SCIENTIFIC DISCUSSION

Quality

ProMeris Spot-On for Cats is a product containing metaflumizone (18.43% w/w) in a non-aqueous solution, designed as a topically applied treatment to control fleas on cats. The product is prepared as a ready to use liquid in single use pipettes in two different filling volumes (0.80 and 1.60 ml) to cover the recommended minimum dose of 40 mg/kg b.w. of metaflumizone to cats. The objective of the formulation is to commercialize a cosmetically attractive, topical application of the active substance to the cat. The presentation in two different size pipettes allows control of dose administered according to the size of the animal.

The majority of the clinical trials have been performed with the same formulations as the described in section II.A.1.

Choice of active substances

Metaflumizone is a semicarbazone insecticide acting as a neuronal sodium channel antagonist. It has shown excellent against fleas on cats.

Preliminary work focused on developing a product that could be applied on a small animal. Applying a minimum volume of material was desirable, but with a concentration of active substance high enough to be effective. An effective dose is approximately 40 mg/kg of animal weight.

The goal of the formulation development studies was to identify a solvent system that would provide:

- Ease of application.
- Acceptable volume that would result in good coverage of the animals without run-off of the product.
- Pleasant odour of the product
- No animal irritation
- Low residue
- Good drying characteristics

Information was used from previous work (metaflumizone/amitraz dog spot-on formulation) that focused solvent selection.

The initial formulation was developed using one surfactant. Subsequently, it was discovered that this surfactant was under regulatory scrutiny in the EU. Another surfactant was selected in it's place. Stability studies comparing the two were performed in amber glass vials. Both products retain full potency after 6 and, 9 months.

Antimicrobial preservative: The formulation is non-aqueous and it is thus predictable that it has sufficient antimicrobial properties by itself. So, no antimicrobial agent or preservative is needed and used.

Antioxidant: Preliminary stability evaluations have shown that the formulation does not require an antioxidant for stability.

Overages: During pre-formulation studies and preliminary stability evaluations it was observed that the formulation was sensitive to moisture and also showed a tendency to be hygroscopic. Therefore, the manufacturing process was developed such that the residual moisture in the product was minimized. To maintain the quality during shelf-life, 2% overage of the active substance was included and packaging was developed that would provide a barrier against ingress of water.
According to the Applicant, studies have shown no impact of the 2% overages on safety and efficacy.

According to the stability results, the use of 2% overage for the active substance is considered acceptable.

It is remarked that the product presentation is a single dosage package. Therefore, broaching studies or in-use stability studies are not required.

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 package was found to be acceptable in terms of compatibility (no solvents migration) and water permeation.
The bulk manufacturing process is a simple dissolving operation of the active substance 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 (using glass containers, via preliminary scale-up of 7.5 L (in both cases using glass containers) to a pilot scale of 8-9 L (using various packaging concepts).

During the stability studies involving pilot scale batches, it was found that a secondary packaging made from plasticised aluminium was necessary to protect the product from moisture absorption and subsequent degradation of the active substance. The product stability is directly related to storage temperature. The product shows acceptable stability at lower temperature.

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 substance and mixing to dissolve the active substance 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.

Manufacture takes place at ambient temperature. As metaflumizone is sensitive to water, it is necessary to lower the content of the water in the formulation by means of passage through molecular sieve and to store the batch in inert atmosphere (purging with nitrogen). The product is packaged in unit dose pipettes (primary container) moreover the secondary packaging (aluminium bags and thermoformed blister) provides further protection from moisture.

In-process controls

Regarding the manufacturing process, acceptable in-process controls are performed:

Flow charts are enclosed in the dossier, showing the various steps in the manufacturing process and the filling packaging operation, including in-process controls. The bulk solution manufacturing process and the filling packaging operation are adequately described.

The process is a standard manufacturing process. The manufacturing process of three pilot batches of formulation (FD04102 (8.678 kg), FD04103 (8.677 kg) and FD04104 (9.721 kg)) has been validated in the manufacturing plant Wyeth Lederle Italia S.p.A., Fort Dodge Animal Health Division, Catania, Italy.

Three batches of formulation were prepared and each single batch was used to fill two different sizes of pipettes. The validation procedure consisted of comparing the recorded values during the formulation operations with the set points and process variable parameters, verification of the accuracy of the dosage during the filling of the pipettes for all presentations, and analysis of all three batches for final release.

All tests performed as per the present protocol have been satisfactory. The validation data demonstrate that the in-process controls are within limits and the batches fully comply with the release requirements. From the validation data it can be concluded that the production process is well under control resulting in a product of consistent quality.

It has not been considered necessary to ask for production scale validation data as part of the marketing authorisation dossier, taking into account the nature of the product (a true solution) and the standard method of manufacture.

Primary package: A thermoformed pipette made from a laminated. 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 hygroscopic nature of the solvents and the hydrolytic instability of metaflumizone.

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.

The active substance is 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. Declarations of the manufacturers were included in the dossier.

Batch analyses

Analysis results of the three 8-9 L pilot batches are provided. Each batch was packaged in 2 different presentation sizes. Three certificates of analysis for each presentation are included in the dossier. All samples passed the release criteria.

STABILITY

During the first twelve months of storage at various environmental conditions, the product showed a direct relationship between the storage temperature and product stability.

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 ProMeris Spot-on for cats exhibited excellent physical and chemical stability during the twelve months storage period at 4ºC, 25ºC/60%RH and 30ºC/60%RH. The product also showed very good stability when stored for six months at 40ºC under extreme humidity conditions of 20 and 75%RH. The shelf life was predicted to be 51 months, based on the twelve months stability results.

Regarding the stability results, the proposed shelf-life of 18 months for the product when stored at 25ºC/60%RH is considered acceptable.

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

Safety Assessment (Pharmaco-Toxicological)

ProMeris Spot-On for Cats is a product containing metaflumizone (18.43% w/w) in a non-aqueous solution, designed as a topically applied treatment to control fleas on cats. The product is prepared as a ready to use liquid in single use pipettes in two different filling volumes (0.80 and 1.60 ml) to cover the recommended minimum dose of 40 mg/kg b.w. of metaflumizone to cats.

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 exists 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 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.

Single dose toxicity

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 oral LD_{50} of M320102 (Z-isomer of metaflumizone) in Sprague-Dawley rats is >5000 mg/kg.

The acute dermal toxicity of metaflumizone in Sprague-Dawley rats is >5000 mg/kg.

One study was carried out to establish the toxicity of metaflumizone after inhalation. 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.

Repeated dose toxicity **((RLD Comment – These could be shortened to match the dog))**

28 days / 13 weeks oral toxicity study in albino rats with Metaflumizone. No mortality was observed during the study. No clinical signs were observed that could be attributed to the test substance. One female from the 100 mg/kg-group died during blood collection at D29. In the 4-week groups food consumption and body weight were statistically significant reduced in the 500 and 1000 mg/kg groups. In the 100 mg/kg-group a slight decrease was observed but this was not statistically significant. A slight decrease was observed in total erythrocytes, haemoglobin and hematocrit in the females of the 500 and 1000 mg/kg-groups. A significant increase in AST-levels was seen in females of the 1000 mg/kg-group, Non-significant increases were also seen in 1000 mg/kg-group males and 500 mg/kg-group males and females. Elevated cholesterol levels were seen in females at each dose, but without dose response, and in males at 1000 mg/kg. Liver weights were increased in the 500 and 1000 mg/kg-groups. Gross pathology revealed no treatment related effects. Histology revealed extramedullary hemopoiesis in the spleen and hepatocellular hypertrophy in the 500 and 1000 mg/kg-groups. In the 13-week group a slight decrease was observed in females but this was not statistically significant. After reduction of the dose males did not show any reduction in body weights and food consumption anymore. Haematology and organ weights did not show any effect of treatment. Gross pathology revealed no treatment related effects. 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 sings of intoxication.

M320102 (Z-isomer of metaflumizone) - Subchronic toxicity study in Sprague-Dawley rats; One female from the 1000 mg/kg-group was found dead on D7 and one female from the 300 mg/kg-group was moribund and sacrificed the same day. Clinical signs included a low general condition, decreased motor activity, ataxia, tonic convulsions, piloerection, absence of defecation, wet anogenital area and aberrant head posture. Similar signs were observed now and then in a small number of females from the 300 and 1000 mg/kg-groups. All animals from these groups and 5 males and 3 females from the 100 mg/kg-group showed light brown discoloration of the faeces, but this was considered to be a consequence of substance administration rather than a toxicological effect. Impaired body weight gain was observed in 300 and 1000 mg/kg-group females, but without dose response. Gross pathology did not show clear treatment related abnormalities, but histopathology revealed some increase in extent of vacuolisation of the *zona fasciculata* of the adrenal cortex of 300 and 1000 mg/kg-group females. A slight necrosis of lymphocytes in the paracortex of mesenteric lymph nodes was also observed. Observed differences in organ weight were observed but due to body weight differences. Adrenal weight was increased in 300 and 1000 mg/kg-group females and this could have been related to treatment.Opthalmoscopy revealed no abnormalities. The "functional observational battery" revealed no abnormalities, except for one female from the 1000 mg/kg-group that showed impaired coordination and an unsteady gait. The No Observed Adverse Effect Level of M320102 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.No mortality was observed. No signs of local irritation due to treatment were observed.

Body weight gain was slightly impaired in 300 and 1000 mg/kg-group males and statistically significant impaired in the females form these groups. Clinical chemistry analysis revealed increased plasma sodium, phosphate, calcium and cholesterol levels in 1000 mg/kg-group females. Histopathological examination revealed minimal to slight multifocal hyperplasia was seen in areas of treated skin in both substance-treated and control animals. Some abnormalities were also observed in 300 and 1000 mg/kg-group females, including lymphocyte necrosis/apoptosis in mesenteric lymph

nodes, increased vacuolisation in *zona fasciculata* cells of the adrenal cortex and diffuse atrophy of mesenteric and mandibular lymph nodes. Abnormalities in the spleen were only seen in 1000 mg/kg-groups. Abnormalities tended to be dose-related. 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.

Sub-chronic/chronic oral toxicity study in Beagle dogs: administration via gelatin capsules for 3 and 12 months. During the first 3 months retarded body weight gain was seen in the highest dose-group in 1 male and 3 females, due to impaired food intake. Poor body condition required the sacrifice of 1 male and 2 females on D57. In addition, hematology revealed a decreased mean corpuscular haemoglobin concentration (MCHC), along with hypochromasia. Similar effects were seen in some animals from the 30 mg/kg-group. 2 males and 3 females from the highest dose group had to be sacrificed between 3 and 12 months. No substance related effects were seen in the 6 mg/kg and 12 mg/kg-groups. In the 30 mg/kg and 60/40/30 mg/kg dose-groups impaired food consumption and retarded weight gain were the predominant signs, along with a decrease in MCHC and a slight hypochromasia. Clinical chemistry, organ weights, gross pathology, urinalysis and ophthalmoscopy did not reveal any findings that were considered treatment related. Histopathology findings in those animals sacrificed prematurely revealed atrophy of the thymus, testes and prostate. These findings were considered to be related to the weight loss. 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.

Studies on the effects on reproduction

ProMeris is indicated for use in cats. Since the product is not indicated for breeding animals and the cat 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; Throughout the study parents and pups were observed daily and parental animals for their mating and reproductive behaviour. Poor general state at the highest dose was the major reason for pup mortality poor nursing behaviour, and peri- and post natal mortality. No adverse treatment effects were seen in low and mid-dose animals. Oestrus cycle data, sperm parameter evaluation and gross histological examinations revealed no effects of treatment. 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; No substance related effects on dams or foetuses were seen for doses of 15 and 40 mg/kg/day. At 120 mg/kg body weight was statistically significant decreased form gestation day 6 to 8. It remained relatively lower for the remainder of the study period with corrected body weight about 22% below controls. Gross pathology of dams and foetuses revealed no abnormalities. 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; One dam from the 100 mg/kg-group was sacrificed in a moribund condition and 2 dams were sacrificed following abortion. 4 300 mg/kg-group dams showed general signs of intoxication. At 30 and 100 mg/kg no substance related effects were observed. At 300 mg/kg mean foetal body weight was lower. The incidence of incomplete ossification of sternebrae was also higher in the 300 mg/kg-group. 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

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.

- genotxic 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 effect 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

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 were 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 and the test animals. It was concluded that metaflumizone doe not have a dermal sensitising effect in the Guinea pig.

Microbiological Studies

Metaflumizone is not known to have antimicrobial qualities. The substance is indicated for use in the cat, 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

User safety was adequately addressed in accordance with the User Safety Guideline. Many exposure scenarios were considered and the exposure estimates could be compared to relevant toxicity end points. Several risks for the user have been identified. The highest risk was identified for children who get direct access to the pipettes. This risk was adequately mitigated by the child-resistant packaging. In addition, a risk for children stroking the cat immediately after treatment was identified. The warning to treat cats in the evening and to not allow children to play with treated animals until the application site is dry is considered adequate to mitigate that risk.

Ecotoxicity

Phase I Assessment

Metaflumizone Spot-On for cats will be used for the control of fleas of kittens and adult cats. 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 ingredient is an ectoparasiticide it is recommended to minimize the potential environment impact as much as possible. Unlike dogs, cats do normally not access water. For this reason no safety phrase is recommended.

Conclusion on Part III

Submitted data on the acute toxicity of metaflumizone, administered via various routes, indicate a very low acute toxicity. Only one animal died, but it is not likely that death was caused to the substance.

It is observed that data on the administration of metaflumizone by the parenteral route has not been included. Metaflumizone was administered by the parenteral rout in mutageniticy studies.

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.

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 mutagenicity potential.

In relation to the use in the cat it can be concluded that:

- 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.
- Metaflumizone is not likely to possess mutagenicity potential.
- metaflumizone can be regarded as non-irritating to the eye and skin.
- Results indicate the absence 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.

It is consequently not likely that metaflumizone will lead to toxic effects in the cat when used as proposed.

Furthermore it can be concluded that, given the proposed use of the product, no Phase II environmental risk assessment is necessary.

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)

Pharmacodynamics

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 relatively low contact activity.

Metaflumizone was tested for its nematocidal and ectoparasiticidal activity. No anthelmintic activity was observed. Insecticidal activity was only obtained by ingestion, not contact.

In vitro testing of metaflumizone against the cat flea *Ctenocephalides felis* in an artificial membrane feeding system showed that mortality after 4 days was 100% for concentrations of 10 and 100 mcg/ml

and 24 hours exposure. Continuous exposure with 1 mcg/ml also resulted in 100% mortality after 4 days. Results indicate that metaflumizone is a relatively slow acting insecticide.

Metaflumizone is active against the cat flea, when topically applied to dogs in a dose of 20 mg/kg BW.

Secondary pharmacodynamic effects.

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.

- 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 is less active by contact.
- Metaflumizone effectively controls fleas.
- A minimum effective level for efficacy (e.g. a LC_{50} for fleas) has not been mentioned. The optimal treatment dose has been established experimentally.

Pharmacokinetics

Pharmacokinetics of metaflumizone after a single topical application of metaflumizone to cats at 40 mg/kg.

The purpose of this study was to evaluate the pharmacokinetics of metaflumizone in cats after a single topical application. The study was conducted to determine if metaflumizone was measurable in the blood of cats following topical application at the proposed minimum commercial dose rate of 40 mg/kg of active substance and, if present, to define the pharmacokinetic profile in blood. All cats were treated on D0 with a 20% spot-on formulation of metaflumizone at a dose of 40 mg/kg BW. Dose volume was 0.2 mL/kg BW, applied at the base of the skull on the skin of the neck as a single dose.

Blood samples were collected prior to treatment and approximately 5 and 10 hours and 1, 2, 3, 5, 7, 10, 14, 21, 28, 42 and 56 days after treatment. Levels were below the LOQ of 50 ng/ml and frequently not detectable (< 1.1 ng/mL). Only 1 male cat had a level of 57.8 ng/mL on D3. No adverse events were observed. The results of this study demonstrate that the bioavailability of metaflumizone was very low when administered as a spot-on formulation to cats. Metaflumizone was not quantifiable in plasma at a dose of 40 mg/kg BW. Results indicate that dermal absorption of a metaflumizone spot-on formulation is virtually absent.

Hair coat distribution of metaflumizone after a single topical application to cats at 40 mg/kg of metaflumizone spot on. The aim of the study was to determine the concentration of metaflumizone in the hair of cats following a single topical application of the spot-on at the at the proposed minimum commercial dose rate of 40 mg of active substance/kg body weight and to define the concentration of the active substance at different sites of the cats over a period of 56 days. All cats were treated on D0 with a 20% metaflumizone spot-on formulation with 0.2 mL/kg BW to provide the minimum dose of 40 mg metaflumizone/kg BW. Application site was the skin on the back of the neck at the base of the skull. Hair samples were collected at 4 different sites; middle of the back, top of the tail/lumbar zone, leftthorax, right thorax. Samples were collected just prior to treatment and 1, 2, 7, 14, 28, 42 and 56 days post treatment. Collected hair samples were analysed for metaflumizone, using a validated HPLC-method. Animals were observed for adverse effects at 4 and 8 hours after application.

Metaflumizone was widely distributed over the hair coat within 1-2 days after administration. Peak levels for middle back and right thorax zones were reached by D2. Levels decreased gradually during the 56-day study period and considerable variation in levels was observed between animals for all zones. No adverse effects of treatment were observed. The results indicate that metaflumizone is widely distributed in the hair coat of the cat the day after administration, with maximum levels in the lumbar and thorax right side zones.

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. GLP / Regulatory basis. In accordance with GLP, incl. Dir. 2004/10/EC and GICP and AFSSA procedures. 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 pharmacokinetic data indicate that

- metaflumizone was not quantifiable in plasma at a dose of 40 mg/kg BW. Therefore, dermal absorption of metaflumizone does not seem to occur, when administered as a spot-on formulation to cats;
- metaflumizone was widely distributed over the hair coat of the cat within 1-2 days after administration, with peak levels reached by D2. Levels decrease gradually in time.

Tolerance in the target species of animal

To define the safety of metaflumizone in cats 4 laboratory studies were conducted, including oral exposure.

Safety evaluation of a topically applied spot-on formulation of metaflumizone in adult cats.

The objective of this GLP study was to evaluate the safety of the test substance in adult cats upon a single topical application of 1x, 3x or 5x the proposed commercial dose compared to placebo treated cats. The duration of the study was 28 days. The day of treatment was designated as D1.

Cageside observations for morbidity, mortality, injury and availability of food and water were made twice daily. Detailed clinical examinations were carried out on D1, at 15 minutes and 1, 2 and 3 hours post treatment, and from D2 to D28, twice daily about 4 hours apart, except on days that physical and neurological examinations were carried out. Physical and neurological examinations were carried out prior to treatment, 4 and 24 hours after treatment and on D8 and D22. Blood samples for haematology and clinical chemistry were collected once prior to treatment, at 24 hours post treatment and on D8 and D22. Body weight and food consumption were recorded. Personnel collecting data was blinded to treatments. Metaflumizone was dosed according to body weight range. For the 1x group this was 160 mg for cats < 4 kg BW and a volume of 0.8 mL and 320 mg for cats > 4 kg BW and a volume of 1.6 mL. 3x and 5x overdose groups were treated with doses and volumes corresponding to the overdoses: For the 3x group this was 480 mg for cats < 4 kg BW and a volume of 2.4 mL and 960 mg for cats > 4 kg BW and a volume of 4.8 mL. For the 5x group this was 800 mg for cats < 4 kg BW and a volume of 4.0 mL and 1600 mg for cats > 4 kg BW and a volume of 8.0 mL. The placebo treated group received the dose volume of the 5x group (4 mL for cats < 4 kg BW; 8 mL for cats > 4 kg BW)

Salivation was noted in 5 male and 8 female cats from treated groups and 1 male and 3 female cats from the control group, most of them 15 minutes after treatment. Some haematology parameters showed statistically significant changes at various post dose intervals, compared to placebo-treated animals. However, a dose effect was not apparent or changes were considered to be without toxicological relevance. The same holds true for changes in clinical chemistry parameters.

There was no effect on clinical observations, body weight change or food consumption.

Treatment with metaflumizone spot-on formulation caused transient salivation, but no adverse effect on clinical observations. Thus a single topical application of metaflumizone spot-on formulation at 1x, 3x or 5x the proposed commercial dose may be administered with no deleterious effects.

Safety evaluation of a topically applied spot-on formulation of metaflumizone in 8-week old kittens.

The objective of this GLP study is to evaluate the safety of the test substance in 8-week old kittens upon a single topical application of 1x, 3x or 5x the proposed commercial dose compared to placebo-treated kittens. The duration of the study was 28 days. The day of treatment was designated as D1.

All kittens survived for the whole of the study period. Salivation was noted 1 hour post treatment in 3 males and 2 females from the placebo-treated groups, 1 male and 1 female from the 3x-group and 1 male and 3 females from the 5x-group. No significant neurological findings were noted. Treatment had no effect on body weight. Some haematology parameters showed statistically significant changes at various post dose intervals, compared to placebo-treated animals. However, a dose effect was not apparent or changes were considered to be without toxicological relevance. The same holds true for changes in clinical chemistry parameters. Treatment with metaflumizone spot-on formulation caused transient salivation, but no adverse effects from treatment were noted. Thus a single topical administration of metaflumizone spot-on formulation to 8-week old kittens at 1x, 3x or 5x the proposed commercial dose did not result in adverse effects.

Safety evaluation study of repeated treatments with a topically applied spot-on formulation of metaflumizone in cats. The objective of this pivotal GLP study was to evaluate the safety of 20% w/v metaflumizone spot-on formulation in 8-week old kittens upon 7 topical applications over a 14 week period of 1x, 3x or 5x the proposed commercial dose compared to placebo-treated kittens. Seven treatments were applied to simulate a season long use. The length of the study period was 93 days. All kittens survived for the whole of the study period. Clinical findings, body weight, food consumption physiological and neurological parameters were not affected by treatment. Transient salivation, 5-15 minutes after application, was noted in several animals from the placebo-treated and the 3x and 5x treated groups, but not from the 1x-treated group. Some haematology parameters showed statistically significant changes at various post dose intervals, compared to placebo-treated animals. However, a dose effect was not apparent or changes were considered to be without toxicological relevance. The same holds true for changes in clinical chemistry parameters. Urinalysis did not show any treatment related change. Repeated administrations of 1x, 3x or 5x the proposed recommended dose of metaflumizone spot-on given once every 14 days for a total of 7 doses in cats as young as 8 weeks of age did not result in adverse health effects.

Safety evaluation study of oral exposure from auto-or allogrooming metaflumizone spot-on formulation in cats. The objective of this GLP study was to evaluate the safety of 20% w/v metaflumizone spot-on formulation in cats and their behavioural response after a single oral application of 0.1x the recommended dose compared to cats similarly dosed with 0.9% bacteriostatic sodium chloride. This dose estimates the potential oral exposure due to licking after topical application. Immediately following dosing, avoidance behaviour, including head shaking, spitting and/or salivation were noted in all cats orally treated with metafalumizone spot-on formulation. Salivation had resolved at 30-45 minutes post treatment. No effect on clinical or neurological parameters was observed. One cat showed lacrimation fromD2 to 8, but this was not considered to be related to treatment. A statistically significant increase in heart rate was noted on D2 in the group, but increase remained within normal range. This effect did not carry over to Day 8 and was primarily the result of increases in heart rates in two treated males. As other animals showed decreases in heart rate at this interval and the heart rates remained within normal limits, this finding is not considered to be toxicologically significant. Food consumption was not affected by treatment. Control animals did not show any response to treatment. Some haematology parameters showed statistically significant changes at various post dose intervals, compared to the control animals. However, a dose effect was

not apparent or changes were considered to be without toxicological relevance. The same holds true for changes in clinical chemistry parameters. Treatment with metaflumizone spot-on administered orally to cats at 10% of the recommended dose did result in avoidance behaviour but not in physical or neurological adverse effects. Transient salivation was the predominant clinical sign. Considering the avoidance behaviour it is not likely that cats will ingest relevant quantities of the spot-on formulation. Although cats may ingest some product, quantities will be low and animals are likely to avoid oral intake

- A single topical administration of metaflumizone spot-on formulation to 8-week old kittens at 1x, 3x or 5x the proposed treatment dose did not result in adverse effects.
- Changes in haematological and clinical chemistry parameters after topical administration were not likely to be the result of treatment, which is in line with the absence of dermal absorption.
- Simulated seasonal treatment, with 7 consecutive treatments in total did not lead to adverse effects, even if applied at 14-day intervals.
- Salivation was the main reaction to the administration of the substance, but no information on the animal's response to treatment was reported, so it is not known if e.g. animals were attempting to lick the application site. Considering the avoidance behaviour it is not likely that cats will ingest relevant quantities of the spot-on formulation. Although cats may ingest some product, quantities will be low. Cats apparently dislike the product and will avoid oral intake.

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.

Conclusion on the Preclinical Part

Considering the submitted data, it can be concluded that:
- information on the mode of action of metaflumizone has been submitted;
- metaflumizone has insecticidal properties by blocking sodium channels in the insect nerve system;
- the proposed use is in agreement with the mode of action;
- when applied to the skin of the cat absorption of metaflumizone is absent;
- When applied to the skin of the cat metaflumizone is distributed over the body surface.

- levels of metaflumizone on the skin persist for 56 days above the LOQ;

Reference can be made to the clinical studies for dose finding. A minimum effective level for efficacy (e.g. a LC_{50} for fleas) in relation to the concentration profile of metaflumizone on the skin of the cat has not been mentioned as being the basis for the dose selection. The dose is based on experimental studies.

CLINICAL STUDIES

Laboratory trials

Dose determination of a topically applied spot-on formulation of metaflumizone against fleas on cats.

The aim of the study was to evaluate the efficacy of 3 dose levels of metaflumizone in a spot-on formulation against fleas on cats. The product was applied at 3 dose rates (20, 30 and 40 mg active substance/kg BW) in comparison with untreated and positive controls. Efficacy was evaluated against existing infestations and weekly post treatment challenges. All non-treated cats maintained flea infestations throughout the study. Treatment with a single dose of 20 mg metaflumizone /kg BW resulted in < 90% control at any time point in the study. Treatment with a single dose of 30 mg metaflumizone /kg BW did result in > 90% control on D28. Treatment with a single dose of 40 mg metaflumizone /kg BW did result in > 90% control on D2 and D7 at 48 hours. Flea infestation was controlled at a rate of 79% to 89% for 5 weeks post treatment when assessed at 72 hours after reinfestation. Treatment with Advantage provided >90% control for up to 35 days post treatment. Cats did not show abnormal behaviour after treatments. Treatment of cats with a metaflumizone spot-on formulation at 40 mg/kg BW controlled an existing flea infestation within 2 days and provide at least 7 days of residual control at > 90% when assessed at 48 hours after infestation. The 90% level was based on geometric means.

Efficacy of a topically applied spot-on formulation of metaflumizone against fleas on cats.
The purpose of the trial was to evaluate the efficacy of metaflumizone in a spot-on formulation against fleas (*Ctenocephalides felis*) on cats at the proposed commercial dose rate of greater that or equal to 40 mg ai/kg bodyweight. Efficacy against existing infestations and weekly post-treatment challenged was evaluated. The results of this study showed that metaflumizone spot-on at the proposed commercial dose rate of $\geq$ 40 mg ai/kg bodyweight was >90% effective against an existing flea infestation on cats and provided 93-98% protection against subsequent flea challenge for 6 weeks post treatment. The product was safe for use on cats.

Efficacy of a topically applied spot-on formulation of metaflumizone against fleas on cats.
The purpose of the trial was to evaluate the efficacy of metaflumizone in a spot-on formulation against fleas (*Ctenocephalides felis*) on cats at the proposed commercial dose rate of greater that or equal to 40 mg ai/kg bodyweight. Efficacy against existing infestations and weekly post-treatment challenged was evaluated. The results of this study showed that metaflumizone spot-on at the proposed commercial dose rate of $\geq$ 40 mg ai/kg bodyweight was >99% effective against an existing flea infestation on cats and provided >95% protection against subsequent flea challenge for 8 weeks post treatment. The product was safe for use on cats. Two cats reacted to application by licking of the lips and grooming the flanks for several minutes or attempting to lick the neck area. Reactions resolved spontaneously.

Dose confirmation of a topically applied spot-on formulation of metaflumizone on cats.
The aim of the study was to evaluate the efficacy of metaflumizone in a spot-on formulation against fleas on cats. The product was applied at a minimum of 40 mg active substance /kg BW in comparison with a placebo formulation and nontreated control. Efficacy was evaluated against existing infestations and weekly post-treatment challenges. All untreated animals maintained flea infestations throughout the study. Treatment with the placebo did not result in efficacy against fleas. Counts between untreated and placebo-treated animals was not different except for D7. All treated cats had a wet fur on the application site for 4 hours after treatment. On D1 animals appeared normal. Treatment of cats

with a single dose of metaflumizone spot-on at a minimum of 40 mg/kg BW controlled an existing flea infestation within 24 hours after treatment and also provided at least 6 weeks of residual control at >95%.

Dose confirmation of a topically applied spot-on formulation of metaflumizone against fleas on cats.
The aim of the study was to evaluate the efficacy of metaflumizone in a spot-on formulation against fleas on cats. The product was applied at a minimum of 40 mg active substance /kg BW in comparison with nontreated controls. Efficacy was evaluated against existing infestations and weekly post-treatment challenges. Treatments were applied as a spot-on to the skin on the neck at the base of the skull. Following treatment substantial run-off was noted in 5 cats. As a result salivation was seen in 1 cat for 2 hours. Treatment of cats with a single dose of metaflumizone spot-on at a minimum of 40 mg/kg BW controlled an existing flea infestation within 24 hours after treatment and also provided at least 5 weeks of residual control at >95%.

Dose confirmation of a topically applied spot-on formulation of metaflumizone against fleas on cats.
The aim of the study was to evaluate the efficacy of metaflumizone in a spot-on formulation against fleas on cats. The product was applied at a minimum of 40 mg active substance /kg BW in comparison with nontreated controls. Efficacy was evaluated against existing infestations and weekly post-treatment challenges. Treatment of cats with a single dose of metaflumizone spot-on at a minimum of 40 mg/kg BW controlled an existing flea infestation within 48 hours after treatment and also provided at least 4 weeks residual control at >95%.

Efficacy of two topically applied spot-on formulations of metaflumizone against fleas on cats.
The aim of the study was to evaluate the efficacy of two spot-on formulations of metaflumizone against fleas on cats. The products were applied at the proposed commercial dose, a minimum of 40 mg active substance /kg BW in comparison with a nontreated control group. Efficacy was evaluated against existing infestations and weekly post-treatment challenges. The only difference between formulations A and B was the surfactant. Surfactants account for 5% w/v of the formulation. Treatments were applied as a spot-on to the skin on the neck at the base of the skull. Treatment of cats with a single dose of metaflumizone at a minimum of 40 mg/kg BW as 2 spot-on formulations, with different surfactants, both controlled an existing flea infestation within 48 hours after treatment and provided residual control for 6-7 weeks. The surfactant was replaced for regulatory reasons. This dose-confirmation studies was carried out to confirm the equivalence of formulations.

Dose confirmation of a topically applied spot-on formulation of metaflumizone against fleas on cats.
The aim of the study was to evaluate the efficacy of metaflumizone in a spot-on formulation against fleas on cats. The product was applied at a minimum of 40 mg active substance/kg BW in comparison with nontreated controls. Efficacy was evaluated against existing infestations and weekly post-treatment challenges. Treatment of cats with a single dose of metaflumizone spot-on at a minimum of 40 mg/kg BW provided control of existing flea infestation within 24 hours after treatment and at least 48 days of residual control at >95% when assessed at 48 hours post infestation.

Field trials

One field trial was carried out in the EU.

Evaluation of the efficacy and persistence of 20% metaflumizone as a spot-on formulation in cats naturally infested with fleas in a multicentric clinical field study in the EU. The purpose of the trial was to evaluate the efficacy and persistency of 20% metaflumizone in a spot-on formulation administered at a minimum dose rate of 40 mg metaflumizone/kg body weight for the treatment of natural infestations with fleas (*Ctenocephalides felis*) in cats presented as veterinary patients in Europe. The test product was tested for non-inferiority to the comparator reference product based on percentage of flea-free animals at various times post-treatment as well as geometric mean flea counts.

The reduction in parasite counts compared to D0 was also evaluated. A total of 173 cats, infested with fleas, were enrolled in the study as primary patients. Cats, mainly European shorthair, were 5 weeks to 16 years of age. Body weights varied from1.1 to 10.4 kg. There were 149 male and 145 female cats included; 205 cats were neutered. Cats were randomly allocated to one of the 2 treatment groups, in a way that the 20% metaflumizone -treated group contained about twice as much animals as the positive control group (comparator reference product-treated); 115 versus 58 respectively. An additional 121 cats from patient-households were also treated; 73 with 20% metaflumizone and 48 with Frontline the comparator reference product. In total 188 (primary and secondary patients) cats were treated with 20% metaflumizone and 106 cats (primary and secondary patients) with the comparator reference product. The study was conducted in 16 veterinary clinics in Germany and 8 veterinary clinics in France.71 cat households were enrolled in Germany and 102 in France. The first day of the study, also being the day of the treatment, was designated D0. To enter the study a cat had to be infested with at least 5 fleas on D0. Animals that were considered eligible were clinically examined and flea counts were carried out, before a treatment was applied. Based on a significance level of 5%, a power of 80, a randomisation ratio of 2:1 and assuming that 52% would be free of fleas after treatment with a non-inferiority delta of 23%, a minimum sample size was calculated, being 97 animals for the 20% metaflumizone-group and 56 animals for the comparator reference product. Clinical examinations and flea counts were then carried out on D14, 28, 42 and 56. The first cat from a household was included in the "flea efficacy population". The veterinary surgeon was blinded to the identity of the product. Exclusion criteria were: households with more than 4 cats; cats <10 weeks of age and/or < 2 kg BW; pregnant/lactating cats, or with planned breeding within 2 months; a pre-existing medical or surgical condition; cats that had been bathed/shampooed 48 before. Test product. 20% metaflumizone in a dose of $\geq$ 40 mg/kg BW as a spot on formulation. Volumes: 0.8 mL for cats $\leq$ 4 kg BW; 1.6 mL for cats > 4 kg BW. The comparator reference product was applied as recommended by the manufacturer. Treatments were applied as a spot-on to the skin on the neck at the base of the skull.

Flea counts per animal. The "primary patient" was the statistical unit. Counts were transformed by natural logarithmic transformation {logn (count +1)}, because a skew distribution was expected. Calculation of efficacy was based on "mean count (period 1) - mean count (period j)/ mean count (period 1) x100 = % efficacy at period j". SAS statistical software was used for analysis. A total of 173 cats (115 treated with 20% metaflumizone + 58 with the comparator reference product) was included as primary patients, making up the "flea efficacy population". 13 cats were excluded from this population because of protocol violations (mainly non-compliance), leaving 160 cats for efficacy evaluation.A total of 294 cats (188 treated with 20% metaflumizone and 106 treated with the comparator reference product) represented the "safety population".

Statistical analysis of the results pointed out that the efficacy of 20% metaflumizone was not inferior to that of the comparator reference product. Four animals treated with 20% metaflumizone showed a skin reaction at the application site. One animal showed apathy. Two animals showed colour changes of the fur. Based on the percentage of flea-free cats and the reduction of flea counts, 20% metaflumizone spot-on was non-inferior to the the comparator reference l product at all post-treatment observations. Within the groups, the reduction of flea counts following treatment was highly significant, compared to D0 at all time points for both 20% metaflumizone spot-on and the comparator reference product. 20% metaflumizone was considered safe in the treatment of cats.

The study was well designed and implemented. Reductions did not achieve the 95% level and the number of flea-free animals was low with only 5 out of 10 free of fleas. This was more or less in agreement with the Applicant's assumption on the efficacy level. However, the results apply to the reduction after a single treatment and reinfestation from the environment is likely to have occurred. It is expected that the number of flea-free animals will increase when treatment is repeated at the recommended interval of 4 weeks, regarding the efficacy levels from the experimental studies.

Conclusion on the Clinical Part

Preclinical studies justify the dose of a minimum of 40 mg metaflumizone/kg BW as the recommended treatment dose.

One dose-finding study was carried out. From the 3 doses used (20, 30 and 40 mg metaflumizone/kg) the highest dose appeared to be the most effective and was selected as the treatment dose. In fact a dose higher than 40 mg/kg should have been studied for its efficacy to find out if such a dose was not more effective that the selected one. Considering the confirmation studies, in which doses of more than 40 mg/kg were applied, and the pharmacokinetic studies, it is observed that a dose, higher than 40 mg/kg, is not more effective but may provide a longer residual effect.

The dose-confirmation studies also indicate that a high level of control is achieved at $\geq$ 40 mg/kg. Variation in efficacy, in reduction levels and residual efficacy, is present, likely due to e.g. differences in flea strains and male/female ratio, infestation levels, differences in cats and, for fleas, the interval between last feeding and infestation.

Replacing the surfactant Tergitol NP-13 (bovine derived) by Synperonic NCA 830 did not affect efficacy.

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 the study periods. Compared to field conditions, parasite levels were relatively high.

It is concluded that data justify the selection of a dose of $\geq$ 40 mg metaflumizone as the recommended treatment dose against flea infestations in cats.

When compared to an authorised product on the basis of the efficacy against flea infestations in cats under field conditions, metaflumizone spot on was not inferior in terms of flea-free animals and reduction of flea numbers. Metaflumizone was considered safe in the treatment of cats.

Overall Conclusions and Benefit Risk Assessment

ProMeris Spot-On for Cats is a product containing metaflumizone (18.43% w/w) in a non-aqueous solution, designed as a topically applied treatment to control fleas on cats. The product is prepared as a ready-to-use liquid in single use pipettes in two different filling volumes (0.80 and 1.60 ml) to cover the recommended minimum dose of 40 mg/kg b.w. of metaflumizone to cats.

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.
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.

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 modified 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 period of 6 months for metaflumizone, under the recommend storage condition, are justified. The studies are on-going. New stability studies with the finished product and done with the modified packaging capable of protecting the formulation from ingress of moisture and packed under adequate forming, filling and sealing operations, justify the proposed shelf-life of 18 months when stored at 25ºC/60%RH.

The active substance is 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 (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 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 cat when used as proposed.

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.

There are no concerns for environmental toxicity with this product and no phase II environmental risk assessment was necessary.

Studies were carried out to assess the efficacy of metaflumizone and to determine the dose rate at which the optimal efficacy was achieved. The proposed use of the product was found in agreement with the mode of action of the active substance.

The dose-confirmation studies also indicated that a high level of control is achieved at $\geq$ 40 mg/kg. Variation in efficacy, in reduction levels and residual efficacy is present, likely due to e.g. differences in flea strains and male/female ratio, infestation levels, differences in cats and, for fleas, the interval between last feeding and infestation.

When compared to an authorised product on the basis of the efficacy against flea infestations in cats under field conditions, metaflumizone spot on was not inferior in terms of flea-free animals and reduction of flea numbers. The claim for flea allergy dermatitis (FAD) can be supported since adequate clinical data were submitted in relation to this claim.

The risk benefit assessment can, therefore, be summarised as follows:

Risk.
- sometimes a less than 100% efficacy .

Benefit.
- very low toxicity
- new active substance, with no history of resistance in non-veterinary use
- absence of dermal absorption
- adequate length of a residual efficacy period of 4-6 weeks
- high reproducibility of efficacy over animals

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