method has been validated with respect to specificity, linearity, accuracy, repeatability, intermediate precision, stability of the solutions, limit of quantification and robustness.

The validations presented for the minor component and degradation products analysis are complete and perfectly in agreement with the present requirements.

Analysis results of the three 45 L pilot batches (FD04102, FD04103, FD04104) are provided. Each batch was packaged in 5 different presentation sizes. Three certificates of analysis for each presentation are included in the dossier. All samples passed the release criteria.

STABILITY

Amitraz

Controlled batches

Stability studies were performed on three 2000 kg batches, manufactured in March 2004.

Packaging
The amitraz was placed into a HDPE bag and closed with a twist tie. The HDPE bag containing amitraz was placed inside of a second HDPE bag, a 5g sachet of silica gel desiccant placed inside of the second bag (i.e. not in contact with the API), and the second bag closed with a twist tie. The double bag was placed inside of a metal container. A second sachet of silica gel is placed on top of the sealed bags prior to closing the metal container.

Studied characteristics and methods of analysis
The batches were checked for appearance (method M-2392), water content (modified USP <921>), and identity, assay and related substances according to BP.Vet.

Results
Interim results indicate that the three lots of amitraz remained within specification (2004 BP.Vet.) for at least 10 months when stored at 4ºC/ambient humidity. After 1 month, amitraz content remained within specification at 30°C/60%RH, whereas one out of three batches failed to meet the assay specification at 40°C/75%RH.

Test articles stored for 12 months at 4ºC/ambient temperature passed British Pharmacopoeia specification (potency, appearance, water and identity) except the criteria for any other secondary peak under related substances (≤ 0.1%) which were passed up to 10 months.

Conclusion
Amitraz can safely be stored at 4ºC/ambient humidity for approximately nine months.

Metaflumizone

Claimed re-test period and storage conditions

Re-test period: 12 months
Storage conditions: No restrictions
Packaging material: Glass bottles

Study set-up: storage conditions, parameters tested, analytical methods, validation.

The above mentioned claim is based on a stability study in glass bottles. This study was performed with only one batch (PF-01-02-Ve-1-3) and only assay was checked. No significant changes were found after 12 months at 20ºC and at 30ºC.

A report on the six months stability in PE bags/fiber drums [25ºC/60% and 40ºC/75% moisture climate room] of metaflumizone technical material pilot scale batches were provided. Interim results on 6-month time points indicate no significant changes in the content of the active ingredient as well as the impurity level. Consequently, a six-month re-test period was considered justified.

The applicant has provided tables showing the levels of impurities initially present in the product and also the levels observed after storage of nine months at 25ºC/60% RH in three batches of smallest, middle and largest five packaging sizes of the batches manufactured in 2005 with improved packaging containing a desiccant strip.

Although some of the degradation products are seen at levels of >1.0% relative to active ingredient, when their initial levels are considered, it is evident that the increase in their concentration during the storage period is below 1% relative to active ingredient and therefore, they do not warrant control at this point.

Monitoring of microbiological quality is considered not necessary as proliferation of microbes is not to be expected due to non-aqueous nature of the product.

Proposed shelf-life and storage conditions

According to the SPC, a shelf-life of 18 months for all presentations is proposed when stored at or below 25°C.

Stability studies

Three 50 L batches of the present product were manufactured in May/June 2005 at Fort Dodge Animal Health in Catania, Italy. Metaflumizone and amitraz had been supplied by the proposed active substance manufacturers.

Each of these batches was then packaged, in unit-dose pipettes of thermoformable-laminated plastic, in the sizes of 0.67, 1.33, 3.33, 5.33 and 6.66 ml. The product-filled pipettes were then individually packaged in plasticized aluminium blisters.

All the batches were then stored at various environmental conditions (including VICH conditions) to evaluate its long-term stability. Results over 12 months have been submitted. The study is ongoing up to 36 months.

Samples were investigated for appearance, container integrity, metaflumizone content, amitraz content, water content, density, metaflumizone degradation products, amitraz degradation productsdeliverable mass and weight loss.

The complete schedules on stability study sampling times and tests have been provided. The scheme can be considered acceptable.

Conclusion

During the first six months of storage at various environmental conditions, the product showed a direct relationship between the storage temperature and product stability. Also, the product in 1arger package sizes was generally more stable compared to the smaller package sizes.

There was minimal weight loss from any of the packages at any of the storage temperatures. Weight gain was also negligible demonstrating effective control of moisture ingress.
The present product exhibited acceptable stability during the twelve-month storage period at refrigeration temperature of 4ºC, 25°C and 30°C as none of the sub-batches showed the levels of active substance below the specified limits.

The batches showed more variable results at 40ºC/75% RH, wherein larger sized packages fared better than the smaller sizes. Nevertheless, the product was within specifications at the 40°C/20% and 75% RH for six months as per VICH guidelines. These results showed a dramatic improvement over the initial stability study demonstrating the importance of the package configuration in protecting the finished product.

Overall Conclusion on Part II

The dossier provides a suitable description of the active substances and the chosen formulation, and demonstrated that production of both the active substances and the product leads to a consistent quality. Analytical methods are well described and validation data confirm their suitability.

Safety

Dogs weighing less than 50 kg are treated with a single pipette with a volume of 0.133 ml/kg bw within weight bands and heavier animals with two pipettes at 4 week intervals through out the flea season. In a multi animal household, all pets should be treated.

ProMeris Duo Spot-on for Dogs is applied to a single spot on the skin of the dog between the shoulder blades. The applicator tube should be removed from the package and, holding the tube upright, the tip of the tube should be bent to break the tip along the fracture line. The top of the tip will fold back against the tube. The tip of the applicator should be pushed through the dog's hair to the skin surface and the tube squeezed to expel the entire contents. The product should not be applied to the surface of the dog's hair coat.

Metaflumizone
Metaflumizone is a semicarbazone sodium channel blocker insecticide, chemically derived from the pyrazoline family, with the same mode of action. Pyrazolines were reported to have a high insecticidal efficacy, with low mammalian toxicity. Indoxacarb, a substance derived from the pyrazolines, was the first insecticide on the market for agricultural use.

Metaflumizone blocks sodium channels by selectively binding to the slow-inactivated state, within the sodium channel pore. The binding site is thought to exist in all open and inactivated states, but as the substance binds only very slowly, the effect is only measured in the slow-inactivated state. In the *Xenopus* oocyte a concentration of 0.1 mcM of metaflumizone was capable of depressing the sodium current under depolarising conditions.

The voltage dependent sodium channel blocking action is similar to that of local anaesthetics. In the insect metaflumizone disrupts nerve function, resulting in paralysis.

Metaflumizone is a mixture of E and Z isomers (ratio 9:1). It has not been used before in veterinary medicine. Metaflumizone has no anthelmintic activity. The insecticidal activity occurs primarily after ingestion; it has lower activity by contact.

Amitraz
Amitraz is a formamidine acaricide and currently approved for use in both food producing and companion animals for many years. Although the exact mode of action is not known, amitraz is classified as an inhibitor of monoamine oxidase, which is responsible for the degradation of the neurotransmitters norepinephrine and serotonin. The mode of action probably also involves interaction with octopaminergic receptors in the tick nervous system, causing an increase in neural activity.

Amitraz can be regarded as a well known substance, with proven efficacy and a reasonable level of safety.

Although the mode of action of amitraz is not fully clarified, it remains one of the more potent substances for the control of infestations, caused by ticks and mites. In mammals amitraz is know to have alpha$_2$-adrenergic effects. Amitraz is not stable to acid. After oral ingestion it is likely to be degraded; its metabolite is known to have alpha$_2$-adrenergic effects and also possesses high affinity for octopaminergic receptors in mites, ticks and some insects.

Amitraz can lead to the occurrence of adverse effects through its major metabolite BTS 27271. It is observed that the formulation can protect amitraz from degradation that subsequently lead to signs of intoxication.

Amitraz is of low toxicity when administered orally to rats and mice, with LD_{50} values of 400-938 mg/kg and >1600 mg/kg respectively. Signs of intoxication are CNS depression, lethargy, hypothermia and hyperexcitability. With regard to intoxication, the dog is a relative sensitive species. A NOEL of 0.25 mg/kg/day was established for oral intake on the basis of subchronic toxicity studies.

Amitraz has no teratogenic potential and is non-carcinogenic.

Dermal absorption of amitraz from the healthy skin is minimal to absent. Only if inflamed, absorption may occur.

The use of amitraz, as proposed now in combination with metaflumizone, is in line with the authorised use in the dog as an acaricide.

Metaflumizone
No reports have been submitted on the secondary effects of metaflumizone. Its mode of action has not yet been fully elucidated. However, it is likely that the sodium current blocking effect in the insect nerve system is the only relevant mode of action.

Amitraz
Secondary pharmacological effects for amtiraz are based on its alpha$_2$-adrenergic effects. Pharmacological effects observed in mammals are related to this mode of action.

Justification of the combination
The cat flea is considered to be the one of most important ectoparasite species of domestic dogs and cats. Ticks are less common. Both parasite species pose a health risk and are a cause of nuisance. Fleas and ticks are often found as common infestations. Ticks may also transmit various infections.

Metaflumizone and amitraz were combined to make a low volume topical spot-on product for the simultaneous control of ticks and fleas in dogs. Studies were carried out to assess the efficacy for each active substance as a combination and to determine the dose rates at which the optimal efficacy was achieved. The possibility of synergy and/or interference was also studied.

The study addressed the efficacy and acceptability of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas and ticks on dogs. The purpose of the study was to examine the efficacy of a metaflumizone /amitraz combination against fleas and ticks on non-Beagle dogs. The efficacy of the individual compounds was also assessed to investigate possible synergy and/or interference in the combination formulation. On Day 8 dogs were infested with 50 unfed adult brown dog ticks (*Rhipicephalus sanguineus*). On Day 7 dogs were infested with 100 unfed cat fleas (*Ctenocephalides felis*). On D-5 each dog was examined and fleas and ticks were removed and counted. 83% of the animals having the highest tick counts were included in the study. Metaflumizone alone controlled fleas, but was not effective against ticks. Amitraz alone had little effect against fleas. Results demonstrated that there was neither synergy nor interference of activity of metaflumizone by amitraz versus fleas and ticks. Treatment of dogs with a combination of metaflumizone and amitraz in a spot-on formulation provided a high level of control of fleas for 5 weeks and of ticks for 3-4 weeks.

Data indicated that the efficacies of both substances remain similar when used as alone or in combination. Interaction between amitraz and metaflumizone appears to be absent.

It was observed that the products used were slightly different from the final formulation. The concentration metaflumizone was 20% for the combination as well as the single instead of 15%. The concentration amitraz was 10% instead of 15%. The dose for metaflumizone was twice that recommended now, 40 mg/kg instead of 20 mg/kg.

Finally, 2 tick species were used, *Rhipicephalus sanguineus* and *Dermacentor variabilis*. Reductions were slightly different for between species, with persistent efficacy against *Dermacentor variabilis* lasting for 4 weeks and for 3 weeks for *Rhipicephalus sanguineus*. The study results indicate that metaflumizone is the active substance against fleas and amitraz against ticks. Interaction was not observed. Levels of control were comparable to those for the fipronil/methoprene combination.

Conclusion on pharmacodynamics

- Metaflumizone blocks sodium channels by selectively binding to the slow-inactivated state, within the sodium channel pore.
- The insecticidal activity depends on this mode of action and occurs primarily after ingestion; it has lower activity by contact.
- Metaflumizone controls fleas, but is not effective against ticks.
- Amitraz is a well known substance, with proven efficacy against arthropods and a reasonable level of safety in mammals.
- Amitraz control ticks, but has little effect against fleas.
- Neither synergy nor interference of activity of metaflumizone by amitraz versus fleas and ticks.
- Treatment of dogs with a combination of metaflumizone and amitraz in a spot-on formulation provided a high level of control of fleas for 5 weeks and of ticks for 3-4 weeks.

Metaflumizone

Metaflumizone was orally administered to rats in doses of 30 and 1000 mg/kg, once or for 14 days. Absorption, distribution, metabolism and excretion were studied. Radiolabelled metaflumizone was used to study metabolism, with labels attached at various positions.

The study consisted of several phases including single oral dose pharmacokinetics, single dose biliary excretion, single dose mass balance, single dose organ and tissue distribution and repeated dose mass balance studies.

Pharmacokinetic parameters were calculated. Availability was low and most of the substance was eliminated via the faeces and very small amounts through the urine. The absorbed fraction was extensively distributed, resulting in a high V_d. Metaflumizone was extensively metabolised and more than 10 different metabolites were identified in plasma, bile urine, liver and kidney. For a dose of 30 mg/kg residues could still be detected after 7 days.

Amitraz

The pharmacokinetics of amitraz has been studied after oral administration to rats and dogs. After oral application amitraz is readily absorbed, metabolised and rapidly excreted via the urine.

Metaflumizone/amitraz

Two studies have been carried out on the pharmacokinetics of the combination.

Pharmacokinetics of metaflumizone and amitraz after a single topical application to dogs at 20 mg/kg of metaflumizone and amitraz. The aim of the study was to evaluate the pharmacokinetics of metaflumizone and amitraz in dogs after a single topical administration of a combination of metaflumizone/amitraz. The study was conducted to determine if either metaflumizone or amitraz were measurable in the blood of dogs following topical application at the proposed dose. All dogs were treated on D0. Blood samples were collected prior to application and after approximately 5 and 10 hours and at D1, 2, 3, 7, 10, 14, 21, 28, 42 and 56 post treatment. Levels of metaflumizone and amitraz were determined in plasma, using a validated HPLC method with a LOQ of 50 ng/mL for both substances. The LOD for amitraz was 3. 2 ng/mL and 1 ng/mL for metaflumizone. A 15% metaflumizone/15% amitraz spot on formulation was used at a dose of 20 mg/kg for each substance, topically applied on the skin between the shoulder blades. Amitraz was generally not detectable in plasma. On only 2 occasions (24 hours and 2 days post treatment) the amitraz levels were above the LOD but not quantifiable. Metaflumizone was detectable in plasma, but not quantifiable. Measurable levels were recorded for 33% of the male dogs on D7, 10 and 14, 67% of the male dogs on D21 and

all male dogs on D28 and 42. Only 33% if the female dogs had a quantifiable level on D42. Measurable levels generally ranged from 50-100 ng/mL. 17% of the dogs had a measurable level on D56. The results of the study indicate that systemic bioavailability of both metaflumizone and amitraz is was low, when topically administered as a spot-on formulation. Dermal absorption of metaflumizone was slightly higher in male dogs. The results did not allow a calculation of pharmacokinetic parameters.

Hair coat distribution of metaflumizone and amitraz after a single topical application to dogs at 20 mg/kg of metaflumizone and amitraz. The aim of the study was to determine the concentrations of metaflumizone and amitraz in the haircoat of dogs over a 56 days period, following topical application at the proposed minimum dose rate of 20 mg/kg BW for each active substance. All dogs were treated on D0. Hair samples were collected from 5 different sites of the dog: neck, middle of the back, top of the tail/lumbar zone, right thorax and left thorax. Samples were taken prior to treatment and at 1, 2, 7, 14, 28, 42 and 56 days after treatment. Samples were analysed for both metaflumizone and amitraz, using a validated HPLC method. High levels of substance were observed in some hair samples, making dilution of the final extract necessary at a higher rate than applied during validation. This may have caused an underestimation of amitraz levels of about 17% and of metaflumizone levels of about 28%. Vomiting was recorded in approximately 17% of the animals 4 hours after treatment. Maximum levels at the 5 sampling sites generally occurred at 7-14 days post treatment, after which levels gradually decreased. Variations in results were observed, which were likely to be due to heterogeneity of hair samples and the difficulty to homogenise pooled samples.

The results demonstrated that both metaflumizone and amitraz were distributed on the haircoats of dogs. Both substances remained quantifiable over a 56-days period.

An HPLC analytical method with UV detection to assay metaflumizone and amitraz in dog plasma and to assay metaflumizone in cat plasma was developed and validated. The method is based on a solid phase extraction for cat and dog plasma. After evaporation to dryness, the sample extract is reconstituted, vortexed, subjected to ultrasonification and centrifugation and finally separated with a Zorbax SB-C18 column and a mobile phase, consisting of a mixture of acetonitrile/methanol/formic acid. Mean extraction recoveries for metaflumizone and amitraz in dog plasma were 82% and 76% respectively for the E-isomer. For metaflumizone in cat plasma this was 86%.

An HPLC analytical method with UV detection to assay metaflumizone and amitraz in dog hair and to assay metaflumizone in cat hair was developed and validated. The method is based on a liquid extraction in acetonitrile for cat and dog hair. The supernatant, after evaporation to dryness and reconstitution in sample diluent, was separated with a Zorbax SB-C18 column and a mobile phase, consisting of a mixture of acetonitrile/methanol/formic acid. Before separation an additional n-heptane washing step was necessary for the cat hair in order to eliminate interferences. Mean extraction recoveries for metaflumizone and amitraz in dog hair were 55% and 100% respectively for the E-isomer. For metaflumizone in cat hair this was 87%.

Submitted data indicate that

- Metaflumizone is not readily absorbed after oral administration to rats.
- Amitraz is absorbed when given orally to dogs, but rapidly eliminated.
- Dermal absorption of both metaflumizone and amitraz is negligible, when topically applied to healthy dog skin. Based on a LOQ of 50 mcg/ml amitraz was generally not detectable in plasma; metaflumizone was detectable in plasma, but not quantifiable.
- Both metaflumizone and amitraz are well distributed over the dog's body, when topically applied to the skin between the shoulder blades and persist for 56 days in quantifiable levels.

Metaflumizone
The acute oral toxicity of metaflumizone was studied in albino mice and Sprague-Dawley rats. The acute oral LD_{50} of metaflumizone in Albino mice is >5000 mg/kg. The acute oral LD_{50} of metaflumizone in Sprague-Dawley rats is >5000 mg/kg.
The acute dermal toxicity of metaflumizone in Sprague-Dawley rats is >5000 mg/kg.
The acute inhalation LC_{50} of metaflumizone in Wistar rats is >5.2 mg/L.
Submitted data on the acute toxicity of metaflumizone, administered via various routes, indicate a very low acute toxicity.

Amitraz
The following studies concern the acute toxicity of amitraz.
The acute oral LD_{50} of amitraz in Albino Wistar rats is >500 mg/kg.
The acute dermal LD_{50} of amitraz in New Zealand White rabbits is >5000 mg/kg.
The acute inhalation LC_{50} of amitraz in albino Wistar rats is >2.1 mg/L. The observed clinical signs are commonly seen in inhalation experiments.

The combination of metaflumizone and amitraz
The following toxicity studies have been conducted with the combination.

Acute oral toxicity/LD_{50} in rats. The acute oral LD_{50} of a 15% w/v metaflumizone/15% w/v amitraz spot-on formulation was concluded to be > 500 mg/kg but less than 5000 mg/kg. Clinical signs and mortality are likely to have been caused by amitraz. Considering a reported oral LD_{50} of about 800 mg amitraz/kg in male rats it is not surprising that a dose of 5000 mg amitraz/kg can be lethal. For the major metabolite of amitraz (BTS 27271) a LD_{50} of 100-200 mg/kg has been reported in mice.

Acute dermal toxicity/LD_{50} in rabbits. The acute dermal LD_{50} of a 15% w/v metaflumizone/15% w/v amitraz spot-on formulation was concluded to be > 5000 mg/kg. Clinical signs and mortality are likely to have been caused by amitraz. An LD_{50} of > 200 mg/kg has been reported for the rabbit and topical application.

Acute inhalation toxicity/LC_{50} in rats. The acute inhalation LC_{50} was concluded to be > 0.57 mg/L, but < 2.32 mg/L. The observed clinical signs are commonly seen in inhalation experiments. The high level of mortality in the 2.32 mg/L-group was due to a combination of a low particle size and a high concentration.

Repeated dose toxicity

Metaflumizone
28 days/13 week oral toxicity study in albino rats. The No Observed Adverse Effect Level is less than 100 mg/kg, based on the reduced body weight gain and decreased food consumption. Although the acute toxicity of metaflumizone is low, repeated administration does affect the physiological functioning of rats and females appear to be relatively more sensitive to intoxication. A clear mode of action cannot be indicated, but interference with sodium channels is not likely. This is also illustrated by the absence of specific signs of intoxication.

Subchronic toxicity study in Sprague-Dawley rats; administration by gavage for 3 months. The No Observed Adverse Effect Level of M320102 (a metabolite of metaflumizone) was concluded to be 1000 mg/kg for males and 100 mg/kg for females. Regarding the NOAEL for metaflumizone it is observed that M320102 tends to be less toxic for males, but equally toxic for females. It should be pointed out that repeated administration of large quantities of chemical substances, even if relatively non-toxic, may lead to abnormalities, incl. adrenals, which are not specifically caused by the substance as such. Metaflumizone is a mixture of E and Z isomers in a ratio of 9:1, so it is obvious that the NOAEL is fixed by the E isomer.

Subchronic toxicity study in Wistar rats; dermal application for 3 months. The No Observed Adverse Effect Level was 100 mg/kg/day for both male and female Wistar rats. Apparently some metaflumizone was absorbed through the skin. Those signs that were observed complied with the signs seen in other repeated dose toxicity studies.

Subchronic/chronic oral toxicity study in Beagle dogs; administration via gelatine capsules for 3 and 12 months. The No Observed Adverse Effect Level for both male and female dogs was concluded to be 12 mg metaflumizone/day for 3 and 12 months. In contrast to the title, this study was designed and implemented as a 12-month study, with an in between evaluation after 3 months.

All (sub) chronic toxicity studies indicate an interference with haematology. Considering the absence of anaemia and the structure of the metaflumizone molecule and its metabolites, it is conceivable that such interference concerns the heme composition. It is obvious that such an effect is not elicited after a single dose. This would also imply that toxicity is related to the continuous presence of the substance rather than specific receptor affinity.

Data also indicate that females, rats and dogs, are relatively more sensitive to intoxication.

Amitraz

The repeat dose toxicity of amitraz has been investigated in the dog within the target animal safety studies.

Amitraz is considered as a well known substance. There is no need to demand study data. However, the Applicant could have summarised the major conclusions for repeat-dose toxicity of amitraz. The occurrence of adverse effects, due to amitraz, is possible and the Applicant has included a warning statement in the SPC.

It should also be pointed out that skin permeability between dogs may vary, e.g. under conditions of FAD, and that dermal absorption can occur.

Signs of adverse effects of amitraz and its metabolite(s) are rather specific and depend on alpha$_2$-receptor stimulation. Although the pharmacokinetic data, based on application of the spot-on formulation on the skin, indicated that amitraz levels in plasma were below the LOQ, this does not mean that minimal quantities of amitraz can be absorbed and may lead to the occurrence of adverse effects. This is due to the very high sensitivity of alpha$_2$-receptor to specific agonists.

Studies on the effects on reproduction

The metaflumizone/amitraz spot-on combination is indicated for use in dogs. Since the product is not indicated for breeding animals and the dog is not a food-producing species, reproduction toxicity studies are not deemed necessary.

However, since metaflumizone is a novel substance for veterinary use, data on reproduction toxicity were presented.

Two-generation reproduction toxicity study in Wistar rats; oral administration (gavage). The NOAEL for overall toxicity was concluded to be 20-30 mg/kg/day for the F_0 females, 75 mg/kg/day for males and 50 mg/kg/day for F_1 parental animals. The NOAEL for reproductive performance was concluded to be 20-30 mg/kg/day for the females and 50 mg/kg/day for the males and F_1 parental animals. The lowest NOAEL for development toxicity was concluded to be 20 mg/kg/day for the F_1 pups and 50 mg/kg/day for the F_2 pups. The toxicity of repeatedly administered high doses is not unexpected, but obviously was not taken into account when designing the study, probably because the results of the repeated dose studies were not available. Results do not indicate a specific toxic effect on reproduction. All observed adverse effects were likely to be due to the general toxicity of metaflumizone after repeated administration. It is pointed out that the NOAELs are more or less "study specific", because of the changes in dosing, with the possibility of carry over effects to persist.

Embryotoxicity/foetotoxicity, including teratogenicity

Prenatal developmental toxicity study in Wistar rats, oral administration (gavage). No effect on gestational parameters was observed.
The NOAEL for maternal toxicity was concluded to be 40 mg/kg/day, whilst the NOAEL for prenatal developmental toxicity was concluded to be 120 mg/kg. The results indicated the absence of specific embryotoxic or foetotoxic effects.

Prenatal developmental toxicity study Himalayan rabbits, oral administration (gavage). The NOAEL was concluded to be 100 mg/kg/day for both maternal and prenatal developmental toxicity. Incomplete ossification of sternebrae is observed for other substances at high doses. It is not considered to be specific for metaflumizone.

Mutagenicity

Metaflumizone

The applicant has submitted a number of studies that cover the following areas:

- Genotoxic potential in prokaryotes, using the bacterial reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli.*
- Gene mutation potential in mammalian cells *in vitro*, using Chinese hamster ovary cells.
- Clastogenic and aneugenic potential in an in vitro chromosome aberration assay, using Chinese hamster V79 cells in both the presence and absence of Arochlor-induced rat liver S9.
- Clastogenic potential and spindle poison effects *in vivo* in the mouse micronucleus test.
- Genotoxic effects, in the in vivo unscheduled DNA synthesis assay in rat hepatocytes.

These studies were conducted according to usual standards, with positive and negative controls, if applicable. The stability of the test article in these studies was confirmed. Metaflumizone and the Z-isomer were negative in the bacterial reverse mutation assay. Metaflumizone was negative the HPRT locus assay and the in vivo unscheduled DNA synthesis assay.

In the mouse micronucleus test, metaflumizone, administered intraperitoneally in doses up to 2000 mg/kg, led to evident signs of toxicity, with slight inhibition of erythropoiesis. However, the rate of micronuclei was not increased and there was no evidence of impairment of chromose distribution during mitosis.
Metaflumizone was concluded to be clastogenic in the *in vitro* chromosome aberration assay. However, the *in vitro* chromosome aberration assay is known to readily produce a positive effect; other tests did not indicate a mutagenic potential.
Therefore, it is concluded that metaflumizone is not likely to possess mutagenic potential.

Carcinogenicity

Studies were carried out in the mouse and the rat.
18 month oncogenicity study in mice via oral gavage administration
No mortality or clinical signs were observed and no treatment related effects were found in organs when examined microscopically, although increased brown pigmentation was seen in the spleen of animals from the 1000 mg/kg-group. The NOAEL for oncogenicity was concluded to be 1000 mg/kg/day. The NOAEL for general toxicity was concluded to be 250 mg/kg/day. No general toxic effects were reported, which is curious, since much lower daily doses were toxic to rats. Apparently mice are not sensitive to intoxication with metaflumizone, without being clear why. It can be questioned if studying oncogenicity in mice was appropriate.

90 day/24 month toxicity and oncogenicity study in rats via oral gavage administration.

The only test substance-related microscopic finding was a dose related increased incidence of central lobular hepatocellular hypertrophy in the liver of males and females administered 60 mg/kg/day and 300/200 mg/kg/day. Although tumours were found, there were no neoplastic findings that were related to treatment. The NOAEL for oncogenicity was concluded to be 300 mg/kg/day for male and 200 mg/kg/day for female rats. The NOAEL for general toxicity was concluded to be 30 mg/kg/day.

Studies of other effects

Metaflumizone

Neurotoxicity
Acute neurotoxicity study in Wistar rats, single administration by gavage.
Wistar rats each were administered doses of 0, 125, 500 and 2000 mg metaflumizone/kg/day for 14 days. Specific observations for motor activity and reflexes and neuropathological examinations were carried out. No mortality or substance related neurological effects were observed.

Subchronic neurotoxicity study in Wistar rats, administration by gavage for 3 months
Groups of male and female Wistar rats were administered doses of 0, 12, 36, 150 and 300 (males only) mg metaflumizone/kg/day for 3 months. Specific observations for motor activity and neuropathological examinations were carried out. Ophthalmic examinations were carried out as well.

In the 150 mg/kg-group 1 male and 1 female died. General signs of toxicity were observed at the higher dose rates and especially in females. No substance related neurological effects were observed in any group.

Eye irritation potential
Primary eye irritation study in albino rabbits.
38 mg metaflumizone powder was dosed into the conjunctival sac of the left eye of rabbits. No signs of corneal opacity or iritis were observed, although slight conjunctival irritation (redness) was seen after one hour in 2 rabbits. This had resolved by 24 and 48 hours respectively. The test substance was concluded non-irritating to the rabbit eye.

Dermal irritation potential
Primary dermal irritation study in albino rabbits.
Metaflumizone was applied to clipped intact skin of rabbits in a dose of 0.5 g. The test substance was moistened and applied under a gauze patch, secured with a semi-occlusive wrapping and kept in contact for 4 hours. Residual substance was removed ad sites were observed for irritation 1 hour after removal and at 24, 58 and 72 hours. No signs of irritation, erythema or oedema were observed.
Metaflumizone was concluded to be non-irritating to the rabbit skin.

Dermal sensitisation
Maximisation test in Guinea pigs.
Method according to Magnussen and Kligman. Animals received intradermal injections of 5% metaflumizone in 1% methylcarboxycellulose solution, with and without Freund's adjuvant. One week later they received a dermal application of 1 g test substance as a 50% formulation, under a gauze patch and with an occlusive dressing for 48 hours. Dermal reactions were then recorded after 48 hours. 2 weeks after the first test run the animals were challenged again following the same procedure, but with 0.5 ml of a 25% metaflumizone formulation at a new site and with only 24 hours of occlusion. Dermal reaction was recorded at 24 and 48 hours. The intradermal injection caused moderate and confluent to intense erythema and swelling. The dermal application caused incrustation, partially open and intense erythema and swelling in all test animals. The challenge did not cause any skin reaction in either the controls or the test animals. It was concluded that metaflumizone does not have a dermal sensitising effect in the Guinea pig.

Amitraz

Eye irritation
Acute eye irritation in rabbits
0.1 ml of amitraz, equivalent to 44 mg, was dosed into the conjunctival sac of one eye in New Zealand White rabbits. The eyes were examined and scored (Draize technique) at 1, 24, 48 and 72 hours after administration. No corneal opacity or iritis was observed, but conjunctival irritation was observed, but cleared by 48 hours. It is concluded that the test material is a mild eye irritant.

Dermal irritation potential
Acute dermal irritation in rabbits
A dose of 0.5 g amitraz was applied to the intact skin of male and female New Zealand white rabbits under a gauze patch for 4 hours. Residual test substance was then removed and the skin was observed for signs of dermal irritation 1 hour after removal and again at 24, 48 and 72 hours, according to the Draize technique. No erythema or oedema was observed.

Dermal sensitisation potential
Delayed contact dermal sensitisation test – Buehler method
Male and female Hartley albino Guinea pigs were induced with a dermal dose of 0.4 g amtiraz, moistened with distilled water and once a week for 3 weeks. The test substance was kept in contact with the skin for 6 hours, when residual material was removed by rinsing with water. Dermal reactions were recorded at 24 and 48 hours. No erythema was observed. It was concluded that amitraz does not have a dermal sensitising effect in the Guinea pig.

Metaflumizone/amitraz combination

Eye irritation
Acute eye irritation in rabbits
A dose of 0.1 ml of metaflumizone/amitraz spot-on formulation was dosed into the conjunctival sac of one eye in male and female New Zealand White rabbits. The eyes were examined and scored according to the Draize technique after 1, 24, 48 and 72 hours and at D7. No corneal opacity was observed. Iritis was observed in 2/3 animals at 1 hour, but had cleared at 24 hours. Conjunctival irritation was observed in all animals, but had cleared by D7. It is concluded that the test material is a mild eye irritant.

Dermal irritation potential
Acute dermal irritation in rabbits
A dose of 0.5 ml metaflumizone/amitraz spot-on formulation was applied under a gauze patch to the intact skin of male and female New Zealand White rabbits and kept in contact with the skin for 4 hours. Residual substance was then removed and the skin was scored for dermal irritation at 1, 24, 48 and 72 hours and at D7, according to the Draize technique. Erythema and oedema were absent at 1 hour and D1, 2 and 7 but barely perceptible at D3. It is concluded that the metaflumizone/amitraz spot-on formulation is not a dermal irritant.

Dermal sensitisation potential
Delayed contact dermal sensitisation test – Buehler method
Male and female Hartley albino Guinea pigs were induced with a dermal dose of 0.4 ml metaflumizone/amtiraz spot-on formulation, applied once a week for 3 weeks. The test substance was kept in contact with the skin for 6 hours, when residual material was removed by rinsing with water. Dermal reactions were recorded at 24 and 48 hours after the residual was removed. 2 weeks after the third induction animals were challenged at a naive site, using the same procedure as in the induction phase. Dermal reactions were recorded again at 24 and 48 hours. Erythema was absent following the first induction and absent to moderate following the second and third. Following challenge erythema was absent to moderate. It was concluded that the metaflumizone/amtiraz spot-on formulation was a potential sensitizer, as at least 20% of the animals showed moderate erythema after challenge.

Observations in humans

This section concerns cases of amitraz poisoning in humans, mainly children, by the oral and dermal route. Signs were dizziness, unconsciousness and vomiting, lower body temperature and an increase in blood glucose levels. Signs were reversible and no fatalities were reported.

In case of ingestion or exposure initial treatment should be symptomatic and supportive (e.g. artificial ventilation). Cases recover completely and most of them spontaneously.

Microbiological studies (studies on human gut flora and organisms used in food processing)

Both metaflumizone and amitraz are not known to have antimicrobial qualities. The substances are indicated for use in the dog, which is not a food-producing species.

Studies on metabolites, impurities, other substances and formulation

Reference can be made to the pharmacological and toxicological study data.

User Safety

The Applicant provided a user safety risk assessment, which was in general in line with CVMP Guideline EMEA/CVMP/543/03-FINAL. However, several shortcomings were noted with regard to the exposure scenarios and the risk characterisation. Therefore, the Applicant was asked to provide further comment on the scenarios used for risk assessment on the contact with treated animals.

The Applicant considered many exposure scenarios and the exposure estimates could be compared to relevant toxicity end points. Several risks for the user were identified. A high risk was identified for children who have direct access to the pipettes. This risk was adequately mitigated by the child-resistant packaging. In addition, risks for children in contact with the dog after treatment were identified. In the opinion of the CVMP, the proposed warnings can be considered adequate to mitigate those risks. Finally, a warning in relation to the sensitisation potential of the product has been included in the Summary of Product Characteristics.
Ecotoxicity

Phase I Assessment

Metaflumizone/Amitraz Spot-On for dogs will be used for the control of fleas and ticks of puppies and adult dogs. According to the VICH Phase I guidance (CVMP/VICH/592/98-final), for products intended to treat companion animals no Phase II environmental impact assessment is necessary. However, since the active ingredients are ectoparasiticides it is recommended to minimize the potential environment impact as much as possible. For this reason the following safety phrase is recommended:

"For 24-h period after treatment the dogs are not allowed to access streams, ponds or rivers"

It is assumed that within a 24-h period the solvents in the product are evaporated and the coat is dry.

Conclusion on Safety

Amitraz is a well known substance, with a history of use in veterinary medicine.

Metaflumizone is a novel substance in veterinary medicine. It is of low acute oral and dermal toxicity, with LD_{50} values of > 5000 mg/kg for each route.
The lowest No Observed Adverse Effect Level identified in repeated dose oral toxicity studies was 12 mg metaflumizone/kg BW/day in dogs, and the dermal NOAEL was 100 mg/kg BW/day in rats.

Dogs were the more sensitive species and females generally showed greater sensitivity to intoxication. Impaired body weight and effects on haematology were the most sensitive endpoints.
Neurotoxicity was not observed.

Metaflumizone has been shown to cause developmental toxicity, but only in the presence of maternal toxicity.

Metaflumizone has no teratogenic potential and does not pose a mutagenic or carcinogenic hazard.

The metaflumizone/amitraz spot-on formulation has shown dermal contact sensitisation potential in the Guinea pig. It also appeared to be a mild eye irritant, but not a dermal irritant.

Metaflumizone is of very low acute toxicity. Toxicity after repeated dosing does not appear to be induced by the mode of action, but rather by the mere presence of substance in the animal for a longer period of time.

It can be concluded that the applicant has adequately studied the local tolerance of the dog for the product. Observed local effects on the application spot are of a cosmetic nature only. The applicant has included a statement in the SPC concerning the presence of aforementioned effects after treatment.

It was also observed that repeated dose toxicity was not studied in mice. From studies that were carried out in mice, it appeared that they were less sensitive to intoxication, compared to the rat and the dog.

Furthermore it can be concluded that, given the proposed use of the product, no Phase II environmental risk assessment is necessary. For this reason, the following safety phrase has been included in the Summary of Product Characteristics: “Dogs should be prevented from accessing streams and rivers for the 24-hour period following treatment”.

Risks were identified in relation to user safety, in particular for the people applying the products and for children in contact with treated animals. Appropriate warning statements were included in the SPC.

Clinical Assessment (Efficacy)

Tolerance in the target species of animal

Five studies were carried out to asses the safety of a metaflumizone/amitraz combination in dogs. Four studies were carried out in Beagle dogs and one study was carried out in Chihuahua dogs.

Safety evaluation study of a topically applied spot on formulation of metaflumizone and amitraz in adult dogs. The aim of the study was to evaluate the safety of a metaflumizone/amitraz combination in adult dogs upon single topical application of 1x, 3x or 5x the proposed dose, compared to placebo treated dogs.

Animals were ranked by weight, assigned to blocks of four and then randomly allocated to one of 4 treatment groups. Groups received a placebo or the combination of 15% metaflumizone/15% amitraz in 1x, 3x or 5x the recommended treatment dose of 20 mg/kg for each substance, as a single treatment, along the dorsal midline between the scapula. The day of treatment was designated as D1. Clinical observations were made twice daily. Body weights were recorded at D-14 (arrival), D-7 and D-1 and thereafter weekly during the course of the study. Food consumption was measured daily. All animals were examined for neurological disorders, twice prior to treatment and 4 and 24 hours post treatment and on D8 and D22. Persons collecting data were blinded to treatment. Blood samples for the

evaluation of haematology and clinical chemistry were collected once prior to treatment and 24 hours after treatment and on D8 and D22. Test substance: 15% metaflumizone/15% amitraz Spot-on. All animals appeared quiet during the first 24 hours after treatment. Since this included the placebo treated animals as well, it was not considered to be substance related, but probably due to handling. Body weight, body weight change and food consumption were not affected by treatment. Physical and neurological examinations did not reveal any effect from treatment. Haematology and clinical chemistry parameters exhibited statistically significant effects following treatment at 24 hours post dose and for some variables also on D8, but effects were of a transient nature and did not follow a dose-dependent relationship. Glucose levels did show a mild dose dependant increase 24 hours after dosing, but this effect was not seen afterwards. Individual glucose levels exceeded the upper range of expected values in males at 5x and females at 3x and 5x overdosing. Urea nitrogen also showed a slight increase at 24 hours post dose and on D8 in some animals from the 5x group. It is concluded that metaflumizone/amitraz spot-on is well tolerated by adult Beagle dogs up to 5x the recommended treatment dose. Statistically significant differences in haematology and clinical chemistry variables were observed between groups, in particular for the 24 hours post dose interval. Variation due to difference in individuals is a common finding in animal studies, easily confounding interpretation of results when study animal numbers are low. Although a relationship between treatment and variation cannot be excluded, it is difficult to indicate a relationship between substance and effect. Metaflumizone can be detected in plasma after dermal application, but is of low toxicity. Amitraz can not usually be detected, but is capable of inducing alpha$_2$-adrenergic effects at very low concentrations. This would mean that amitraz would be able to elicit such effects at levels of about 50 ppb. Considering the high sensitivity of alpha$_2$-adreno receptors this is likely. The transient rise in glucose blood levels appears to be treatment related, considering the dose dependent nature. It is most likely due to amitraz. However, the handling of the animals can have attributed to an increase as well. Regarding the levels for the 1x treatment groups in relation to those for the placebo controls, the rise of about 20% is moderate, short lasting, reversible and therefore without further consequences for the animal's health, even in diabetic dogs. The aspect of the application site was not reported on and observation not included in the study design / protocol. This concerns the aspect of the skin as well as the cosmetic aspect of treatment.

Safety evaluation study of a topically applied spot-on formulation of metaflumizone and amitraz in 8 week old puppies. It was concluded that metaflumizone/amitraz spot-on is well tolerated by 8-week old Beagle pups up to 5x the recommended treatment dose. The aspect of the application site was not reported on. Moreover, the study protocol did not mention the observation of the application site.

Safety evaluation of repeated treatments with a topically applied spot-on formulation of 15% w/v metaflumizone and 15% w/v amitraz in dogs. Several haematology and clinical chemistry parameters showed statistical significant differences between treated and control animals. Differences were not dose dependent and could not be attributed to treatment. A transient rise in leukocytes, neutrophils, monocytes and fibrinogen was noted at some time points in animals from the 5x group. These increased levels were attributed to mild, transient inflammation. Serum urea nitrogen was increased in treated animals. It was not associated with an increase in creatinine, suggesting a non-renal cause. Repeated administrations of 1x, 3, and 5x the recommended treatment dose of metaflumizone/amitraz spot-on, given once every 14 days for a total of 7 doses in dogs from 10 weeks and on, was well tolerated. A transient rise in leukocytes, neutrophils, monocytes and fibrinogen was noted at some time points in animals from the 5x group. An increase in serum urea nitrogen was noted in all dose groups, but not considered of toxicological significance.

The Applicant attributed the observed rise in leukocytes, monocytes and fibrinogen to inflammation. Being treatment related it can be suggested that the application of the spot-on formulation was irritating, causing a reaction on the application spot. However, it should be pointed out that signs of respiratory infections were observed throughout the study and this could have attributed to the aforementioned rise. Such rise was not seen in the single dose studies.

A small rise in serum urea nitrogen levels has been observed in all tolerance studies and seems to be active substance related. The mode of action is not clear. Serum glucose levels were only higher at the 3rd and 5th dose and for the 3x and 5x groups only.

Safety evaluation study of oral exposure from auto- or allogrooming metaflumizone/amitraz spot-on formulation in dogs. The aim of the study was to evaluate the safety of 15% w/v metaflumizone and 15% w/v amitraz spot-on formulation in dogs and their behavioural responses after a single oral application of 0.1X the recommended dose compared to dogs similarly dosed with 0.9% bacteriostatic sodium chloride. This dose estimates the potential oral exposure due to licking after topical application. Avoidance behaviour was noted in all dogs immediately after dosing of the metaflumizone/amitraz spot-on formulation. This included head shaking, spitting, licking and/or salivation. Beginning 1-2 hours after treatment decreased activity was noticed in 50% of the males and 50% of the females. A lower skin temperature was observed in 25% of the males and 25% of the females and pale gums in 25% of the males and 25% of the females. 25% of the females showing decreased activity also developed ataxia lasting up to 3 hours post treatment. A lower body temperature was also observed until 10 hours post dose. Observed signs were attributed to treatment with metaflumizone/amitraz spot-on. Heart rates remained normal and neurological examinations did not reveal abnormalities. Food consumption was not affected. Control saline-treated animals did not show any avoidance behaviour. Comparisons of several haematology and clinical chemistry parameters between treatment and control groups revealed statistically significant differences. This concerned leukocytes and monocytes on D2 and lymphocytes and eosinophils on D8. Sodium was increased in males and decreased in females on D8 and urea nitrogen was increased in females on D2. Treatment and control groups were also different on D2 for phosphorus, alkaline phosphatase, alanine aminotransferase, creatinine and glucose; on D8 for total bilirubin, creatine kinase and creatinine. Individual values for all parameters remained within expected ranges or were not considered meaningful. Oral administration of metaflumizone/amitraz spot-on to dogs at approximately 10% of the proposed recommended dose had no effect on mortality, body weight, food consumption, heart rate neurological examinations or haematological and clinical chemical parameters. Oral administration of metaflumizone/amitraz spot-on did induce signs of avoidance behaviour, including licking, spitting, head shaking and salivation in all animals. Decreased activity, a cold skin and pale gums were also observed. The adverse effects that were observed after metaflumizone /amitraz treatment are likely to be caused by amitraz. In the treatment group heart rates were lower for all animals, indicating some effect of metaflumizone /amitraz on this parameter as well. Considering the avoidance behaviour it is not likely that animals will lick up the product. But even if the spot-on formulation is ingested, signs are mild and transient.

Evaluation of the toxicity in Chihuahua breed dogs of an insecticidal spot on for dogs containing metaflumizone and amitraz. A 15% metaflumizone /15% amitraz spot-on formulation administered at a dose of 0.67 mL to Chihuahua dogs, with body weights between 0.5 and 2 kg, was well tolerated and safe, when used as recommended. The smallest packaging, for a 5 kg animal, was used, but because of the relatively low body weight of the animals an overdose was produced. The reason for choosing the Chihuahua dog is the fact that this breed has been reported to be relatively sensitive to amitraz intoxication. Pruritis, localised as well as generalised, was observed in all treated dogs, but disappeared in most dogs after 8 hours. It is observed that results for haematology and clinical chemistry between Beagle dogs and Chihuahuas are not consistent. It is likely that study conditions may have affected the results.

Resistance

Metaflumizone.
Metaflumizone is a novel active substance in veterinary medicine, with no previous exposure of fleas to the substance and a mode of action that differs from the currently available active substances used for the control of fleas in dogs and cats.

A substance related to metaflumizone, indoxacarb (DPX-MP062, DuPont) has been used in agriculture for many years. Mild cross resistance with pyrethroids has been observed in tobacco budworm,

diamondback moth and Colorado potato beetle. On the other hand, piperonyl butoxide enhances the activity of indoxacarb against resistant insects.

No cross resistance has been detected between metaflumizone and indoxacarb in tobacco budworm, diamondback moth and Colorado potato beetle. Despite a similar mode of action, by blocking neuronal sodium channels, a difference in receptor affinity is presumed.

As a novel active substance metaflumizone would be expected to be fully effective against fleas in cats and dogs.

As fleas have not been exposed to metaflumizone before, induced resistance is not likely to be present. Any resistance that would be observed would then be the consequence of intrinsic resistance. However, no data on resistance have been submitted. All flea strains used in studies appear to be susceptible.

The availability of a new insecticidal substance with a mode of action that is different from already marketed substances, can be beneficial.

Amitraz.
Amitraz has been authorized for use as an acaricide in both food producing and companion animals for many years. Although the exact mode of action is not known, amitraz is classified as an inhibitor of monoamine oxidase, which is responsible for the degradation of the neurotransmitters norepinephrine and serotonin. The mode of action probably also involves interaction with octopaminergic receptors in the tick nervous system, causing an increase in neural activity.

For tick control in the dog amitraz is used as collars and spot on; for mite control (*Demodex*) it is used as a wash or spray.

While resistance has been reported in ticks on cattle, resistance to amitraz in ticks strains relevant for dogs has not been reported yet, except for 1 publication on the brown dog tick (*Rhipicephalus sanguineus;*Miller *et al*., 2001) in a larval *in vitro* essay. In the field, no resistance to amitraz has been observed for ticks in dogs, but the presence of a natural variability in susceptibility to amitraz cannot be excluded.

In arthropods amitraz is considered to be active primarily by interfering with the octopaminergic receptors in the nerve system. The inhibition of monoamine oxidase is considered to be of less importance, but remains effective.

The Applicant's information on resistance to amitraz is based on a literature search, revealing only two publications on the same topic. Although amitraz has been used for many years as an acaricide to control tick infestations in dogs, no resistance has been reported after use in dogs under field conditions.

Considering the different modes of action, the combination of metaflumizone and amitraz is not likely to show interference that could enhance the induction of resistance. Cross resistance with other acaricidal and insecticidal substances has not been reported.

The combination of metaflumizone and amitraz is considered to provide effective control in dogs of fleas and ticks respectively.

Conclusion on the Preclinical Part

The Applicant has not reported a conclusion on the preclinical part.
Considering the submitted data, it can be concluded that:

- Information on the mode of action of both substances has been submitted;
- Metaflumizone has insecticidal properties by blocking sodium channels in the insect nerve system;

- Amitraz has acaricidal properties by acting on the octopaminergic nerve system of arthropods;
- The proposed use is in agreement with the modes of action;
- When applied to the skin absorption of metaflumizone and amitraz is negligible, with plasma levels almost always below the LOQ;
- Levels of both substances on the skin persist for 56 days above the LOQ;
- When used in combination, efficacies of both substances remain similar when used as singles or in combination. Interaction between amitraz and metaflumizone appears to be absent.

Reference can be made to the clinical studies for dose finding.

CLINICAL STUDIES

Laboratory trials

Dose-confirmation
Three laboratory studies were carried out to confirm the efficacy of the combination of metaflumizone/amitraz against fleas and a number of tick species in dogs, when used at the proposed dose rate. A 4th study concerned efficacy against ticks only. Two further studies were conducted in the EU (Germany and Ireland) to confirm efficacy against European flea and tick strains. In another study persistent activity was evaluated. Studies to evaluate the effects of repeated treatment, water immersion, shampooing and outdoor exposure on the efficacy against ticks and fleas were also carried out.

Dose confirmation of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas and ticks on dogs
The aim of the study was to evaluate the efficacy of 3 dose levels of a metafalumizone/amitraz combination in a spot-on formulation against cat fleas (*Ctenocephalides felis*) and brown dog ticks (*Rhipicephalus sanguineus*) on dogs. The product was applied at 3 dose rates (0.5x, 1x and 2x the proposed minimum commercial dose of 20 mg metaflumizone and 20 mg amitraz/kg BW) in comparison with vehicle-treated controls (at 1x volume) and positive controls. Efficacy was evaluated against existing infestations and weekly post treatment challenges. All non-treated animals maintained ticks throughout the study. Mean counts were similar for vehicle-treated and non-treated animals, except for D14. No adverse effects were noted that could be related to treatment. Results indicate that treatment with metaflumizone /amitraz spot on at the recommended dose rate of 20 mg metaflumizone/kg BW and 20 mg amitraz/kg BW will control existing flea and tick infestations and provide a residual effect for at least one month after treatment.

Efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas and ticks on dogs
The aim of the study was to evaluate the efficacy of a spot-on formulation of metaflumizone in combination with amitraz against European strains of fleas (*Ctenocephalides felis*) and ticks (*Ixodes ricinus*) on dogs at the proposed commercial dose rate of greater that or equal to 20 mg each active substance/kg bodyweight. Efficacy against existing infestations and weekly post-treatment challenges was evaluated. The results of this study showed that metaflumizone/amitraz spot-on at the proposed dose of 20 mg active substance each/kg BW was >99% effective against an existing flea infestation on dogs and provided > 95% protection against subsequent flea challenge for 6 weeks post treatment. It was also 97.4% effective against an existing tick infestation and provided > 90% protection for 5 weeks. The product was safe for use on dogs.

Efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas and ticks on dogs
The aim of the study was to evaluate the efficacy of a spot-on formulation of metaflumizone in combination with amitraz against European strains of fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus*) on dogs at the proposed commercial dose rate of greater than or equal to 20 mg/kg bodyweight. Efficacy against existing infestations and weekly post-treatment challenges was evaluated. No abnormalities were observed at the site of the spot-on application at any time point for any animal. Cosmetic effects, like spiking, clumping, greasy appearance and deposits, were observed

in all animals from both groups. In the placebo treated group effects had resolved by D7. In the metaflumizone/amitraz treated group cosmetic effects had resolved in 25% of the animals by D7, 50% of the animals by D14 and all animals by D21. No abnormal general health observations were noted. The results of this study shows that metaflumizone/amitraz spot-on at the proposed dose of 20 mg active substance each/kg BW was >98% effective against an existing flea infestation on dogs and provided > 95% protection against subsequent flea challenge for 5 weeks post treatment. It was also 95.3% effective against an existing tick infestation and provided > 90% protection for 3 weeks. The treatment of animals with metaflumizone/amitraz spot-on is appropriate and effective for the protection of dogs against infestation with *Rhipicephalus sanguineus* ticks and adult *Ctenocephalides felis* fleas for up to 43 days. The treatment was well tolerated and had no effect locally or systemically.

Efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas (*Ctenocephalides felis)* at various times after infestation on dogs

The aim of the study was to evaluate the efficacy of a metflumizone/amitraz combination in a spot-on formulation against fleas on dogs at various times after post treatment infestations. The product was applied at the commercial use dose in comparison with positive controls and non-treated animals. All non-treated animals maintained infestation throughout the study.

For dogs treated with metaflumizone plus amitraz, fleas kill at various times after treatment was only slight at 8 hours after infestation, but markedly higher at 24 and 48 hours after infestation. Differences in flea control between the 24 and 48 hours assessments were most marked for the later infestations. Notably control increased from 90 to >99% for D42 infestation and from 80 to 97% for D49 infestation. For both control products treatments resulted in >95% reduction at 8 hours post dosing, based on geometric mean flea counts. All treatments resulted in at least 8 weeks flea control at a 95% level when assessed at 48 hours after infestation. The results clearly demonstrate the differences in modes of action between the substances. The relatively longer interval between exposure and lethal effect for the metaflumizone/amitraz formulation is obvious and can be explained by the mode of action of metaflumizone. For metaflumizone efficacy at an adequate level is maintained for over a 5-6 weeks period.

Efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas and ticks on dogs

The aim of the study was to evaluate the efficacy of a metaflumizone/amitraz combination in a spot-on formulation against fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus* and *Dermacentor variabilis)* on dogs. The product was applied at the proposed commercial dose rate in comparison with vehicle-treated controls and positive controls. Efficacy was evaluated against existing infestations and weekly post treatment challenges. All non-treated animals maintained flea and tick infestations throughout the study. There was no significant difference between non-treated and vehicle placebo-treated animals, although geometric mean flea counts were higher for placebo-treated animals. For ticks geometric mean counts were lower for placebo-treated controls, compared to non-treated animals on D1, 2 and 7. Treatment of dogs with a single dose of a metaflumizone/amitraz spot-on at the proposed commercial dose resulted in the control of existing flea and tick infestations within 24 hours after treatment. The treatment provided >90% control of infestations, within 48 hours, of both tick species for up to 3 weeks and >95% control of fleas for up to 4 weeks. Reported efficacy results were based on geometric means.

Efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas and Deer ticks on dogs

The aim of the study was to evaluate the efficacy of a metaflumizone /amitraz combination in a spot-on formulation against fleas (*Ctenocephalides felis*) and deer ticks (*Ixodes scapularis*) on dogs in comparison with non-treated controls. Efficacy was evaluated against existing infestations and weekly post-treatment challenges. All non-treated animals maintained flea and tick infestations throughout the study. Treatment of dogs with metaflumizone/amitraz spot-on provided at least 4 weeks control (>90%) of fleas and at least 4 weeks (>80%) control of Deer ticks. Reported efficacy results were based on geometric means.

The applied doses were almost twice those recommended as minimum doses. *Ixodes scapularis* is not relevant for the EU.

Efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus* and *Dermacentor variabilis*) on dogs

The aim of the study was to evaluate the efficacy of a metaflumizone/amitraz combination in a spot-on formulation against fleas and ticks on dogs in comparison with non-treated controls. Efficacy was evaluated against existing infestations and weekly post-treatment challenges. All non-treated animals maintained flea and tick infestations throughout the study. Treatment of dogs with a single dose of metaflumizone /amitraz spot-on at the proposed dose resulted in 100% control of existing flea, brown dog tick and American dog tick infestations within 48 hours after treatment. The treatment provided >90% control of fleas for 5 weeks, > 88% control for 4 weeks of brown dog ticks and >89% control for 4 weeks of American dog ticks. No adverse reactions to treatment were observed. Reported efficacy results were based on geometric means.

Efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz against Lone star ticks in dogs

The aim of the study was to evaluate the efficacy of a metaflumizone/amitraz combination in a spot-on formulation against Lone Star ticks (*Amblyomma americanum*) on dogs. All non-treated animals maintained flea and tick infestations throughout the study. Treatment of dogs with a metaflumizone/amitraz spot-on combination at the proposed commercial dose rate will control existing Lone Star tick infestations and should provide up to 5 weeks of residual control. There were no adverse reactions to treatment. Reported efficacy results were based on geometric means. The number of tick-free animals per examination was low. Most animals harboured some ticks. The Lone Star tick is not relevant for the EU.

Efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas and ticks on dogs exposed to outdoor conditions

The aim of the study was to evaluate the efficacy of an metaflumizone/amitraz combination in a spot-on formulation against fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus*) on dogs that were exposed to ambient outdoor conditions for approximately 2 hours per day. The product was applied at the commercial use dose in comparison with non-treated controls. Efficacy was evaluated against existing infestations and weekly post-treatment challenges. 12% of the animals from the treated group vomited, but this was not considered to be related to treatment. All non-treated animals maintained flea and tick infestations throughout the study. A single treatment with a metaflumizone/amitraz spot-on formulation of dogs, exposed to ambient outdoor conditions for about 2 hours each day, resulted in control of existing flea and tick infestations. The treatment provided at least 4 weeks control (>90%) of fleas and 5 weeks of ticks. There were no adverse reactions to treatment. The study design does not allow to indicate the outdoor conditions as source for variation, which could have been caused by the infestation and/or the animals as well, e.g. regarding the count and reduction for D28. It would have been conceivable to compare outdoor conditions to indoor conditions or to compare substance hair level profiles and to take into account *in vitro* stability data. Now the Applicant compares the efficacies to a standard (>90% based on geometric means). Reported efficacy results were based on geometric means. In general the results are in agreement with the other studies, but there was a tendency for a 1 week shorter protection period outdoors. Climatological data (Stillwater, Oklahoma; August-October 2003) are submitted but the Applicant apparently did not evaluate them.

Effects of shampooing or water immersion on the efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz against fleas and ticks on dogs.

The aim of the study was to evaluate the efficacy of a metaflumizone/amitraz combination in a spot-on formulation against fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus*) on dogs following weekly simulated exposure to rain and swimming of after shampooing at 14 days post treatment. The product was applied at the commercial us dose and compared with non-treated controls. Efficacy was evaluated against existing infestations and weekly post treatment challenges. Despite some minor ailments, dogs were in good condition. All non-treated animals maintained flea and tick infestations throughout the study, indicating that immersion in water or shampooing on D14 had little

effect on the flea and tick holding ability. For dogs, subjected to water immersions on D2, 9, 16 and 23, the metaflumizone/amitraz treatment resulted in significant lower flea and tick counts relative to non-treated controls. Exposure to water by swimming or rainfall is unlikely to affect the flea and tick control provided by metaflumizone/amitraz spot-on. Similarly, shampooing about 2 weeks after treatment will not markedly affect flea control. However, shampooing may reduce the efficacy of the product against ticks. There were no adverse reactions to treatment. Reported efficacy results were based on geometric means.

Evaluation of repeat applications of a topically applied spot-on formulation of metaflumizone combined with amitraz on dogs.
The aim of the study was to examine the general safety and efficacy of repeated monthly applications of a metaflumizone/amitraz combination in a spot-on formulation for flea (*Ctenocephalides felis*) and tick (*Rhipicephalus sanguineus*) control on dogs. The test substance was applied for 4 monthly treatments at the commercial use dose, and compared with non-treated controls. Efficacy was evaluated against flea and tick challenges at 4 weeks after each treatment. Occasional diarrhoea and emesis were observed on both groups. No skin irritation. All non-treated animals maintained flea and tick infestations throughout the study period. Treatment of adult dogs with metaflumizone/amitraz spot-on for 4 consecutive monthly treatments did not result in any skin irritation and had no effect on the hair coat. Treatments provided excellent control of both fleas and ticks (>95%) on dogs when efficacy was assessed at 4 weeks after each treatment. Reported efficacy results were based on geometric means. Random selection of dogs from group B for reinfestation resulted in 25% of the dogs being reinfested only once, 42% of the dogs twice and 25% of the dogs 3 times. Infestation frequency did not affect efficacy.

Comparative study on the therapeutic and residual efficacy of metaflumizone/amitraz spot on and a comparator reference product against *Haemaphysalis leachi* on dogs.
The aim of the study was to assess and compare the therapeutic and residual efficacy of 15% m/v metaflumizone/15% m/v amitraz spot on for dogs with the comparator reference product when applied as a single treatment against artificial infestations of *Haemaphysalis leachi* ticks on dogs. Therapeutic efficacy, determined at D2, was about 62% for both products. Residual efficacy was >90% up to 42 days for metaflumizone/amitraz spot-on. No adverse effects from metaflumizone/amitraz spot-on treatment were observed.

Supplementary studies were carried out to evaluate efficacy against lice (*Trichodectes canis*) and demodicosis (caused by *Demodex* spp.) and to further evaluate efficacy against ticks. These are summarised as follows.

Efficacy of ProMeris Duo compared to a fipronil containing spot-on and a no treatment control against natural infestations of lice in dogs
The aim of the study was to evaluate the efficacy of ProMeris Duo when applied topically as a spot-on against natural lice infestations compared with a fipronil containing spot-on. Efficacy was assessed on the basis of a comparison of geometric mean lice counts between the treated groups and control group. Lice counts decreased to zero in 25% of the untreated controls, but at least 75% of the animals were infested at each time point. All treated animals had significantly fewer lice, compared to the untreated controls. Both ProMeris Duo and the fipronil containing product effectively controlled lice infestation, with a 100% efficacy at D14 until study ending (D35).

Study on the efficacy of a metaflumizone / amitraz spot-on in the treatment of generalized demodicosis in dogs
The aim of the study was to evaluate the efficacy of three monthly treatments or six treatments at 14 day intervals with metaflumizone/amitraz spot on (ProMeris Duo for dogs) at the proposed minimum commercial dose against *Demodex* mites in naturally infested dogs, suffering form generalized demodicosis. The dogs were allocated to two groups. Each dog served as its own control. Two different treatment regimens were compared for their clinical efficacy. Observations were made at D-1, 28, 56 and 84. Treatment resulted in a rapid reduction in clinical signs and mite counts. For the 28-day regimen (Group 1) mite counts were reduced by 97, 94 and 98% respectively. For the 14-day treatment regimen (Group 2) reductions were >99%. Success rate in terms of mite-free dogs at the end

of the study were 43% and 62% for the 28-day and 14-day treatment regimens respectively. The monthly treatment regimen resulted in a >95% reduction in mite numbers and 43% of the dogs were free of mites at D84.

Evaluation of ProMeris Duo for dogs for the control of demodectic mange in naturally infected animals showing clinical signs of mange in Ohio

The aim of the study was to confirm the efficacy of ProMeris for dogs under clinical use conditions in these animals showing clinical signs of demodicosis. Physical examination was carried out on D0. Skin scrapings were taken from at least 5 areas. Animals were sampled, treated and examined every 28 days, with D168 being the last day. Clinical signs of demodicosis were scored, as an inclusion criterion and for the assessment of treatment efficacy after inclusion. Scores were added to obtain a *Demodex*-induced skin lesion score of 0-21. Efficacy was based on the comparison of geometric means of pre-treatment and post-treatment values for mite counts and lesion scores. Dogs served as their own controls. Treatment reduced mite counts and skin lesion scores. However, 22% of the dogs showed increased mite counts at D84. 64% of the dogs achieved clinical cure, 55% of dogs on D56, 33% of dogs on D112 and 9% of dogs on D140.

Efficacy of a single dose of a topically applied spot-on formulation of metaflumizone combined with amitraz to repel, prevent attachment and kill *Rhipicephalus sanguineus*

Dogs (5.9-9.8 kg body weight; > 6 months old) were treated on D0 as follows: Group A: non-treated; Group B: metaflumizone-amitraz formulation (ProMeris Duo); Group C: fipronil-methoprene formulation Group D: imidacloprid-permethrin formulation. All treatments were according to label instructions. On D7, 14, 21 and 28 each animal was infested with 50 unfed adult *R. sanguineus* ticks. Dogs were examined for ticks at 4, 24 and 48 hours after infestation. Attached ticks were counted. Ticks were removed after the 48-hours examination. Detached ticks were collected and observed for effects of short term acaricide exposure. The number of unattached ticks recovered from Group B and group C animals were similar to those in controls. The number of unattached ticks recovered from Group D was considerably higher, indicating the higher repellence of this product, probably due to permethrin. Little mortality was observed in unattached ticks, except for Group D where mortality in such ticks was high (80-100%). Post treatment geometric mean tick counts for non-treated controls varied from 14.7 to 25.7, with little to no tick mortality. All treatments resulted in lower attached tick counts, compared to untreated controls, but the imidacloprid-permethrin formulation was the only treatment that provided significant reductions at all observations. The level of control for ProMeris Duo was at least > 97% by 48 hours after infestation for at least 3 weeks and 82% by 48 hours at 4 weeks.

Efficacy of a topically applied spot-on formulation of metaflumizone combined with amitraz to repel, prevent attachment and kill American dog ticks *(Dermacentor variabilis)*.

Dogs were infested each with 50 adult *Dermacentor variabilis* ticks on D-2. Dogs were treated on D0 as follows: Group A: non-treated; Group B: metaflumizone-amitraz formulation (ProMeris Duo); Group C: fipronil-methoprene formulation; Group D: imidacloprid-permethrin formulation. All treatments were according to label instructions. Tick counts were carried out about 4 and 24 hours after treatment and at 48 hours, when ticks were removed. Attached ticks were counted. Detached ticks were collected and observed for effects of short term acaricide exposure. Subsequently dogs were re-infested weekly with 50 adult ticks on D7, 14, 21, 28, 35, 42 and 49. An additional tick infestation was carried out on D55 for the groups B and C. The metaflumizone-amitraz formulation provided > 90% control within 24 hours, the fipronil-methoprene formulation after 48 hours, while the imidacloprid-permethrin formulation achieved 87%. All 3 products provided > 90% control after 48 hours for at least 5 weeks.

Efficacy of a single dose of a topically applied spot-on formulation of metaflumizone combined with amitraz to repel, prevent attachment and kill *Amblyomma americanum* and *Dermacentor variabilis*

Dogs (> 6 months of age; 7.5-15.9 kg body weight) were allocated to 4 groups. Groups were on D0 treated as follows: Group A: non-treated; Group B: metaflumizone-amitraz formulation (ProMeris Duo); Group C: fipronil-methoprene formulation; Group D: imidacloprid-permethrin formulation. All

treatments were according to label instructions. Animals were infested with 50 unfed adult ticks of each species on D7, 14, 21 and 28. Tick counts were carried out about 4 and 24 hours after treatment and at 48 hours, when ticks were removed. Attached ticks were counted. Detached ticks were collected and observed for effects of short term acaricide exposure.

A. americanum

All treatments (Groups B-D) resulted in lower geometric mean live attached *A. americanum* tick counts at 4, 24 and 48 hours on D7, 14 and 21. The imidacloprid-permethrin formulation (Group D) was the only treatment that provided significant reductions at 4 hours after infestation on D7 and 14. All 3 treatments provided > 92% control within 48 hours after infestation on D7, 14 and 21. Following the D28 infestation, only the metaflumizone-amitraz formulation (Group B) and the imidacloprid-permethrin formulation (Group D) produced significant reductions in geometric mean tick counts within 24 hours. The numbers of detached ticks were similar for all groups, indicating little repellence at D28.

D. variabilis

All 3 treatments provided reduced tick counts at 24 and 48 hours after infestation, compared to non-treated controls. The metaflumizone-amitraz formulation (Group B) and the imidacloprid-permethrin formulation (Group D) produced significant reductions in tick counts at 4 hours after infestation on D7, but only the imidacloprid-permethrin formulation (Group D) produced significant reductions at D14 and 21 at 4 hours. The control provided by the metaflumizone-amitraz formulation (Group B) ranged from 81-100% up to D28 within 24 hours. For the fipronil-methoprene formulation (Group C) the range was 19-96% and for the imidacloprid-permethrin formulation (Group D) this was 79-92%.

All 3 formulations reduced tick numbers, but with differences in time required to control reinfestation as well as differences in the level of control in relation to the persistence in effect of a single treatment. Results also show a different pattern in response for *A. americanum* and *D. variabilis* to the various active substances.

Compared efficacy of a single dose of topically applied spot-on products to repel, prevent attachment and kill *Ixodes scapularis*

Dogs (> 6 months; 8.5-16.7 kg body weight) were allocated to one of 4 groups and treated on D0 as follows: Group A: non-treated; Group B: metaflumizone-amitraz formulation (ProMeris Duo); Group C: fipronil-methoprene formulation; Group D: imidacloprid-permethrin formulation. All treatments were according to label instructions. On D7, 14, 21 and 28 each dog was re-infested with 50 adult *Ixodes scapularis* ticks. At 3-4 hours and at 12, 24 and 48 hours after infestation, dogs were examined for live attached, unattached and dead ticks. Ticks were removed at 48 hours after infestation. Only live attached tick was incorporated in the results for statistical analysis. Geometric mean tick counts for non-treated dogs (Group A) ranged from 10.7 to 20.3. Relative reductions in geometric mean tick counts for dogs treated with the metaflumizone-amitraz formulation (Group B) were > 90% after 24-48 hours up to D21, but only 80% at D30 (48 hours after D28 infestation). Both the fipronil-methoprene and the imidacloprid-permethrin formulations produced reduction > 90% for the whole study period.

For ProMeris Duo the following results were found:

Tick species	short term efficacy	persistent efficacy
Rhipicephalus sanguineus	> 90% RR* after 48 hours	> 90% for 3-5 weeks
Dermacentor variabilis	> 90% RR after 24 hours	> 90% for 4-5 weeks
Amblyomma americanum	> 90% RR after 48 hours	> 90% for 3 weeks
Ixodes scapularis	> 90% RR after 24-48 hours	> 90% for 3 weeks

* Relative Reduction. All reductions in tick counts were relative to numbers recorded on non-treated animals and calculated on the basis of geometric means per group.

Field trials

A field trial was conducted to confirm the efficacy and safety of the combination of amitraz and metaflumizone.

The efficacy and persistency of 15% metaflumizone/15% amitraz as a spot-on formulation in dogs, naturally infested with fleas and/or ticks was evaluated in a multicentric clinical field study in the EU.

442 dogs (203 males, 239 females) of various breeds, 10 weeks to 14 years of age and weighing 2.1-79 kg, were enrolled as flea and tick patients and randomly assigned to the IVP (investigational veterinary product) treatment group or the comparator-treated control group in a ratio of approximately 2:1. Dogs with concurrent flea and tick infestations were considered as flea household dogs. Animals holding ticks and animals holding fleas were randomised separately. Dogs in the same household were treated with the same product as the primary patient and included in the safety evaluation. Cats in the same household were treated with a commercially available product, but not monitored during the study.

Multi-centre randomised, blinded, controlled, non-inferiority field study
Trial locations: Germany, 19 veterinary clinics in 4 different areas; France, 11 veterinary clinics in 3 areas. The "household" was the experimental unit for flea efficacy. Only one dog per household was included in the evaluation. For tick efficacy evaluation, the dog was considered the experimental unit and up to 3 dogs per household could be included.

Inclusion criteria: fleas: >5 fleas on D0; ticks: >3 viable ticks on D0;
Exclusion criteria: pregnant; planned for mating within 2 months after treatment; lactating, nursed. Dogs with know hypersensitivity to spot-on treatment; dogs with disorders, other than tick/flea infestations. Dogs washed 48 hours before treatment. Prior to treatment on D0 a clinical examination was carried out. Observations were made on D14 ($\pm$2), 28 ($\pm$2), 42 ($\pm$2) and 56 ($\pm$2).At each visit the animals were combed for flea and tick counts and the parasites collected. At each visit animal owners were questioned if any adverse events had occurred. Persons doing the efficacy and safety evaluation were blinded to treatments. 15% metaflumizone/15% amitraz spot-on was tested for non-inferiority to the comparator reference product, based on the percentage of animals cured (defined as no ticks or fleas) and on geometric mean flea/tick counts at each visit, using a Chi-square test. A Chi-square test for superiority of the IVP was also conducted for the percentage of animals cured in the tick efficacy population.

Efficacy was calculated for each observation day, using Abbot's formula. The duration of the efficacy was percentage reductions in flea and tick counts.

IVP: 15% w/v metaflumizone/15% w/v amitraz spot-on. Recommended treatment dose is a minimum of 20 mg/kg of each active substance. Dosing: $\leq$ 5.2 kg, 0.7 ml; 5.2-10.5 kg, 1.4 ml; 10.6-25.5 kg, 3.4 ml; 25.6-40.5 kg, 5.4 ml; 40.6-51 kg, 6.8 ml; >51 kg, 6.8 ml + the appropriate smaller volume size.

Dosing for the comparator reference product was carried out as recommended by the manufacturer. Both products were applied once as a single spot-on treatment, on the skin between the shoulder blades.

Collected ticks were sent to the laboratory for species determination. A number of dogs (9 from the tick-group/ 2 from the flea-group) were excluded, due to protocol violations (e.g. shampooing, removing of ticks).

At D0 a total of 181 dogs were included to study the efficacy against tick infestations. A total of 173 dogs were included to study the efficacy against flea infestations, with 3 dogs in for ticks and fleas. With respect to tick control, metaflumizone/amitraz spot on was non-inferior to the comparator reference product for all observation days and superior at D 14 and 28. For metaflumizone/amitraz spot-on reductions in geometric mean tick numbers and compared to D0 values were >90% on D14, 28 and 56; it was 89% on D42. Efficacy against *Rhipicephalus* spp. was somewhat higher (97.3 -

99.7%), compared to that against *Ixodes* spp. (96.5 - 83.7%). On all observation days reductions for the comparator reference product were <90%. For flea control, metaflumizone/amitraz spot on was inferior to the comparator reference product for all observation days. For metaflumizone/amitraz spot-on reductions in geometric mean flea numbers and compared to D0 values were 88.1%, 85.1%, 87.6% and 86.3% for D14, 28, 42 and 56 respectively. For the comparator reference product, reductions were 97.0%, 94.3%, 93.1% and 92.9% respectively. Efficacy against fleas was notably lower for 2 veterinary clinics, located in France, compared to similar sized centres. The investigators reported very high flea challenges in summer/autumn 2004. Excluding the patients from these 2 centres flea count reductions were as follows: for metaflumizone/amitraz spot-on 91.8%, 88.7%, 91.5% and 92.0% for D14, 28, 42 and 56 respectively. For the comparator reference product reductions were 98.2%, 96.3%, 95.9% and 96.7% respectively.
Adverse events were reported in 12 animals form the metaflumizone/amitraz group. Two animals (0.68%) showed minor application site reactions. Ten animals were presented with adverse events that were not related to the treatment.

Based on the number of cured tick patients and tick numbers metaflumizone/amitraz spot-on was non-inferior to the comparator reference product on at all post treatment observations and superior at D14 and 28. Based on the number of cured flea patients and flea numbers metaflumizone/amitraz spot-on was inferior to the comparator reference product on at all post treatment observations. Metaflumizone/amitraz spot-on was safe in the treatment of dogs infested with ticks and/or fleas.

Multicentric field efficacy trial of efficacy of a metaflumizone/amitraz sport-on against demodicosis in dogs in Europe
The aim was to evaluate the efficacy and safety of the combination of metaflumizone (15% w/v) and amitraz (15% w/v) (ProMeris Duo spot-on) administered at a dose of $\geq$ 20 mg metaflumizone/kg bodyweight and $\geq$ 20 mg amitraz/kg bodyweight for the treatment of generalized demodicosis in dogs presented as veterinary patients in European veterinary practices. Dogs were randomly assigned to one of 2 treatment groups (ProMeris Duo and other authorised product with similar indication). Treatment was administered as a spot-on at 28-day intervals. Dogs were examined at D0 and subsequently another 7 times every 4 weeks (D28, 56, 84, 112, 140, 168 and 196). Skin scrapings were taken every 28 days. Skin lesions (clinical signs) were assessed for erythema, comedones, hyperkeratosis, seborrhoea, scales, alopecia, lesions of deep or superficial pyoderma. Scores were added to calculate a "*Demodex*-induced skin lesion score" (DSLS). Change in clinical signs was evaluated. Animals were treated every 4 weeks until showing 2 series of negative skin scrapings at 4-week intervals after the last treatment. If so, the animal was regarded as parasitologically cured. A 90% reduction from baseline in mite numbers was reached at D56 fro ProMeris Duo. For the comparator product the 90% level was reached at D84. The final parasitological cure rate (D168) was 89% for dogs treated with ProMeris Duo and 75% for dogs treated with the comparator product. Treatment produced a decrease in DSLS for both products. No cure was achieved in 4.5% of animals in the ProMeris group and in 10% of animal for the comparator product group. Adverse events were reported in some animals, but none were considered as treatment-related.

Conclusion on the Clinical Part

A series of studies was conducted that provide an effective profile of a metaflumizone/amitraz spot-on formulation for dogs. Data indicate that the product is well tolerated and effective when used according to the recommended dose range

The studies submitted by the applicant have been well designed and conducted. Studies cover nearly all aspects of the product on safely and efficacy; the submitted information is quite complete.

All studies were carried out according to similar or comparable protocols and as a randomised complete block design. Statistics were based on log-transformed data and ANOVA, using PROC MIXED procedure (SAS 8.2). Studies were carried out according to GLP.

Adequate infestation levels were induced and maintained throughout. Compared to field conditions,

parasite levels were relatively high.

It was observed that reproducibility of efficacy over dogs was high. No dogs appeared as being left untreated.

%Efficacy was calculated as: (geometric mean control geometric mean treated) / geometric mean control x100. Geometric mean + 10^{aml}-1 (aml = arithmetic mean of log transformed counts).

Fleas and ticks were usually from the same supplier.

Dose titration resulted in a minimum dose rate of 20 mg/kg for each substance for adequate efficacy. Based on geometric means and a minimum level for efficacy of at least 95% reduction for fleas and 90% for ticks, persistent efficacy is about 4-6 weeks for Ctenocephalides felis fleas and 3-5 weeks for *Ixodes ricinus* and *Rhipicephalus sanguineus* ticks.

Shampooing of dogs after treatment reduces the efficacy of metaflumizone/amitraz spot-on against ticks, but not on fleas.

In the experimental preclinical studies dosing was as follows: ≤ 4.5 kg, 0.6 ml; 4.6-10.5 kg, 1.4 ml; 10.6-25.5 kg, 3.4 ml; 25.6-60 kg, 8.0 ml.

It was observed that field trial dosing was as follows: ≤ 5.2 kg, 0.7 ml; 5.2-10.5 kg, 1.4 ml; 10.6-25.5 kg, 3.4 ml; 25.6-40.5 kg, 5.4 ml; 40.6-51 kg, 6.8 ml; >51 kg, 6.8 ml + the appropriate smaller volume size. Differences could have been the consequence of another standard in USA-studies, but are not considered relevant as long as a minimum dose of 20 mg of each active substance

The proposed weight bands in relation to pipette sizes are, therefore, considered justified.
The field trial was well designed and conducted. The efficacy of metaflumizone/amitraz spot on against flea infestation was consistently less than that of its comparator reference product. The reason for this was the larger number of flea free animals for the comparator reference product but with some animals still having high numbers of fleas. In the metaflumizone/amitraz spot-on group there were more animals still harbouring some fleas but not many with high numbers. The efficacy of the metaflumizone/amitraz spot-on formulation against ticks was superior to that of the comparator reference product.

Taking the efficacy results from the repeated treatment studies into account, it is likely that a high level of ectoparasite control can be achieved by repeating the treatment.

Overall Conclusions and Benefit Risk Assessment

ProMeris Duo Spot-On for Dogs is a product containing amitraz (14.34% w/w) and metaflumizone (14.34% w/w) in a non-aqueous solution, designed as a topically applied treatment to control fleas and ticks on dogs. The product is prepared as a ready to use liquid in single use pipettes in five different filling volumes (0.67, 1.33, 3.33, 5.33 and 6.66 ml) to cover the recommended minimum dose of 20 mg/kg b.w. of both amitraz and metaflumizone to dogs.

Amitraz is described in the British Pharmacopoeia (Veterinary). Metaflumizone is not described in any Pharmacopoeia (Ph.Eur., USP or JP). Information provided in the dossier is consistent to justify the quality of batches of amitraz and batches of metaflumizone.

Adequate validation of methods used to control the active substance is submitted. Excipients used in the manufacture of the product are considered quite common for use in a spot-on and their quality specifications have been sufficiently laid down.

The method of preparation is detailed for the bulk solution and the approximate number of pipettes of each size intended to be produced out of the bulk solution is laid down.

Primary and secondary packaging has been developed in order to protect the formulation from ingress of moisture. All immediate packaging materials comply with Directive of 2002/72/EC. The package appears to be senior friendly and a study has been provided that sufficiently proved the child resistance characteristics of the package.

The testing monographs for the final product contain specifications and tests for appearance, container/closure integrity, identification, assay, impurities, ratio Z/E of metaflumizone, uniformity of dosage units, water content, density and deliverable mass. Microbiological controls are unnecessary. Determination of the contents of the active substances and their impurities are performed by HPLC. Validations of the methods are enclosed to confirm their suitability.

Stability studies have been performed according to VICH guidelines. The recommended retest periods of 9 months for amitraz and 6 months for metaflumizone, under the recommend storage conditions, are justified. The studies are on-going. Stability studies with the finished product done under adequate forming, filling and sealing operations, justify the proposed shelf-life of 18 months when stored at 25ºC/60%RH. The studies are on-going.

The active substances are synthetic and free of any animal material. The excipients are also of non-animal origin. The product complies with the TSE Note for Guidance (EMEA/410/01 Rev.2.) and Council Directive 2001/82/EC, as amended.

Metaflumizone is a semicarbazone sodium channel blocker insecticide, chemically derived from the pyrazoline family, with the same mode of action. Pyrazolines were reported to have a high insecticidal efficacy, with low mammalian toxicity. Metaflumizone blocks sodium channels by selectively binding to the slow-inactivated state, within the sodium channel pore. The voltage dependent sodium channel blocking action is similar to that of local anaesthetics. In the insect metaflumizone disrupts nerve function, resulting in paralysis.

Metaflumizone is a mixture of E and Z isomers (ratio 9:1). It has not been used before in veterinary medicine. Metaflumizone has no anthelmintic activity. The insecticidal activity occurs primarily after ingestion; it is inactive by contact. Data on the acute toxicity of metaflumizone, administered via various routes, indicated a very low acute toxicity. Repeated administration, however, did affect the physiological functioning of rats and females appeared to be relatively more sensitive to intoxication. A clear mode of action cannot be indicated, but interference with sodium channels is not likely. This was also illustrated by the absence of specific signs of intoxication. However, all subchronic toxicity studies indicate an interference with haematology. Considering the absence of anaemia and the structure of the metaflumizone molecule and its metabolites, it is conceivable that such interference concerns the haem composition.

Furthermore, metaflumizone is not likely to possess mutagenicity potential and the substance can be regarded as non-irritating to the eye and skin. There is no indication of specific embryotoxic/foetotoxic effects or specific toxic effect on reproduction. Observed adverse effects were likely to be due to the general toxicity of metaflumizone after repeated administration. In conclusion, it is not likely that metaflumizone will lead to toxic effects in the dog when used as proposed.

Amitraz is a formamidine acaricide and has been approved for use in both food producing and companion animals for many years. Although the exact mode of action is not known, amitraz is classified as an inhibitor of monoamine oxidase, which is responsible for the degradation of the neurotransmitters norepinephrine and serotonin. The mode of action probably also involves interaction with octopaminergic receptors in the tick nervous system, causing an increase in neural activity.

Amitraz is considered a well known substance. Signs of adverse effects of amitraz and its metabolites are rather specific and depend on alpha$_2$-receptor stimulation. Although the pharmacokinetic data, based on application of the spot-on formulation on the skin, indicated that amitraz levels in plasma were below the LOQ, minimal quantities of amitraz can still be absorbed and may lead to the

occurrence of adverse effects. This is due to the very high sensitivity of alpha$_2$-receptor to specific agonists.

Submitted data indicate that metaflumizone is not readily absorbed after oral administration to rats, whereas amitraz is absorbed when given orally to dogs, but rapidly eliminated. The dermal absorption of both metaflumizone and amitraz is negligible, when topically applied to healthy dog skin. Based on a LOQ of 50 mcg/ml amitraz was generally not detectable in plasma; metaflumizone was detectable in plasma, but not quantifiable. Both metaflumizone and amitraz are well distributed over the dogs body, when topically applied to the skin between the shoulder blades and persist for 56 days in quantifiable levels in the dog hair coat.

Risks were identified in relation to user safety, in particular for the people applying the products and for children in contact with treated animals. Appropriate warning statements were included in the SPC.

Given the proposed use of the product no phase II environmental risk assessment is necessary. A warning statement to prevent access of dogs to streams, ponds and rivers for 24 hours post treatment is included in the product literature.

Five studies were carried out to assess the safety of a metaflumizone/amitraz combination in dogs. Four studies were carried out in Beagle dogs and one study was carried out in Chihuahua dogs. The reason to choose the Chihuahua dog is the fact that this breed has been reported to be relatively sensitive to amitraz intoxication. The metaflumizone/amitraz combination was well tolerated and safe, when used as recommended.

Metaflumizone and amitraz were combined to make a low volume topical spot-on product for the simultaneous control of ticks and fleas in dogs. Studies were carried out to assess the efficacies for each active substance as combination and to determine the dose rates at which the optimal efficacy was achieved. The proposed use of the product is in agreement with the modes of action of the active principles; neither synergy nor interference of activity of metaflumizone by amitraz versus fleas and ticks could be shown.

Supplementary data were submitted concerning the extension of the indication for use with a claim for treatment of demodicosis (caused by *Demodex spp*) and lice infestation (*Trichodectes canis*), which were assessed and were considered justified.

A well designed and conducted field trial was reported. In line with the results of the experimental studies, the efficacy of metaflumizone/amitraz spot on against flea infestation was consistently less than that of its comparator reference product whereas the efficacy against ticks was superior to that of the comparator reference product although such a difference did not appear from the experimental studies. Taking the efficacy results from the repeated treatment studies into account, it is likely that a high level of ectoparasite control can be achieved by repeating the treatment.

The risk benefit assessment can therefore be summarised as follows:

Benefit

- Low toxicity of the substances (for target animal)
- Negligible dermal absorption
- High level of control for tick infestations
- Adequate length of residual efficacy period of up to 6 weeks for fleas and 4 weeks for ticks, making it a suitable combination
- High reproducibility of efficacy over animals
- Simultaneous occurrence of both infestations, justifying the combination
- A large proportion of the target population infested, justifying the combination

Risk

- Possibility of skin sensitisation
- Mild eye irritant
- Relatively lower level of efficacy against fleas, when compared to a product with a similar application, but still above 90% relative reduction.
- User safety, in particular for children in contact with treated animals and users taking Monoamine Oxidase Inhibitor (MOAI)-containing medication

Based on the original and complementary data presented the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of ProMeris Duo were considered to be in accordance with the requirements of Directive 2001/82/EC, as amended.