SCIENTIFIC DISCUSSION

1. SUMMARY OF THE DOSSIER

PRAC-TIC is a veterinary medicinal product for dogs containing pyriprole, an ectoparasiticite of the phenylpyrazole class. Pyriprole interacts with a receptor involved in transmitting neural impulses (GABA receptor), resulting in uncontrolled activity of the nervous system and the death of fleas or ticks.

PRAC-TIC is available in four strengths (56.25 mg, 137.5 mg, 275 mg and 625 mg) and is presented in packs/containers of 3, 6 and 30 pipettes.

The approved indication is the treatment and prevention of flea (*Ctenocephalides canis* and *C. felis*) and tick (*Ixodes ricinus, Rhipicephalus sanguineus, Dermacentor variabilis, Ixodes scapularis, Dermacentor reticulatus, Amblyomma americanum*) infestations in dogs and the use as part of a treatment strategy for the control of Flea Allergy Dermatitis.

The product is applied monthly as a spot-on between the shoulder blades directly on the skin of the animals at a minimum recommended dose of 12.5 mg pyriprole/kg bw.

The most common side effects in dogs are local reactions at the place where the product has been applied: fur discolouration, hair loss and itchiness, also cosmetic effects of the substance (greasy appearance and clumpiness). A brief period of hypersalivation might occur if a dog licks the application area. As a precaution, humans should avoid direct contact with the dog until the application site is dry and should wash if they come into contact with the product.

2. QUALITY ASSESSMENT

PRAC-TIC spot-on solution is a non-aqueous solution, presented as single dose spot-on containers of four different filling volumes (0.45, 1.1, 2.2 and 5.0 ml) to cover the recommended minimum dose of 12.5 mg/kg b.w. pyriprole for dogs of 2 kg bodyweight and upwards. Pyriprole is a novel active substance in veterinary medicine.

Composition

PRAC-TIC spot-on solution contains 125 mg/ml pyriprole. The product also contains 1 mg/ml butylhydroxytoluene (E321) to prevent oxidation and diethylene glycol monoethyl ether as solvent. PRAC-TIC is available in four strengths (56.25 mg, 137.5 mg, 275 mg and 625 mg).

Container

The finished product is presented in bright yellow plastic spot-on pipettes sealed with an aluminium laminated pale yellow lidding foil. There is a score line near the end of the neck for opening the container.

Three pipettes are contained in a child resistant soft tempered aluminium foil/PVC blister (aluminium multi-layer foil sealed with an aluminium lidding foil). Opening is by cutting the aluminium platine and tearing the foil. Each strength is available in pack sizes of one cardboard carton containing 1, 2 or 10 blisters, each containing 3 pipettes.

Clinical Trial Formula(e)

The pivotal target animal safety studies and pharmacokinetic studies, bioavailability and clinical studies were conducted with the final formulation.

Apart from this formulation, several formulae have been used in target animal studies and full details are given. Minor changes in active substance/preservative/solvent ratio did not have a significant impact on the pharmacokinetics profile.

Development Pharmaceutics

Solubility characteristics of the active substance were given and justified the use of non-aqueous solvents. As the formulation is a solution, polymorphism of the active ingredient was not considered relevant for the finished product.

Initially a solution containing 10% of the active substance was developed, however dose confirmation studies confirmed the necessity to increase the concentration to 12.5%.

Both the excipients are described in the Ph. Eur., and are well-established excipients for use in topical dosage forms. The solvent (diethylene glycol monoethyl ether) was selected for its ability to dissolve pyriprole at the desired concentration and for its spreading behaviour. Use of an antioxidant (butylhydroxytoluene) was justified due to the sensitivity to oxygen of both the active substance and the solvent. The antioxidant efficacy of BHT was sufficiently demonstrated. The concentration (0.1% w/v) is commonly found in pharmaceutical products of these characteristics.

The chosen package has also been justified, taking into account the presentation of the product in single dose containers jointly with the hygroscopicity of the solvent. The foils in contact with the product comply with standard quality requirements for food-contact products, which in the case of topical products can be considered as sufficient. An outer secondary pack in form of Alu packaging is used to add further protection from moisture.

The bulk manufacturing process is simple, and consists of dissolution of the BHT and the active substance in the solvent, followed by filtration. Analytical results of five pilot batches (150 l) are summarised in the dossier.

Filling and packaging are also standard processes. Four batches of bulk solution were filled into pipettes of different sizes, and all except the first were within the specifications. An explanation for the failure is given and justified.

Manufacturing process and in-process controls

The proposed batches sizes are 200 l and 400 l.

The bulk solution manufacturing process is carried out under nitrogen and is adequately described. The amounts of components of the formulation are charged and stirred. No manufacturing overages are included in the formulation. Neither heating nor cooling is necessary. The solution is filtered over a clarification depth filter and barrelled into stainless steel drums.

The bulk solution is visually checked for clarity as an in-process control, and after the filtration, a weight check is performed to record the yield. Individual batches of bulk solution may be filled into pipettes of different sizes. The forming and filling of the pipettes is considered as a standard process using a thermoform machine and is satisfactorily described. In-process controls concerning physical parameters, delivered volume and a leakage test are performed in the pipettes.

The packaging of the pipettes into platines (containing three pipettes) and the packaging of one or two platines into the secondary packaging follow this step. When packaging the pipettes into platines in process control of aspect jointly with a vacuum test to control that no liquid enters the platine cavity are performed.

The process is a standard manufacturing process. Analytical results of five pilot batches (150 l) prove that the manufacturing process is suitable. Complete validation protocols are provided. The process will be fully validated (at both the 200 l and 400 l batch sizes) prior to distribution and commercialisation on three commercial batches.

Control of Starting Materials

Active substance

The active substance (pyriprole) is not described in any pharmacopoeia and detailed information on quality control and analytical methods has been provided.

Testing instructions for pyriprole include appearance, identity by IR and HPLC, colour and clarity of solution, residual solvents, loss of drying, residue on ignition, heavy metals, assay by HPLC and related substances.

Identity is confirmed by comparison of the IR spectrum with the reference spectrum and by agreement of the HPLC retention times of the reference and the samples. Colour and clarity of a 5% solution in the solvent for the finished product are checked to ensure consistent finished product appearance. Residual solvents (2-propanol and methylcyclohexane) are detected by headspace gas chromatography with flame ionization detection, based on Ph. Eur. 2.4.24. The method is satisfactorily described and data of validation are provided. The LOQ for both solvents is according to the VICH requirements. The non-specific volatile impurities are controlled by the loss on drying test according to the Ph. Eur. method. Standard USP tests (considered equivalent to Ph. Eur.) for heavy metals and residue on ignition are carried out to determine insoluble organic impurities. Content of pyriprole and related substances are determined by a binary gradient HPLC with UV detection. System suitability test is made in accordance with USP, which was considered according to the requirements of the current CVMP guidelines. The specification limit for content of pyriprole (98.0-102.0%) and related substances have been set based on the analysis of a number of development batches and the results of

9 months stability studies. The method used for assay by- and degradation products has been proved as suitable and stability indicating. The limit of quantitation has been established as < 0.05% for AHC 2071719 and AHC 2000148, the two specified impurities. These impurities have been quantified against their own by-product standards. Other potential impurities are quantified using the response factor of the active ingredient.

The manufacturing process of pyriprole is adequately described. The proposed size for a production batch of active substance is 250 kg ± 70 kg.

The active substance is stored and shipped in metallic drums lined with polyethylene bags sealed with a plastic closure to protect the material from humidity. Quality of the starting materials is confirmed and specifications are provided. General methods used to assay appearance, infrared spectrum, loss of drying and sulphated ash are provided. Certificates of analysis of all the raw materials used in the manufacture of the active substance have been provided and confirm the proposed specifications.

Control tests performed on intermediate products are stated. The chromatographic methods used to assess the purity are considered suitable to control the process. In-Process controls and their analytical methods are described and fit for purpose.

The chemical structure of pyriprole was confirmed using LC-MS, NMR-Spectra, IR-Spectra and elemental analysis. No chirality was found. Solubility of pyriprole in water is 1.3 mg/l and in Diethylene Glycol Monoethyl Ether (the main solvent of the final product) is 29.4% (w/w). The substance is crystalline and shows no hygroscopicity. Its melting point is 120ºC.

All potential impurities arising from the route of synthesis are well described and were found in the pilot batches in amounts below the VICH reporting limits.

Eight pilot batches (sizes ranging from 5.32 to 45.02 kg) were release tested confirming the proposed specifications.

Excipients

Both excipients are described in the Ph.Eur. Certificates of analysis of representative batches are given and they confirm compliance with the respective current monographs.

Packaging Material

The final product is packaged in yellow plastic pipettes with an aluminium lidding foil. The pipettes are packed in an aluminium multilayer foil sealed with an aluminium lidding foil (platines/blister). Their specifications and routine test are adequately described in the dossier. The foils were adequately described. Certificates of analysis and IR spectra for the different layers are supplied. It was shown that no migration of non-volatile residues from the foil occurs. These foils comply with standard quality requirements for food-contact products. Results of stability studies confirm the suitability of this package.

Specific measures concerning material of animal origin with respect to TSE

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.

Control tests on the intermediate products

The bulk solution is tested routinely to comply the same specifications as the finished product for appearance, identity and content of BHT by GC, TLC identity of pyriprole and identity and content of pyriprole by HPLC. This bulk solution is barrelled in stainless steel drums. On receipt at the filling site, the bulk solution is tested for identity (TLC).

A stability period of 12 months has been demonstrated for this bulk. The start of shelf life of the dosage form is set according to the "Note for Guidance on Start of Shelf-Life for the Finished Dosage Form" (EMEA/CVMP/453/01).

Control tests on the finished product

The specification for the final product packed in four different pipettes contain tests and limits for appearance, uniformity of dosage, identity and content of BHT by GC, microbial limit test for total aerobic count, combined yeast and moulds and the specific microorganisms *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Enterobacteriaceae. Identity of the active substance is confirmed by two methods (by HPLC and TLC) and content determination of pyriprole is determined by HPLC. The test methods have been validated according to VICH guidelines.

Ranges for pyriprole content has been fixed at 95.0-105.0% of the declared value for both release and shelf life purposes. These ranges are in accordance with the current guidelines. There was no justification for wider limits during shelf-life as updated stability data confirmed no trend in assay results obtained during testing under VICH conditions (during shelf life at 25°C/60%; for 12 months 30°C/65% and for 6 months at 40°C/75%RH). This is confirmed by the results for total degradation products, which do not exceed 0.5% under those conditions. A slight decrease in assay results and increase in impurities is only observed after 18 months at 40°C/75%RH, which is a combination of testing period/conditions that is much more stringent than foreseen by VICH and not representative for product on the market.

Analyses tests in five bulk pilot scale batches and batches used in target animal studies were provided.

Stability Tests on the Active Substance(s)

A primary stability study performed in three batches of pyriprole stored in simulated commercial containers was performed in accordance with the guideline VICH GL3 "Guideline on stability testing of new veterinary drug substances and medicinal products". Data for up to 12 months are available. The results show that the active substance is chemically and galenically stable. Two specific substances remained within the range of 0.2%. Three unknown related substances were also detected, but they do not exceed 0.1%.

Additional results from stress test proved stable in solid state against light and temperature stress (50ºC and 75ºC). It did not adsorb water when stored in open glass containers at 40ºC/75%RH for 21 days. In solution/suspension (7 days), one unknown related substance appeared at pH 7 and 9 at a level of 0.3%, another one mainly in 0.1 NaOH and in more concentrated peroxide solution, at a maximum level of 0.5%. The active ingredient appears sensitive to hydrolysis under neutral and alkaline conditions and to oxidation.

Stability Tests on the Finished Product

The shelf life for the bulk product is 12 months. The product is chemically and galenically stable and met the specifications up to 12 months of storage in partly emptied bulk containers.

The shelf life for the finished product is 24 months as supported by the stability data presented. A total of 12 final product batches have been tested (i.e. 3 of each strength, according VICH conditions).

Identity tests are not performed during the stability studies. A weight control is added to monitor changes at various storage conditions. Other parameters tested include Pyriprole content, related substances, BHT content, dosage uniformity, appearance and microbial limit tests.

Only 18 months data are available at the time of authorisation but this justifies a shelf-life of 24 months for the product, without any special precautions for storage. Extrapolation data are not considered necessary in view of the behaviour of the batches during the reported time.

In addition to the long term and accelerated testing, freeze and thaw test (7, 14, 21 days at -21ºC followed by storage at 25ºC/60%RH until fully thawed), temperature stress (50 and 75ºC, up to 21 days) and light stress (1.2 million lux hour) tests have been performed. Temperature stress test has been performed in closed glass vials containers. A moderate degradation of the product (2.5%) only was noted when stored at 75ºC, jointly with a decrease of BHT content to 72.3% of the declared content. Light stress test was made in the 0.45 ml presentation, only in its primary pack. 1.3% of degradation products were reported. As the product is sold in a light resistant secondary pack, the product should be stored in the original container. Freezing stress was developed in the whole pack, only in the 5.0 ml presentation. No change of the product was noted at -22ºC where it remained liquid.

Based on the results, the agreed storage conditions for the product are “Store in the original container in order to protect from light” and “Do not store above 30°C.”

Overall Conclusion on Quality assessment

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

The active substance is not described in any Pharmacopoeia but sufficient information is provided to justify the quality of batches. The specifications and test methods are considered suitable to control the active substance. Validation of methods used to control the active substance is submitted.

Both of the excipients used in the manufacture of the product are well established for use in topical dosage forms and are described in European Pharmacopoeia.

The method of preparation for the finished product is appropriately described, jointly with the planned validation on an industrial scale.

The testing monographs for the final product contain specifications and test for appearance, uniformity of dosage, identity and content of BHT by GC, microbial limit test for total aerobic count, combined yeast and moulds and the specific microorganisms *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Enterobacteriaceae. Identity of the active substance is confirmed by two methods (HPLC and TLC) and content determination of pyriprole is determined by HPLC. Validations of the methods are enclosed to confirm their suitability.

Stability studies have been performed according to VICH guidelines. Data presented (12 months for the active substance and 18 months for the final product) justify the proposed stability period of 2 years for both the active substance and the final product. Additional data to confirm the proposal for the finished product have been submitted.

3. SAFETY ASSESSMENT

Pharmacokinetics

Pharmacokinetics of pyriprole was studied in rats and dogs. In dogs, three pivotal GLP studies were presented to document the pharmacokinetics of pyriprole following intravenous or topical administration.

Percutaneous distribution and absorption

Following topical administration, pyriprole is rapidly distributed in the hair coat of dogs within one day after application. Tests in dogs with different hair lengths showed that the product when applied directly to the skin is distributed over the dog in the greasy layer of the skin. Pyriprole is then stored in the sebaceous glands and slowly released on to the dogs skin. Over-animal distribution, storage and slow release are, therefore, independent of the fur length of the animals.

In vitro data on the percutaneous absorption of a single dose of radiolabelled pyriprole in the final Spot-on Solution were provided using a model with human, rat and dog skin membranes. Absorption of pyriprole through mammalian skin is low and species-specific differences were observed.

Total percutaneous absorption (dermal delivery + absorption) through human skin was 3.6-fold lower than through dog skin and 2.7-fold lower than through rat skin. The total absorbed dose over a period of 24 h and thus the systemically available and toxicologically relevant dose was even 9-fold lower through human than through rat skin.

In vivo, following topical administration to dogs, the parent compound pyriprole is absorbed by the skin with a bioavailability of about 50 %. The absorption rate is slow (half-life of absorption of about 21±14 days) and the metabolic clearance in the liver is high (31 ± 4.6 ml/kg/min); therefore, no pyriprole was quantifiable in blood *in vivo*. About 30-45% of the topically applied dose of radiolabelled pyriprole was recovered 3 weeks after application in skin and hair together. The recovered dose mainly consisted of the parent substance with low levels of metabolites.

Metabolism

Pyriprole is rapidly metabolised by the liver; skin metabolism of pyriprole is not very significant. Parent compound is found unchanged in skin 3 weeks after the spot-on administration. In rats, 7 metabolites were identified in the plasma. The metabolite profile of pyriprole is similar in rats and dogs and independent from the route of administration.

Pyriprole is rapidly converted via hepatic metabolic pathways into its sulfone and sulfoxide derivatives. The two main metabolites are considered responsible for the secondary pharmacodynamic and toxicological effects.

The enzymes involved in the formation of both metabolites in the skin have not been identified. However similarly to other phenylpyrazoles (fipronil), a CYP3A4-mediate metabolic pathway is assumed.

Distribution

The pyriprole volume of distribution (Vss) is very large (11814±4390 ml/kg) indicating a high affinity for the different tissues.

In dogs, following topical or intravenous application, the parent compound is not found in plasma but the two main metabolites were detected. The quantity of the main metabolites was higher in females than in males. The highest concentrations in plasma were observed for one metabolite with a terminal half-life of about 24 days. The other metabolite was detected with an estimated terminal half-life of 21±13 days and 4.5±2.8 days after topical and intravenous application, respectively.

Excretion

The major pathway of excretion is via faeces (55-59% of intravenous dose in 14 days), followed by urine (12-20% of intravenous dose in 14 days).

Accumulation

Pyriprole is to be applied regularly at a dosage interval of 1 month. As the terminal half-life of the main metabolite is long (about 24 days), it is expected that repeated pyriprole application would lead to some metabolite accumulation. However, pyriprole hair concentrations did not display any accumulation in hair during multiple topical dose administration.

Toxicological studies

Toxicity studies were performed using the active ingredient pyriprole or the final product. However, observed neurological effects are attributed to the activity of the systemically available metabolites, which are the major moieties in circulation after intravenous and topical application in dogs as well as after oral and dermal application to rats. They act as potent inhibitors of the GABA-gated chloride channels resulting in adverse signs related to transient effects on the nervous system (impaired neuronal muscle control), such as incoordination, muscle tensions, ataxia, sensory induced convulsions and seizures.

Single dose toxicity

GLP compliant studies were carried out in rats (oral, dermal) and rabbits (dermal). After single dermal administration, pyriprole toxicity is low in rats and moderate in rabbits. After a single oral dose, pyriprole toxicity is considered moderate in rats.

Following single oral administration, no clinical signs and no mortality were observed in the animals given 200 mg pyriprole/kg apart from a reduced body weight gain of the males during first week of the study. At the 2000 mg/kg dose-level, 2/3 males were found dead on day 2 and hyperactivity, piloerection and dyspnoea were noted. At necropsy, no apparent abnormalities were observed. It was concluded that pyriprole is moderate toxic after a single oral dose administration.

In rats, after an acute dermal exposure (dose 2000 mg/kg bw) the only sign was reduced gain or slight body weight loss between day 1 and day 8. No cutaneous reactions were observed. In rabbits, following acute dermal exposure (400 mg/kg bw), deaths and clinical signs (hypoactivity, dyspnoea and tonic-clonic convulsions) were observed.

Repeated dose toxicity

Three GLP-compliant repeat dose toxicity studies were carried out in **rats** over 28 days (oral, dermal) and 90 days (oral).

In a 28-day oral toxicity study (0, 0.3, 2, 20 mg/kg bw), target organs were liver and thyroid. Liver weights were significant increased in female rats from 2.0 mg/kg. At necropsy, liver enlargement and necrosis of hepatocytes was observed at 20.0 mg/kg. Thyroid follicular-cell hypertrophy was found from doses of 2.0 mg/kg. Based on these data, the oral NOEL for rats (subacute) was considered 0.3 mg/kg/day.

In a 90-day oral toxicity study (0, 0.1, 0.3, 3 mg/kg bw), target organs were liver and thyroid. In doses from 0.3 mg/kg, multifocal diffuse follicular hypertrophy and minor diffuse liver hypertrophy were observed. Based on these data, a NOEL of 0.1 mg/kg day was identified in rat following daily oral (feeding) administration for 13 weeks.

In a 28-day dermal study in rats with daily semi-occlusive application for 24 hours (0, 60, 180, 600 mg/kg bw), no local signs were observed. Minimal increased in the liver weight was

observed with absence of histopathological changes at 600 mg/kg. The systemic NOEL was 60 mg/kg/day.

The CVMP concluded that in **rats**, the liver is the main target organ after repeated oral or dermal administration. The findings observed were increased liver weight, hepatocellular hypertrophy and thyroid follicular hypertrophy. The predominant toxic effect is characterised by the disturbance of the thyroid-pituitary axis due to increased liver metabolism of thyroid hormone. However, it was noted that rats are particular sensitive to phenylpyrazoles. Since observations made in dogs identified the *nervous system* as major target organ, it was concluded that the data in rats would only be supportive.

Reproductive toxicity, including teratogenicity

The effects of pyriprole on reproductive toxicity were studied in two OECD and GLP-compliant studies in rats, a one-generation reproduction study and a prenatal developmental (teratogenicity) study.

The one-generation **reproduction study** was designed to provide general information concerning the effects of pyriprole on male and female reproductive performance and indicators of developmental neurotoxicity of the offspring. Increased post-implantation and postnatal loss, reduced pup body weight, reduced brain weight and delayed onset of development were seen only at doses causing significant parental toxicity (15 mg/kg). NOEL for the parental animals and the F1 offspring was considered to be 0.3 mg/kg bw/day. No effect indicative for developmental neurotoxicity was observed.

In a rat **teratogenicity study**, pyriprole was administered orally by gavage once daily from day 6 through to day 20 post coitum at dose levels of 0, 4, 8 and 16 mg/kg bw. The study did not show any teratogenicity potential. The highest concentration tested (16 mg/kg bw/day) induced clear signs of maternal toxicity, which are considered causative for the lower stage of development of the foetuses in this dose group. The NOEL for maternal toxicity was 4 mg/kg bw/day. The NOEL for foetal toxicity was 8 mg/kg bw/day.

No data were submitted for the target species. The CVMP therefore concluded to add recommendation in the SPC and package leaflet:
”The safety of the veterinary medicinal product has not been established during pregnancy and lactation, nor in breeding animals, although in laboratory animal studies no indications of relevant effects on reproduction or foetal development were observed. Use only accordingly to the benefit/risk assessment by the responsible veterinarian”.

Mutagenicity

Mutagenicity was investigated *in vitro* (*Salmonella typhimurium*; Chromosome aberration test with cultured human peripheral blood lymphocytes) and *in vivo* (Micronucleus Assay in Bone Marrow Cells of the Mouse with Pyriprole). The available studies indicate that pyriprole is not mutagenic in the bacterial gene mutation assay and are not clastogenic *in vitro* or *in vivo*. The submitted test battery satisfies the ICH/VICH guidance.

The CVMP concluded that no evidence of mutagenicity was identified in a series of studies conducted according to the relevant guidelines.

Carcinogenicity

No studies on the carcinogenicity of pyriprole were conducted. Since pyriprole did not show any genotoxic potential in the gene mutation assay with bacterial cells, in the *in vitro* chromosome aberration study and in the *in vivo* micronucleus assay, it was concluded that pyriprole is non-mutagenic and non-clastogenic.
Like fipronil, pyriprole causes a perturbation of the pituitary-thyroid status as indicated by changes in the levels of triiodothyronine (T3), thyroxine (T4) and the thyroid stimulating hormone (TSH) and

histopathological changes in the liver and the thyroid as observed in the repeat-dose oral toxicity studies in rats. This correlation is considered to be causative for the observed induction of thyroid tumours by fipronil in rats. Nevertheless, there is sufficient scientific evidence that this is only of limited relevance for humans. According to European Chemicals Bureau ECBI/49/99 Add. 1 Rev. 2 non-genotoxic substances like fipronil producing thyroid tumours in rodents with low or medium potency by a clearly established perturbation of the thyroid hormone axis, in general do not need to be classified as potential human carcinogens.

The CVMP, therefore, agreed that there is no need to perform a carcinogenicity study.

Studies of other effects

Dermal irritation
One study was submitted investigating the acute dermal irritation in rabbits (single dose of 500 mg (≅ 200 mg/kg bw) for 4h under semi-occlusive dressing). Some signs of systemic toxicity, consistent with that observed after an acute dermal exposure (hypoactivity, dyspnoea and tremors) were observed in one out of three animals on days 3 and 4; however, no reaction were noted on the skin. The CVMP, therefore, concluded that pyriprole is non-irritating to skin.

Eye irritation
One study was submitted investigation the acute eye irritation in rabbits (single dose of 100 mg (about 45 mg/kg bw) into conjunctival sac of one eye). Serious signs of systemic toxicity, consistent with the ones observed after an acute dermal exposure (hypoactivity, dyspnoea, tremors, convulsions and lateral recumbency) were recorded in all animals. Slight conjunctival reactions, iritis and corneal opacity were observed in some of the animals. The CVMP, therefore, concluded that pyriprole is slightly irritating to the eye. An appropriate warning has been included in the SPC and product literature.

Skin sensitization potential
A number of studies were provided investigating the skin sensitisation potential in mice using the local lymph node assay in doses up to 13 mg/kg. No systemic clinical signs were observed and no cutaneous reactions or relevant lymphoproliferation was noted. The CVMP concluded that pyriprole does not appear to be a skin sensitising agent.

Immunotoxicity
There were no adverse effects on the lymphoid organs and changes in the cellularity of lymphoid tissue, bone marrow or peripheral leukocytes observed in the repeat dose toxicity and target animal safety studies. Thus no additional studies of the effects of pyriprole on the immune system were considered necessary.

Phototoxicity
UV-light stability studies demonstrated that pyriprole is photostable and no degradation occurs after light exposure of 3.6 MLxh for 21 days. In the clinical field trials no adverse effects to skin were reported which could be associated with phototoxicity.

Observations in humans

Pyriprole is not authorised for use in humans and no data for humans were submitted. However, the applicant provided *in vitro* studies, demonstrating that percutaneous absorption through human skin was 3.6-fold lower than through dog skin and 2.7-fold lower than in rat skin.

In addition, neurotoxic effects of pyriprole are attributed to its major metabolite in circulation, SCG-475. However, an approximately 5-fold lower activity of SCG-475 was determined at the human receptor than at the rat receptor. Thus, a significantly (5-fold) higher exposure to humans would have to occur in order to induce the same degree of neurotoxic effects as observed in laboratory animals.

User Safety

The user safety was assessed taking into account the pharmacological and toxicological profile of the product and experimental exposure data. Both, professional users (veterinarians and veterinary nurses) and non-professional users (pet owners and persons/children) might be exposed to the product. The most relevant routes of skin exposure are considered the accidental spillage, and when stroking or otherwise handling treated animals post application. Oral exposure could occur in toddlers by accidental ingestion or by hand-to-mouth transfers. Due the low vapour pressure of the product and the method of application, inhalation exposure is considered to be minimal.

Accidental spillage:
The CVMP considered a worst case scenario of spillage of a complete content of 2.6 maximum strength (625 mg) pipette (13 ml) in connection with the results of the acute dermal study with the product in rabbits (LD_{50} >2000 mg/kg and no clinical signs were observed) and the slower penetration through human skin. The CVMP concluded that there is no risk to the user after accidental spillage of PRAC-TIC.

Post-application dermal exposure:
The applicant provided a detailed user risk assessment based on the risk of repeated dermal exposure for adults (pregnant women) and for toddlers via direct contact e.g. stroking the recently treated dog. The post-application dermal exposure took account of the applied dose, the fraction of active ingredient available on the pet, the amount of product which can be dislodged and the surface area of the body that comes in contact with treated dog.
The CVMP concluded that the warnings included in the SPC and product literature would sufficiently address any risks associated with potential post-application dermal exposure.

Risk to toddlers after oral exposure
A detailed user safety assessment has been provided taking into account oral exposure in children by accidental ingestion or by hand-to-mouth transfer. Taking into account the pharmacological and toxicological profile of the product, the experimental exposure data and the packaging (child proof container), the Committee agreed that the warnings in the SPC and product literature would sufficiently address any risks associated with potential oral exposure.

The CVMP concluded that topical exposure due to spillage or to residues on treated animals and oral exposure do not represent an unacceptable health risk to the user taking into account the recommended user warnings:

- As a precautionary measure, direct contact with the treated animals should be avoided and children should not be allowed to play with treated animals until the application site is dry. It is therefore recommended to treat animals in the evening. Recently treated animals should not be allowed to sleep in the same bed as their owners, especially children.
- People with known hypersensitivity to the phenylpyrazole class compounds or any of the excipients should avoid contact with the veterinary medicinal product.
- Avoid skin contact with the pipette content. Wash hands or other exposed body parts after use with soap and water. If accidental eye exposure occurs, the eyes should be rinsed carefully with water.
- Do not smoke, eat or drink during application.

Ecotoxicity

An Environmental Risk Assessment (Phase I) was performed in compliance with the relevant VICH guideline. The product will only be used in a non-food producing animal species and will only be used to treat a small number of individual animals. In accordance with the VICH Topic GL6 (Ecotoxicity Phase I) Guideline on Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products – (CVMP/VICH/592/98-final), a Phase II environmental risk assessment was, therefore, not required.

However, the possible exposure of surface water and the ensuing risk was assessed and the CVMP noted that pyriprole may adversely affect aquatic organisms. The CVMP concluded that there is an acceptable risk to the aquatic environment even in the unlikely case that the pet owner allows his dog to enter a pond shortly after treatment. However, an appropriate disposal warning was included in the SPC and package leaflet that the product should not be allowed to enter surface water, as it may be harmful for aquatic organisms.

Overall Conclusion on safety assessment

Absorption of pyriprole through mammalian skin is low and species-specific differences were observed. The *in vivo* metabolism of pyriprole after repeat oral and dermal administration was studied in rats; the metabolic profile is basically identical in rats and dogs and independent from the route of administration. In dogs, pyriprole absorption is slow (half-life of absorption about 21±14 days), and has a high metabolic clearance (31 ± 4.6 ml/kg/min), therefore no parent compound is quantifiable in blood after a spot-on administration. The two main metabolites are considered responsible for pharmacodynamic and toxicological effect. Pyriprole has a large volume of distribution indicating a high affinity for different tissues. The major pathway of excretion is via faeces.

Pyriprole exerts a moderate toxic effect after a single oral dose administration in rats. After single dermal administration toxicity is low in rats and moderate in rabbits. A subacute (28 days) oral toxicity feeding study in rats established a NOEL of 0.3 mg/kg/day. A NOEL of 0.1 mg/kg day was identified in rats following daily oral (feeding) administration for 13 weeks. In a repeated dose dermal study in rats with daily semi-occlusive application for 24 hours during a period of 28 days, no local signs were observed. The systemic NOEL was 60 mg/kg/day.

In rats, the liver is the main target organ after repeat administration (increased liver weight, hepatocellular hypertrophy, thyroid follicular hypertrophy). The predominant toxic effect is characterised by the disturbance of the thyroid-pituitary axis due to increased liver metabolism of thyroid hormone. However, in dogs, observed effects are attributed neurological to the activity of the systemically available metabolites, which are the major moieties in circulation after intravenous and topical application in dogs as well as after oral and dermal application to rats. They act as potent inhibitors of the GABA-gated chloride channels resulting in adverse signs related to transient effects on the nervous system (impaired neuronal muscle control), such as incoordination, muscle tensions, ataxia, sensory induced convulsions and seizures.

Pyriprole is non-irritating to the skin, slightly irritating to the eye, and does not appear to be a skin sensitising agent. There was no information relating to experience in humans.

The effects of pyriprole on reproductive toxicity were studied in a one-generation reproduction study in rats and in a prenatal developmental (teratogenicity) study in rats. No effects indicative for developmental neurotoxicity was observed and pyriprole did not show any teratogenicity potential in rats. The NOEL for maternal toxicity was 4 mg/kg bw/day. The NOEL for foetal toxicity was 8 mg/kg bw/day. No data were submitted for the target species. No evidence of mutagenicity was identified in a series of studies conducted according to the relevant guidelines. Carcinogenicity studies were not considered necessary.

The user safety of the product is sufficiently addressed and the user risk management procedures detailed in 4.5 of the SPC are appropriate.

In accordance with the VICH Topic GL6 (Ecotoxicity Phase I), a Phase II environmental risk assessment is not required. The possible exposure of surface water and the ensuing risk was assessed. Although pyriprole may adversely affect aquatic organisms, there is an acceptable risk to the aquatic environment when PRAC-TIC 12.5% Spot-on solution is used according to the labelling.

4. CLINICAL ASSESSMENT (EFFICACY)

Pharmacodynamics

Pyriprole belongs to the phenylpyrazole class. The applicant provided several studies on the general mode of action of this class, which is attributed to their interaction with ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA). This leads to a non competitive blockage of pre- and post-synaptic transfer of chloride ions across cell membranes resulting in uncontrolled activity of the central nervous system.

Although the exact mode of action of pyriprole has not been fully investigated, the CVMP considered it comparable to that of other well described molecules of similar chemistry, such as fipronil. In addition, several studies were provided demonstrating similar effects of pyriprole and fipronil and their main metabolites. As pyriprole is quickly metabolized after systemic absorption, the neurotoxic effects of pyriprole are attributed to its major metabolite in circulation.

Species specific differences in the affinities of phenylpyrazoles between GABA receptor chloride channel sites have been demonstrated, with selective toxicity to insects. In addition, the applicant provided a study demonstrating an approximately 5-fold lower activity of the main metabolite at the human GABA receptor than at the rat GABA receptor.

Following contact (rather than systemic) exposure, **insects** are killed within 24-48 hours post-exposure. The applicant provided a laboratory study demonstrating the intrinsic toxicity of pyriprole in comparison with fipronil using susceptible and multi-resistant adult cat flea (*Ctenocephalides felis*) strains. Following administration of either pyriprole or fipronil, the fleas were transferred into test tubes and dead insects were counted 24h and 48h after treatment. Pyriprole was similarly effective as fipronil against susceptible cat flea strains.

In **dogs** and **rats**, direct effects of pyriprole are associated with transient effects on the nervous system, such as incoordination, muscle tension, ataxia, sensory induced convulsions and seizures. Secondary pharmacological effects (histopathological changes in the liver and thyroid) were only observed in rodents, which are known for species-specific sensitivity towards disturbances of the pituitay-thyroid axis following increased elimination of T4. The target animal safety studies with PRAC-TIC indicate that there is no effect on the pituitary-thyroid status in dogs. The CVMP, therefore, concluded that the relevant safety-related effects are associated with neurotoxicity in dogs.

Formulation selection

The final formulation selection was based on the efficacy results (obtained in pilot studies with different test formulations and the dose determination studies) and on comparison of different formulations on their cosmetic appearance on dogs (clogging of hair at the application site).

In vivo study results indicated that *Ixodes ricinus* is less sensitive than *R. sanguineus* and therefore, tick efficacy studies selecting the final formulation were performed with the least susceptible tick, *I. ricinus*. Initial test formulations contained 10% w/v pyriprole and animals were dosed at a dose of 10 mg/kg. However, the results were not conclusive (efficacy against *I. ricinus* was below 90% on day 36) and a higher dose was selected (12. 5 mg/kg) and the concentration of pyriprole was increased in the final formulation up 12.5% w/v (efficacy against *I. ricinus* was more than 90% until Day 35).

The applicant provided several studies comparing the impact of different spot-on formulations on the cosmetic appearance of the coat of the dogs. All animals showed cosmetic effects to varying degrees. Using the final formulation, clumpiness of hair and greasy deposits were seen up to 48 hours post-administration. Reference to these effects is made in section 4.6 (adverse reactions) of the SPC and product literature:

"The following local reactions may be observed at the application site: fur discoloration, local alopecia, pruritus, greasiness and a clumpy appearance of the hair. Those signs will disappear within 48 hours. "

Dose determination

In the pivotal dose determination study, three doses were evaluated (6.25; 12.5 and 25 mg pyriprole/kg bw after topical administration) in their efficacy against the least susceptible tick species, *I. ricinus*.

Thirty two animals were randomly distributed in four treatment groups and 50 adult *I. ricinus* were placed on each dog at days +7, +14, +21 and + 28. The primary parameter of efficacy was the number of live attached ticks plus live free ticks. Ticks were counted and removed 48 hours after each infestation on days 7, 14, and 21. After infestation of day 28, ticks were counted 48 hours after the infestation, and counted and removed 72 hours after the infestation. The results showed insufficient efficacy of the lowest dose tested and a similar efficacy profile between 12.5 and 25 dose range.

The CVMP noted some deficiencies in the study design (not in accordance with the requirements of guideline EMEA/CVMP/005/00). However, taking into account the results of the efficacy studies, it was concluded that a dose of 12.5 mg/kg was sufficiently supported.

Tolerance in the target animal

The applicant provided 5 tolerance studies investigating the safety of the final spot-on formulation in dogs of different age groups, following single administration (1x, 2x or 10x the recommended dose) or repeated monthly doses (1x, 3x and 5x the recommended dose) up to six months. The design of the target animal safety studies was appropriate and the selected parameters and data analysis were adequate to identify pyriprole specific effects in the target animals.

Treatment followed the recommended route and safety was evaluated regularly by detailed clinical assessments, bodyweight measurements, diet consumption, site of spot-on assessments, analysis of parameters for haematology, clinical chemistry, veterinary and neurological examinations and by the results of histopathological examinations and organ weights at necropsy. Urinalysis was performed regularly in the margin of safety study and the acute topical overdose study.

In all treatment groups, application of the product caused a clogged appearance of hair at the treatment spot for up to 24 hours (cosmetic effect). Tolerance was good following a single dose of 1x, 2x or 3x the recommended dose.

In some animals, treated repeatedly once a month with 3x the recommended dose for six consecutive months, mild neurological signs were observed such as slight incoordination and unsteadiness. Those signs disappeared within 3 hours following administration.

Repeated topical administration of 5x the recommended dose was usually well tolerated. The following transient adverse effects were observed in some animals: tremors, ataxia, panting and convulsions. These signs disappeared within 18 hours following administration.

A single topical administration at 10x the recommended dose to beagle dogs caused adverse effects, affected mainly the nervous system (inducing muscle tensions, seizures and convulsions becoming evident at +10 hours, inability to control limbs; weakness, unsteadiness). Vomiting was seen in a few animals and a significant loss of appetite was noted starting at study day 3. There were no mortalities and the adverse effects were reversible within 48 hours, with the exception of the loss of appetite.

The CVMP concluded that in dogs, the prominent adverse effects of pyriprole and its major metabolites correlate with the interaction at the GABA receptor resulting in neurological signs such as impaired muscle control in form of tremors, imbalance and convulsions. Unlike rats, no disturbance of

the pituitary-thyroid axis was observed in dogs. Appropriate warnings have been included in the SPC and product literature.

Resistance

The CVMP noted that pyriprole has a similar mode of action to that of an authorised ectoparasiticide (fipronil), where resistance has been documented in some insect species. However, no European flea strain with resistance to fipronil has been isolated. In addition, good efficacy against fleas was shown in the field trials with PRAC-TIC conducted in several European regions and no efficacy failure due to resistance of pyriprole against this population of fleas was noted.

No information was provided concerning resistance of tick species against pyriprole (or phenylpyrazoles).

Dose confirmation studies

Fleas

The applicant provided 3 non-GLP-compliant studies to confirm the selected dose of a spot-on application with 12.5% w/v pyriprole for the treatment and prevention of infestations with adult fleas (*Ctenocephalides felis*). Absence of data for *C. canis* was justified with data demonstrating no difference in the effectiveness of another phenylpyrazole (fipronil) against *C. felis* or *C. canis*, and also since *C. canis* was included in the field studies.

Dogs were infested with cat fleas at different time points (depending on the study) and efficacy was determined on basis of the percentage reduction of flea counts in the treated groups compared to an untreated control group. Tests were conducted up to 60 days. The number of live fleas was determined by combing the dogs.

The study results confirmed the efficacy of the selected dose in the prevention and treatment of flea infestations for at least 30 days.

Fleas and ticks

The applicant provided a GLP compliant controlled, randomised, masked study to determinate the speed of kill of adult fleas (*Ctenocephalides felis*) and adult ticks (*Ripicephalus sanguineus*) following topical administration of pyriprole 12.5% spot-on. Dogs were infested with fleas and ticks on study day -1. The number of live fleas was determined by combing the dogs and ticks were counted at 12, 24, 36 and 48 hours after the treatment.

The study results demonstrated that fleas were killed within 24 hours and ticks within 36 hours post-exposure.

Ticks

The applicant provided 11 laboratory studies investigating the efficacy of the final pyriprole formulation 12.5% w/v against adult ticks on dogs. The ticks species included in these studies were relevant ticks species in Europe (*Ripicephalus sanguineus, Ixodes ricinus Dermacentor reticulatus)* but also American ticks *(Ixodes scapularis, Dermacentor variabilis, Amblyomma americanum).*

Dogs were randomly assigned to the different treatments groups (different pyriprole formulations or placebo) and repeatedly (3-5 times at various study days) infested with ticks (male and female) of one species over a period of up to 30 days. In one study *(D. reticulatus)*, dogs were pre-infested with ticks on Day –1 before treatment in order to demonstrate also curative efficacy.

Ticks from the fur of the treatment and placebo control animals were counted (by hand or comb) and removed, 48 hours after each tick infestation. Efficacy was determined on the basic of percentage reduction in live ticks counts in the treated groups compared to placebo treated group. Others

parameters monitored were bodyweight, clinical observations, general health, behaviour, appetite, application site reactions, cosmetic effect, mortality, hair and skin.

Study results from one study investigating the curative effect demonstrated an effect of more than 93% at day 2 against an existing tick infestation (*D. reticulatus*). This was also demonstrated in the combined ticks & flea study (see above) with *R. sanguineus* (efficacy of more than 99% at day 2).

Concerning the preventative effect, results showed satisfactory efficacy rates up to 30 days against infestations with *I. scapularis, R. sanguineus, I. ricinus and D. reticulatus* of more than 96%, 99%, 99% and 98%, respectively. Treated animals showed in general no live attached engorged ticks whereas untreated animals showed live attached engorged ticks.

Efficacy against two further, American tick species, *Dermacentor variabilis* and *Amblyomma americanum,* was investigated in 4 additional GCP-compliant dose confirmatory studies carried out in accordance with CVMP guideline (EMEA/CVMP/005/00). The efficacy against these tick species was over 90% at all time points up to 30 days post treatment.

Based on the results of these laboratory studies, the CVMP concluded that PRAC-TIC is effective in the selected dose in the prevention and treatment of tick infestations in the above tick species for up to 30 days. However, no laboratory study was presented for *Ixodes hexagonus* and, therefore, it was not accepted as a target tick.

Testing for water stability

Pyriprole is liposoluble and shampooing dissolves fatty acids in the fur. Therefore, the impact of immersion of dogs in water and / or shampooing on the efficacy of the product was investigated.

The effect of regular weekly water immersion was tested in 2 studies against fleas and ticks and no effect on the efficacy was seen.

The impact of shampooing was demonstrated in studies against fleas and ticks.

However, the influence on the effect of shampooing prior to treatment has not been studied. Therefore, a statement was added to the SPC and product literature, “Dogs should not be bathed or shampooed 48h before treatment”.

In ticks, shampooing 24 hours post treatment reduced the efficacy on Day 30 as compared to the non-shampooed group; however, efficacy still remained above the 90% threshold. Shampooing 48 hours post treatment or immersion in water 8 hours did not affect the efficacy against ticks. In fleas, shampooing had no negative effect on treatment. However, the shampooing effect was only tested 24 hours post-treatment. Appropriate recommendations have, therefore, been added to the SPC and product literature: “Immersion of the animal in water or shampooing within 24 hours after treatment may reduce the efficacy of the veterinary medicinal product. However, weekly immersion in water did not affect efficacy against fleas and ticks.”

Testing for photo stability

Unlike another phenylpyrazole (fipronil), pyriprole is not susceptible to photo-degradation. Stress tests on the active substance and finished product demonstrated good stability under UV light. Efficacy of the product was tested and confirmed in the two clinical studies, where dogs were exposed to sunlight. No reduction of the persistent efficacy was observed.

Field trials

Fleas

The Applicant provided a GCP-compliant multicentre field study examining the efficacy and safety of PRAC-TIC administered according to the recommended posology to dogs presented in different veterinary clinics in France, Belgium and UK.

The study was randomised, blinded, controlled and included a large number of dogs naturally infested by fleas, of different breeds, aged between 3 months and 16 years and weight between 3 to 64 kg. Dogs were either treated with PRAC-TIC (12.5 mg pyriprole per kg bodyweight) or with a positive control containing selamectin (authorised in EU). Treatment was repeated (with both products) after 30 days.

The inclusion criteria were evidence of flea infestation and / or evidence of flea activity in the form of flea droppings, eggs, flea bites or Flea Allergy Dermatitis (FAD). Dogs were monitored at days 0, 14, 30 and 60 for viable flea count, clinical observations and applications site reactions.

The results of the study demonstrated comparable results in the efficacy of treatment and prevention of flea infestations between the PRAC-TIC and the positive control group. Non inferiority product was demonstrated. The product was well tolerated in all dogs.

Significant improvement for the clinical parameters with regard to FAD parameters (pruritus and skin lesions) were noted. The CVMP, therefore, agreed that the product could be used as part of a treatment strategy for the control of Flea Allergy Dermatitits (FAD).

Ticks

The Applicant provided a GCP-compliant multicentre field study examining the efficacy and safety of PRAC-TIC administered at a minimum dose of 12.5 mg/kg for the treatment of dogs presented as veterinary patients in different veterinary clinics in France and Germany.

The study was randomised, blinded, controlled, and included dogs naturally infested by ticks, different breeds, age between 3 months and 15 years and weight between 4 to 69 kg.

Dogs were either treated with PRAC-TIC (12.5 mg pyriprole per kg bodyweight) or with a positive control containing fipronil (authorised in EU). Treatment was repeated twice (with both products), after 30 and 60 days.

Viable and dead ticks were counted prior to the treatment and dogs infested with at least one tick were included in the study.

The parameters monitored were tick count (viable and dead, attached and not attached), clinical observations including applications site reactions at Days 0, 7, 14, 21, 30, 45, 60 and 90. Body weight was controlled at Day 0, 30, 60 and 90 and blood haematology and serum chemistry was performed at beginning and end of the study (Day 0 and 90).

The efficacy was based on the number of live ticks counted at a single time-point or over a time period consisting of several samples. The results were compared between the two groups (test and control) and non-inferiority analysis was performed. Safety of the test product was assessed on the basis of general health, clinical observations, application site reactions and results of blood samples.

Ticks included in this study were *Dermacentor reticulatus*, *Ixodes hexagonus*, *I. ricinus*, and *R. sanguineus*.

Non-inferiority on the predefined primary endpoint (total viable tick count at single days) could not be established, due to the high variance between animals. In order to prove non-inferiority on viable tick

counts, around 2,700 dogs would have needed to be included in the study. However, non-inferiority of pyriprole against fipronil was shown regarding viable tick count reduction (efficacy). This endpoint was considered the most suitable endpoint to demonstrate efficacy of the product.

The efficacy of both products in this field trial is below 90% on most time-points. However, the CVMP recognised that under field conditions, results might not be as robust as those derived from laboratory studies. The CVMP acknowledged the lower efficacy rate in the field trial; however, taking into account the result of the dose confirmation studies, efficacy in the prevention and treatment of tick infestations was considered comparable to another product, authorised in Europe.

Overall conclusion on efficacy assessment

The mode of action of pyriprole is attributed to actions at the GABA receptors (non competitive blockage of pre- and post-synaptic transfer of chloride ions) resulting in uncontrolled activity of the central nervous system. As pyriprole is quickly metabolised after systemic absorption, the neurotoxic effects of pyriprole are attributed to its major metabolite in circulation. Species-specific differences have been demonstrated, with selective toxicity to insects. Following contact (rather than systemic) exposure, insects are killed within 24 - 48 hours post-exposure. In dogs and rats, direct effects of pyriprole (i.e. adverse reactions) are associated with transient effects on the nervous system. Secondary pharmacological effects (histopathological changes in the liver and thyroid) were only observed in rodents.

The final formulation selection was based on efficacy results and on comparison of different formulations on their cosmetic appearance on dogs. The pivotal dose selection study was conducted with three doses testing efficacy against the least susceptible tick species, *Ixodes ricinus*. A dose of 12.5 mg/kg was selected.

Tolerance to the product in dogs has been shown good following repeated monthly application of the recommended dose for up to 6 months. The prominent adverse effects of pyriprole correlate with the interaction at the GABA receptor, resulting in neurological signs such as impaired muscle control. Unlike rats, no disturbance of the pituitary-thyroid axis was observed in dogs.

Fifteen studies were performed to confirm the dose selected using *Ctenocephalides felis* and mainly European ticks; however, four studies were against US ticks (*Ixodes scapularis* and *Amblyomma americanum)*. The studies confirm the efficacy of the proposed dose of 12.5 mg pyriprole.

The impact of immersion of dogs in water and / or shampooing on the efficacy of the product was investigated and appropriate recommendations have been included in the product literature. Pyriprole is not susceptible to photo-degradation and no reduction of the efficacy was observed in UV light tests or under field conditions (when the product was exposed to sunlight).

Two field trials have been presented, one with ticks and other with fleas. A large number of dogs of different breeds, between 3 months to 16 years and weight between 3 to 69 kg were enrolled in the studies. The studies were performed under GCP. The design of the studies was considered suitable for the efficacy assessment at the therapeutic treatment dose. All studies were run as multicentre, randomised, blinded and controlled studies. The monitored parameters were tick count (viable and dead, attached and not attached) and viable flea count. Clinical observations, applications side reactions and body weight were monitored in both studies. Blood haematology and serum chemistry parameters were monitored in the ticks study. Selamectin and fipronil were used as positive controls in ticks and fleas studies, respectively. The efficacy was based on the number of live ticks and number of flea counts at a single time-point or over a period consisting of several samples time. The results were compared between the two groups (test and control) and non-inferiority analysis was performed. Statistical primary efficacy were non-inferiority analysis for total viable ticks count at single days and for average AUC levels for total viable ticks count time/profiles AUC/{t}. The statistical unit was the experimental group for central values of group and individual animal in the other cases. The results support the efficacy of the product.

5. RISK BENEFIT ASSESSMENT

The quality aspects of the product have been well documented and comply with current guidelines. Suitable descriptions of the active substance and the chosen formulation have been provided, demonstrating that production of both the active substance and the product leads to a consistent quality. Analytical methods are well described and data of their validation confirm their suitability. Both of the excipients used in the manufacture of the product are well established for use in topical dosage forms. The method of preparation for the finished product is appropriately described, jointly with the planned validation on an industrial scale.

Stability studies have been performed according to VICH guidelines. The studies are on going, but based on the submitted data (12 months for the active substance and 18 months for the final product, with additional confirmatory data for the latter) the applicant has justified the proposed retest period of 24 months and shelf life of 24 months.

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.

Following topical administration, pyriprole is rapidly distributed in the hair coat of dogs within 1 day after application. Absorption of pyriprole through mammalian skin is low and species-specific differences were observed. Following percutaneous absorption, the parent compound is rapidly metabolised in the liver into pharmacologically active metabolites. The major pathway of excretion is via faeces.

Pyriprole exerts a moderate toxic effect after a single oral dose administration in the rat. Following dermal administration, toxicity is low in the rat and moderate in rabbits. Pyriprole is non-irritating to the skin, slightly irritating to the eye and does not appear to be a skin sensitising agent.

Pharmacodynamic and toxicological effects are attributed to the major metabolites, which act as potent inhibitors of the GABA-gated chloride channels. Insects such as fleas and ticks are killed within 24 - 48 hours post-exposure due to contact (rather than systemic) exposure. In dogs and rats, adverse reactions following systemic exposure are associated with transient neurological signs (incoordination, muscle tension, ataxia, sensory induced convulsions and seizures). Secondary pharmacological effects (histopathological changes in the liver and thyroid) were only observed in rodents, which are known for species-specific sensitivity towards disturbances of the pituitary-thyroid axis. Appropriate warnings regarding neurotoxicity have therefore been included in the SPC and product literature.

Pyriprole did not show reproductive toxicity in laboratory animals; however, no data were submitted for the target species and an appropriate warning has been included in SPC and product literature.

Data on human exposure have not been provided; however, taking into account the lower percutaneous absorption of the parent compound through human skin and an approximately 5-fold lower activity of the active metabolites at the human GABA receptor than in the rat; the CVMP agreed that user safety of the product is sufficiently addressed and the user risk management procedures detailed in the SPC and package leaflet are appropriate.

The product is for use in individual dogs and a Phase II environmental risk assessment is not required. However, pyriprole may adversely affect aquatic organisms and an appropriate disposal warning has been included in the SPC and package leaflet.

The product was well tolerated in dogs following repeated monthly application of the recommended dose for up to 6 months.

No information on pyriprole resistance has been provided; however, no European flea strain with resistance to fipronil has been isolated yet and good efficacy against fleas was shown in the field trials

with PRAC-TIC conducted in several European countries. The CVMP, therefore, concluded that the risk of resistant parasites was minimal.

The final formulation selection was based on efficacy results and on comparison of different formulations on their cosmetic appearance on dogs. The pivotal dose selection study was conducted with 3 doses testing efficacy against the least susceptible tick species, *Ixodes ricinus*. A dose of 12.5 mg/kg was selected and confirmed by 15 dose confirmation studies in fleas (*Ctenocephalides felis*) and ticks. Most of the tick studies were preformed with European ticks; however, 4 studies were against US ticks (*Dermacentor reticulatis, Dermacentor variabilis* and *Amblyomma americanum)*. The studies confirm the efficacy of the proposed dose of 12.5 mg pyriprole.

The impact of immersion of dogs in water and / or shampooing on the efficacy of the product was investigated and appropriate recommendations have been included in the product literature. Pyriprole is not susceptible to photo-degradation and no reduction of the efficacy was observed in UV light tests or under field conditions (when the product was exposed to sunlight).

Two GCP compliant multicentre field trials were conducted in veterinary practices in different geographic regions in Europe, one with ticks and the other with fleas. The design of the studies was considered suitable for the efficacy assessment at the therapeutic treatment dose. The monitored parameters were tick counts (viable and dead, attached and not attached) and viable flea counts. Selamectin and fipronil were used as positive controls in tick and flea studies, respectively. The results support the efficacy of the product in the treatment and prevention of flea and tick infestations and also the use as part of a treatment strategy for the control of Flea Allergy Dermatitis.

Based on the original and complementary data presented, the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of PRAC-TIC Spot-on solution for dogs were considered to be in accordance with the requirements of Council Directive 2004/28/EC, as amended.