PRODUCT PROFILE

Name:	Stronghold
Active substance/s:	Selamectin
International Non-proprietary (INN) Name:	Selamectin
ATCvet code:	QP 54AA05
Therapeutic indication:	Treatment and prevention of *Ctenocephalides spp*. infestations in cats and dogs Use as part of a treatment strategy for flea allergy dermatitis in cats and dogs Prevention of heartworm disease caused by *Dirofilaria immitis* in cats and dogs Treatment of ear mites (*Otodectes cynotis*) in cats Treatment of sarcoptic mange (*Sarcoptes scabiei* infection) in dogs Treatment of adult intestinal roundworms (*Toxocara cati*) and intestinal hookworms (*Ancylostoma tubaeformae*) in cats Treatment of adult intestinal roundworms (*Toxocara canis*) in dogs. Treatment of biting lice in dogs (*Trichodectes canis*) and cats (*Felicola subrostratus*).

SCIENTIFIC DISCUSSION

1. INTRODUCTION

Stronghold is a topical product containing selamectin intended for use in cats and dogs.

The product is intended for the treatment and prevention of flea infestations caused by *Ctenocephalides spp.* in cats and dogs; use as part of a treatment strategy for flea allergy dermatitis in cats and dogs; prevention of heartworm disease caused by *Dirofilaria immitis* in cats and dogs; treatment of ear mites (*Otodectes cynotis*) in cats; treatment of sarcoptic mange (*Sarcoptes scabiei* infection) in dogs; treatment of adult intestinal hookworms (*Ancylostoma tubaeforme*) and adult roundworms (*Toxocara cati*) in cats, treatment of adult roundworms (*Toxocara canis*) in dogs, treatment of biting lice infestations in dogs (*Trichodectes canis*) and cats (*Felicola subrostratus*).

2. OVERVIEW OF PART II OF THE DOSSIER: ANALYTICAL ASPECTS

PART II A

QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

1. Composition of the veterinary medicinal product

Selamectin is a semi-sythetic compound of the avermectin group. It is synthesised from the fermentation-derived cyclohexyl-B_1 avermectin, doramectin, which is already authorised for veterinary medicinal use. Virtually insoluble in water, it is presented as either a 6% w/v or 12% w/v solution in an alcoholic vehicle containing 0.08% butylated hydroytoluene.

2. Container

The product is supplied in unit-dose, flexible, polypropylene tubes. Tubes delivering 15 or 45 mg selamectin contain the 6 % solution; those delivering 30, 60 120 or 240 mg selamectin contain the 12 % solution.

3. Development Pharmaceutics

The formulation was defined following clinical, stability and processing studies of solutions containing a range of concentrations of selamectin in alcohol based solvent systems with alternative antioxidants.

The question of antimicrobial preservation has not been discussed within the dossier. However, it is considered that the non-aqueous nature of the vehicle and the inherent antimicrobial activity of the alcoholic vehicle render the product relatively invulnerable to contamination or spoilage.

Flexible polypropylene tubes are the primary containers of the product. To minimise the potential loss of the solvent vehicle and the potential uptake of water vapour, tubes are individually sealed within aluminium foil sachets.

II B DESCRIPTION OF METHOD OF PREPARATION

Manufacturing formula

The manufacturing formula has been presented.

Manufacturing process

The manufacture of a 400 litre batch of each strength of solution is provided. Apart from the nitrogen gassing of vessels to minimise fire risk, manufacture is entirely conventional. Allowance is made for the varying potency of selamectin, and the weight of alcohol being appropriately adjusted. Butylated hydroxytoluene (BHT) is added and dissolved with simple stirring, followed by the active substance. The bulk is mixed, and filtered through a 10 µm polypropylene filter cartridge into a holding tank, having rejected the first 2 litres of filtrate. After analytical clearance the bulk is filled into the polypropylene containers and heat sealed, using heated nitrogen gas. A filling overage ensures that the nominal dose volume can be removed from the tubes. In-process controls include the assessment of the bulk solution before and after filtration for Clarity, Content of active substance and Density (in order to define the target filling weight). Fill Weight (and hence, Fill Volume) and Legibility of Batch Number are monitored throughout the filling process. Additionally, visual inspections and pressure testing for Efficiency of Sealing and absence of Leakage are conducted throughout filling.

Validation of the process

Substantial validation work has been carried out on 19 development batches. Data have been summarised showing a homogeneous product is achieved by the proposed manufacturing method: analysis of active substance and antioxidant content at 12 points throughout manufacture and filling of 3 small scale batches (70-100 litres) produced on two sites show the process to be reproducible and the product homogeneous. The product has been shown to be satisfactory when held in bulk for 12 days before filling and when filling has been extended over 6 days. The manufacturing process is therefore considered robust. There is no reason to expect that scale-up will markedly affect the validity of these results, but the Applicant has undertaken similarly to examine the first three full-scale batches of each strength of solution.

II C CONTROL OF STARTING MATERIALS

1. Active substance(s)

Selamectin is a semi-synthetic avermectin, derived by chemical modification of Doramectin which is long established and well characterised. The data on the manufacture and control of the drug substance provided in the dossier is considered to be clearly presented, of a high standard and comprehensive. The synthetic route is chemically feasible under the specified reaction conditions and the product is obtained in good yield with a high degree of purity. Detailed conditions for the synthesis have been included in the dossier. The quality of the avermectin starting material, reagents and solvents is generally controlled by analysis. Some raw materials, such as gases, are accepted on the basis of the manufacturer's guarantee. Methods employed are well documented and, where appropriate, are accompanied by good validation data.

Quality control during manufacture is maintained by high performance liquid chromatography (HPLC) to check the various stages in the synthesis. The Applicant has been thorough in considering actual and potential impurities, as evidenced by the comprehensive manner in which these compounds have been identified and catalogued. Methods for assaying the intermediates and their impurities are considered fit for their purpose and are accompanied by sound validation data.

The structure of the active substance is largely determined by the identity of the starting material and by the method of synthesis. Nuclear magnetic resonance (NMR), infra-red (IR) and mass spectrometry data are well-documented, of high quality and fully support the assigned structure.

No pharmacopoeial monograph is available for selamectin, which instead is controlled by an in-house specification.

Other Ingredients

In the absence of a pharmacopoeial monograph, the solvent employed in the manufacture of the finished product is examined against a Manufacturer's Specification.

For other ingredients, the requirements of the European Pharmacopoeia have been met. Full accounts of any non-compendial methods employed in the evaluation of the other ingredients have been detailed in the dossier.

Containers

Polypropylene tubes are purchased with a Certificate of Analysis from their manufacturers. Identity of the material of construction, shape and dimensional checks are also verified. Tests of extractables, non-volatile residue and heavy metals are not routinely performed on receipt, but are certified by the supplier to meet USP requirements. Testing has demonstrated that the tubes meet the requirements of the European Pharmacopoeia (3rd Edition) in respect of polyolefin materials used for the manufacture of containers. Additionally it has been shown that the tubes do not absorb the active substance or yield extractives into the solvent vehicle. Additional tests using the solvents and stabilisers employed in the formulation have indicated that no exceptional extraction from the containers or absorption into them occurs. On the basis of this evidence the reliance primarily on the supplier's Certificate may be justified. Detailed specifications for the 1 ml and 2 ml tubes with spike caps have been provided with letters of certification from the suppliers and are considered satisfactory.

Cold form aluminium foil is used to form the blister. 20 µm aluminium foil, coated on one side with heat sealable lacquer forms the backing of the pouch. Both foils are accepted on the basis of supplier's Certificates of Analysis, supplemented with visual examination and dimensional checks on receipt. This is considered acceptable for this barrier layer which is not in contact with the product.

II D CONTROL TESTS CARRIED OUT AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

In-process controls are applied as indicated under method of preparation above. There are no intermediate products.

II E CONTROL TESTS OF THE FINISHED PRODUCT

1. Specifications of routine testing

The Release Specifications for the two formulations are identical.

Full details of the test methods and their validations are detailed in the dossier. The assay methods have been soundly validated. The same HPLC assay of the active substance is employed for Identity Testing, Batch Release and Stability Testing of the finished product, and uses the same chromatographic system as that for assay and stability assessment of the drug substance alone. Details of the characterisation of the Reference Standard of selamectin have been given.

The Committee considered that the selamectin content limits, which are applied to the delivered dose and not to the solution filled into the container (which is clearly very tightly limited) represent a very high order of control.

Uniformity of filling is assessed during the determination of mean dose volume, where the Percentage Relative Standard Deviation (%RSD) of the dispensed dose volume is calculated.

For the BHT assay, specificity has been shown in the presence of the excipients and of selamectin and its related substances. Linearity has been demonstrated over the range 10 -200 % of the nominal concentration. Reproducibility of injection, accuracy, precision (repeatability and intermediate precision) and robustness of the method have been satisfactorily demonstrated and a limit of quantitation established. The analytical method used for the assay has been shown to distinguish

between BHT and related compounds, such as butylated hydroxyanisole and therefore forms an acceptable basis for an identity test for BHT.

The proposed release limit for total impurities includes the drug substance impurities and is therefore derived from the selamectin drug substance impurities specification.

The comprehensive batch analysis referred to above have been obtained on 7 batches of the 6 % solution, each of 50 or 80 litres and filled into 0.25 and 0.75 ml tubes. Six batches of 12 % solution, each of 100 litres have been filled into 0.25, 0.5, 1.0 and 2.0 ml tubes. Data indicate that the manufacturing process is reproducible and that compliance with the proposed finished product specifications is routinely achievable.

2. Scientific data

II F STABILITY

1. Stability tests on active substance(s)

In the assessment of drug substance stability, forced degradation studies were conducted which indicated that selamectin is stable to heat in the solid state and in solution. In acid and basic solution degradation is observed, yielding identified impurities detectable using the validated stability-indicating HPLC method. The substance is essentially light stable, slight photodegradation being only observed under intense conditions. In common with other avermectins, selamectin is sensitive to oxidation, again yielding readily detectable degradation products.

The formal stability studies have been undertaken in accordance with the Veterinary International Co-operation on Harmonisation (VICH) guidelines for both the Stability Testing and Photostability Testing of New Drug Substances and Products, except that separate, rather than combined, challenges of exposure to fluorescent and ulta-violet (UV) light were conducted. In the long-term study, 3 batches of selamectin have been stored at 30°C/60% relative humidity (RH) and at 25°C/60%RH for up to 18 months. Samples were kept and in containers representative of the proposed packaging. These tests have been supplemented by accelerated studies under storage at 45°C/75%RH for 6 months, 60°C for 3 months and 25°C/85%RH for 3 months. Tests were also conducted on unprotected material in intense UV light, 200 watts/m², and fluorescent light, 1.2 million lux hours. Stored materials have been examined for appearance, assay, impurities and water content. The results of these heat and humidity studies show an unchanged assay (on an anhydrous basis), negligible change in impurity profile or content, but increasing water content. In some samples, water content rose, but remained within the limit in the raw material specification.

In the light of these findings, the proposed retest interval of 24 months for the drug substance stored in tightly closed containers below 30°C is acceptable.

2. Stability tests on the finished product

The results of forced degradation studies on the product have been summarised. These have shown that thermal degradation can be induced in extreme conditions (50°C for 12 weeks) only in samples where the antioxidant is omitted. Photodegradation is induced only under intense fluorescent light. From these studies it may be anticipated that under normal conditions of handling and storage solutions protected from light by the aluminium blister pack will show no significant degradation. In other stressing studies reported in the dossier, the product has been shown resilient to 7 days exposure to sub-zero temperatures (-20°C) and to 3 cycles of -20°C to ambient over 21 days. The test programme for the packaged finished products has been conducted in accordance with the VICH stability guidelines, using the recommended accelerated, intermediate and long-term test conditions.

The results demonstrate that the formulation is inherently stable, presenting a virtually unchanged appearance, assay and impurities profile across the range of storage conditions. BHT Content shows a temperature-related progressive but slight decline, consistent with its role as a sacrificial antioxidant.

The Applicant is committed to continuing assessment on the stability programmes through their 36 month protocols.

On the basis of the presented data, the Committee concludes that the product is inherently physically and chemically stable. The extrapolation of the shelf-life for the products to 24 months from date of manufacture, when stored below 30°C is considered justified. In view of the potential for alcoholic solutions to absorb moisture, advice to store the product in a dry place is given. The formulation of the product is fundamentally sound. Controls proposed are adequate and appropriate. Good physical and chemical stability has been demonstrated.

Shelf-life

As discussed in Section IIF above, the product may be expected to have a shelf-life of 24 months from date of manufacture under the following storage conditions:

STORE IN THE UNOPENED FOIL PACKAGE AT OR BELOW 30°C
STORE IN A DRY PLACE

In use shelf-life
Not applicable to these unit-dose presentations.

3. OVERVIEW OF PART III OF THE DOSSIER: SAFETY AND RESIDUES

PART III A SAFETY

3.1 PRECISE IDENTIFICATION OF THE SUBSTANCE CONCERNED BY THE APPLICATION

The application is for a spot-on product for the treatment and prevention of diseases caused by endo- and ecto-parasites in cats and dogs. The product contains the active substance selamectin, which is a semi-synthetic compound of the avermectin class. The product is supplied in various sized plastic tubes, the largest of which contains 2 ml of 12% solution (240 mg of active substance). This is designed to treat a dog weighing between 20.1 and 40 kg.

As the product is intended for use in companion animals only, there is no requirement for residue data.

3.2 RELEVANT PHARMACOLOGICAL STUDIES

3.2.1 Pharmacodynamics

The Applicant has submitted a number of published papers on the mode of action of the avermectin class of compounds. No pharmacodynamics studies are reported specifically for selamectin, the justification being that all avermectins possess the same mode of activity.

It is proposed that the action of the avermectins is due to specific binding to the post-synaptic gamma-aminobutyric acid (GABA) receptor-chloride ion channel complex. This binding leads to an increase of the density of the available high affinity GABA receptors and stimulation on GABA release from the presynaptic end of the GABA synapse.

Very little metabolism was observed in dogs. Minor metabolites resulting from hydroxylation at several sites on the molecule were identified. Quantitatively, there was more metabolism observed in cats than in dogs, but the primary metabolic route was different. The major metabolite in cats was oxidation at the
C-24 position with the formation of a carboxylic acid. The more polar nature of carboxylic acid metabolites, in general, lead to reduced pharmacological activity and enhanced elimination from the body.

Thus it is very unlikely and there is no evidence to suggest that the metabolites identified in cats and dogs would contribute any pharmacological activity with this product.

3.2.2 Pharmacokinetics

The pharmacokinetics of selamectin have been evaluated in the rat, cat and dog following oral, intravenous and topical administration.

Rat:

Oral:-
A group of Sprague-Dawley rats was administered a single oral dose of 5 mg/ml selamectin in sesame oil at a dose volume of 2 ml/kg. The results indicate that selamectin is rapidly absorbed, with C_{max} being achieved within 4 hours and exceeding 1 μg/ml in all animals. Selamectin was rapidly eliminated with a half-life of 10.3 hours.

Intravenous:-
The plasma pharmacokinetics of selamectin were evaluated in male and female Sprague-Dawley rats following a single intravenous dose of 0.1 mg/kg. A significant difference was observed between male and female animals; in males the plasma concentration was below the limit of quantification 2 hours after dosing, but was detected in the majority of females up to 24 hours. The area under the curve (AUC) for females was 865±165 ng.hr/ml, the volume of distribution was approximately 2.23±0.37 l/kg, clearance was 115.67±22.03 ml/kg.hr and half-life was approximately 16.5 hrs. No values could be derived for males due to the limited points on the elimination phase of the curve.

Topical:-
A 6% formulation (12 mg/kg) of the commercial product was applied topically to the shaved skin of groups of 5 rats and the animals collared to prevent ingestion of the product during grooming. The results show that the product is slowly absorbed, with C_{max} being achieved within 24 hours and slowly eliminated with a half-life of elimination of 60 hours.

Dog:

Topical:-
A group of three male and three female beagle dogs received 1 ml of tritiated 12% formulation (120 mg/kg) topically to a single site at the base of the neck and to the front of the scapulae. Urine, faeces and pan rinse samples were collected daily and shed fur specimens obtained from filtering the urine were pooled for each dog. Cage washes were collected on days 15, 35 and 42 and assayed for radioactive content. After 42 days, the animals were euthanased and tissues assayed for radioactive content.

It is noted that approximately the same percentage of radioactivity is present in shed fur as in faeces.

Intravenous, topical and oral:-
A group of 6 male and 6 female beagle dogs was treated sequentially with intravenous doses of 0.05 mg/kg, 0.1 mg/kg and 0.2 mg/kg selamectin, 24 mg selamectin/kg topically and 24 mg selamectin/kg orally, in order to establish the pharmacokinetic order and profile of selamectin in the target species.

After single, 30 minute intravenous infusions to each dog at dose levels of 0.05, 0.1 and 0.2 mg/kg, the mean maximum plasma concentrations at the end of the infusion and the areas under the plasma drug concentration-time curves (AUC_{∞}) were linearly related to the dose level. The mean systemic clearance and residence time were independent of the dose level administered. The systemic clearance in dogs represented approximately 1-2% of cardiac output. Within individual animals the clearance of drug was similar at each dose level, some inter-animal differences were noted.

After infusion, plasma concentrations declined apparently biphasically, with a mean terminal phase half-life of approximately 14.0 hours. Mean terminal phase half-lives were independent of dose level; inter-animal differences were noted.

Mean maximum observed plasma concentration was approximately 86.5 ng/ml and was reached approximately 3 days after a single topical dose. For the oral dose C_{max} was approximately 7630 ng/ml and was reached approximately 8 hours after dose administration. Mean terminal half-life was 45.7 hours for oral administration.

Dose-related differences in clearance and steady-state volume of distribution were not statistically significant ($p \geq 0.807$) and the 90% confidence intervals were contained within ±20% of the overall parameter means. The similarity in clearance between dosages established clearly the linearity of the pharmacokinetics of selamectin over the dose range 0.05-0.2 mg/kg. Sex-related differences of C_{max} and AUC_t after the topical doses were also not statistically significant ($p \geq 0.462$) but the 90% confidence intervals were not contained within ±20% of the overall parameter means. After the oral doses, sex-related differences of C_{max} and AUC_∞ were not statistically significant ($p \geq 0.198$).

Metabolism:-
Radio-labelled selamectin was eliminated in the faeces (18-20% of the dose) and urine (1-3%) following a single topical dose. Approximately 99-100% of the radioactivity in dog urine was associated with selamectin. No metabolites were detected in dog urine. The majority of the radioactivity in the faeces was associated with selamectin and represented approximately 39% and 64% of the radioactivity in female and male dog faeces respectively. Major metabolites (>20% of the total radioactivity) were not detected in the dog faeces. However, several metabolites constituting less than 10% of the total radioactivity were detected in the faeces samples. The majority of the metabolites in the faeces were primarily oxidation products of the O-desmethyl selamectin. Additionally, O-desmethyl selamectin was also detected in the faeces samples.

Cat:

Topical:-
A group of three male and three female cats received 0.75 ml of tritiated 6% formulation (60 mg/kg) to a single site at the base of the neck and to the front of the scapulae. Urine, faeces and pan rinse samples were collected daily and shed fur specimens obtained from filtering the urine were pooled for each cat. Cage washes were collected on days 15, 35 and 42 and assayed for radioactive content. After 42 days, the animals were euthanased and tissues assayed for radioactive content.

A very low percentage of radioactivity was found in the shed fur in cats when compared to that recovered in dogs.

Intravenous, topical and oral administration:-
A group of 6 male and 6 female cats was treated sequentially with intravenous doses of 0.05 mg/kg, 0.1 mg/kg and 0.2 mg/kg selamectin, 24 mg/kg selamectin topically and 24 mg selamectin/kg orally, in order to establish the pharmacokinetic order and profile of selamectin in the target species.

The mean systemic clearance and mean residence time were independent of the intravenous dose level of selamectin administered. However, interindividual differences were noted.

Following intravenous dosing, the maximum plasma concentration and AUC were linearly related to the dose level administered and plasma concentrations declined apparently polyexponentially. For dosages up to 0.2 mg/kg, mean systemic clearance, mean residence time and terminal half-life were independent of dose. Comparison of equivalent doses administered orally and topically shows that C_{max} for oral dosing was reached more rapidly than for the topical dose and that the mean terminal half-life was shorter. The mean relative bioavailability following topical administration was approximately 68%.

Metabolism:-
The major route of elimination in cats was in the faeces (48-60% of the dose) with approximately 1-3% of the dose recovered in the urine. The majority of the radioactivity in the faeces was associated with the parent compound (26-43%). Selamectin accounted for more than 95% of the radioactivity observed in the urine of male cats and 58% in the female cat urine. The major metabolite in cat faeces and urine had undergone oxidation of the C-24 methyl group of selamectin to carboxylic acid and represented approximately 14-30% of these samples. The C-24 oxidative product of the O-desmethyl glycoside was present in small quantities (approximately 3% of the total radioactivity) in cat faeces.

In Vitro Dermal Absorption:-
In an *in vitro* dermal absorption study, the objective was to measure the transfer of selamectin across dog, cat, rat and human cadaver skin. The results demonstrated that maximum transfer of material occurred within the first 8 hours for skin from all species evaluated and equilibrium was achieved within 24 hours for cat, rat and cadaver skin and 48 hours for dog skin. At the end of the 72 hour exposure period, only 3, 5, 2 and 3 μg equivalents of selamectin were detected in the reservoir fluid following application of the dosing solution to dog, cat, rat and cadaver skin. This shows that most of the dosing solution remains on the skin and is not absorbed. This study demonstrates that very little of the dose is absorbed, therefore there is a risk of exposure to the animal owner.

3.3 TOXICOLOGICAL STUDIES

3.3.1 Single oral dose toxicity

The Applicant has submitted the results of two studies with low numbers of animals one in rats and one in mice. The studies were performed to the requirements of the GLP guidelines.

In the rat there were no mortalities in any of the groups, either treated or control. Diarrhoea was present in both treated groups and the control group, appearing 0.25-5 hours after dosing and being present after 3 hours in controls, 27 hours in 800 mg/kg group and 4 hours in 1600 mg/kg group. Additional clinical signs in 1600 mg/kg group included dyspnoea, hunched posture, chromodacryorrhea, partially closed eyes, decreased activity and piloerection.

In the mouse there were no mortalities in any of the groups, either treated or control, therefore an LD_{50} could not be calculated. Diarrhoea was present in treated and control groups, although the incidence was higher in the treated groups. Onset occurred 0.25-2 hours after dosing and being present after 1 hour in controls, 2 hours in 800 mg/kg group and 4 hours in 1600 mg/kg group. Diarrhoea was the only clinical sign present in mice treated with selamectin at the dose levels used.

The low numbers of animals per group used in both studies did not provide sufficient statistical confidence in the results, selamectin was clearly of low acute oral toxicity.

3.3.2 Repeated dose toxicity

The Applicant has submitted a number of pilot studies and three-month oral toxicity studies in rats and dogs. All the studies were performed to Good Laboratory Practice (GLP) guidelines.

A 14-day rat oral gavage pilot study was conducted. However, a No Observed Effect Level (NOEL) could not be determined in this study because effects were observed at the lowest oral dose of 80 mg/kg body weight.

In a 3-month oral gavage study in the rat, three animals died during the study, 2 high dose males and 1 intermediate dose male - these deaths were not treatment related. Changes in white blood cell parameters and red blood cell parameters were observed in the high dose group males and females - these were associated with inflammatory tissue changes. Sporadic changes in serum chemistry parameters were observed in the intermediate and high dose groups but were not regarded as treatment related due to the random nature of their occurrence. Slight increases in mean absolute liver weights in the males and females in the high dose group, females in the intermediate dose group, slight increase

in mean absolute adrenal gland weights in high dose males and females. A correlative increase in mean relative liver and adrenal gland weights was observed. Microscopic findings included treatment-related fatty change in the liver, lymphatic dilatation in the lamina propria of the jejunum and duodenum and in the ileum and colon, adrenal gland hypertrophy and inflammation in the urinary bladder and renal pelvis of the kidney.

A NOEL of 5 mg/kg/day orally was derived from the results of this study.

A 14-day oral gavage pilot study in the dog was conducted.

In a 3-month oral gavage study in the dog no mortalities were reported. The only reported clinical signs were emesis (more frequent in treated than control animals), salivation and loose stools (described as light coloured and oily in appearance in mid and high dose groups). The serum chemistry and liver changes observed with 80 mg/kg orally for 2 weeks were not observed with 40 mg/kg orally for 3 months.

A NOEL of 15 mg/kg/day orally was derived for this study.

In a 4-week topical study in the dog, no deaths occurred and there are no reports of treatment related changes to the parameters measured. At 168 hours after the third dose, the plasma concentration was lower after administration of 80 mg/kg than after 48 mg/kg. No explanation for this effect is provided, but it seems likely to be due to experimental error. It is noted that no vehicle control group was included in the study. Presumably because no irritation was observed at the site of application, histopathological examinations of treated and untreated skin were not made.

A NOEL of >80 mg/kg/week orally was derived from this study.

3.3.3 Tolerance in the target species

Tolerance in the target species
In the topical repeat-dose study, dogs received doses of 16, 48, and 80 mg/kg administered at weekly intervals. Given that the relative bioavailability (topical/oral) of selamectin in dogs dosed at 24 mg/kg was 0.069, these topical doses would result in systemic exposure corresponding to weekly oral doses of approximately 1.0, 3.3, and 5.5 mg/kg, respectively. The 3-month repeat-dose study in dogs used oral doses of 5, 15, and 40 mg/kg/day. Systemic exposure in the orally treated dogs clearly far exceeded the topically dosed dogs, especially when one considers that dogs were dosed daily in the oral dose study and weekly in the topical dose study. Plasma concentrations were obtained in each study, and although the timepoints are not directly comparable, the data also supports much greater exposure in the 3-month repeat dose study in which histopathology was performed. For example, the mean plasma concentrations on day 15 at 24 hours after dosing in the oral dose study were 0.343, 1.225, and 2.542 µg/ml, respectively for the 5, 15, and 40 mg/kg/day doses, whereas the mean plasma concentrations at 72 hours after the 4th dose in the topical dose study were 0.088, 0.215, and 0.375 µg/ml, respectively for the 16, 48, and 80 mg/kg doses. Thus, the histopathology reported for oral dosing in the 3-month oral toxicology study represents exposures far above those obtained in the topical repeat-dose study. In addition, histopathology was completed in the 10X dose dogs in the margin of safety study with topical administration. Therefore, no additional histopathology data from topically dosed dogs are necessary.

Systemic exposure in the rat and the clinical exposure in the target species have been calculated for a comparative safety assessment based on margin of exposure. Systemic exposure in the rat reproductive toxicity studies substantially exceeds clinical exposure in commercial use for both the dog and the cat. Furthermore, the systemic exposure at all stages of gestation in the reproductive safety studies, which used three times the commercial dosage, also exceeds the maximum clinical systemic exposure. The safety of selamectin at the commercial dosage in pregnant dogs and cats has therefore been confirmed.

The Applicant reports 14 additional studies performed in the target species, dogs and cats, and these are evaluated in the Part IV assessment report.

3.3.4 Reproductive toxicity (including teratogenicity)

In one study between gestational days 6-17, mean gestational food consumption decreased in female rats treated with 60 mg/kg/day. Maternal body weights were decreased from gestational day 16 to post-partum day 10 in 60 mg/kg animals. Increased incidence of resorptions and dead foetuses was observed in animals treated with 60 mg/kg. An increase in the number of pups born dead in the 40 and 60 mg/kg groups was observed. Sex ratio (% male) was 51, 42, 49, 37% for dose levels 0, 10, 40, 60 mg/kg respectively. At necropsy, discolouration in the vaginal area was noted in one dam and brownish fluid in both uterine horns was noted in another dam, both in the high dose group.

A NOEL of 10 mg/kg/day orally was derived from the results of this study.

In a modified segment I fertility and reproduction study in both male and female rats, food consumption reduced in F_0 males (60 mg/kg/day group) during week 2 of treatment and F_0 females during lactation. Food consumption increased in F_1 males and females in 10 and 25 mg/kg/day groups. Overall mean gestational body weight gains for high dose F_0 females were significantly reduced. Clinical signs were limited to sporadic vaginal discharge in all F_0 generation treated groups. Significant decreases in mean pup weight were noted for both male and female offspring in 60 mg/kg/day group; offspring of 10 and 25 mg/kg/day groups had increased mean body weight when compared to controls. Reduced fertility and litter size occurred at 60 mg/kg/day in F_0 generation. Fetotoxicity was noted in the 25 and 60 mg/kg/day groups as indicated by a significant increase in pre-parturition loss in these groups.

A NOEL of 10 mg/kg/day orally has been derived from the results of this study.

In a rat oral gavage study, mean gestational food consumption was significantly lower in the 40 and 60 mg/kg/day groups between days 6 and 12 of gestation. Mean gestational body weight gains were lower in high dose group throughout dosing period, but not significantly. Sporadic incidences of vaginal discharge and staining (reddish brown), were observed from gestational day 13 to caesarean on gestational day 21 in all treated and control groups. Enlarged right atria were observed in litters in the 40 and 60 mg/kg/day groups. A low incidence of septal defects and common truncus arteriosus and levocardia is reported in the high dose group only. In all groups, including controls, a thick fibrin material was observed in the thoracic cavity of some foetuses. In some instances, both the enlarged right atrium and the fibrin mass were observed in the same foetus.

A NOEL of 10 mg/kg/day orally for maternotoxicity and teratogenicity was determined.

This study was performed in line with the current OECD Guidelines. The incidence of cardiac defects observed in the high dose group was not considered. The incidence of the fibrin mass was the same for the low dose group and the control group and increased with higher dose. The assumption that the NOEL is 10 mg/kg/day is therefore questionable. Similarly, the significance of the vaginal discharge is unclear. However, despite the deficiencies in the Applicant's interpretation of the study, it appears that the NOEL for teratogenicity would be at least 10 mg/kg/day orally.

3.3.5 Mutagenicity

An Ames test, an *in vitro* chromosome aberration test, a mammalian mutation assay test and an *in vivo* micronucleus test were carried out. These tests were in accordance with the relevant OECD guidelines. They included appropriate positive and negative controls and utilised suitable concentrations of test substance. Selamectin was shown not to possess mutagenic activity in the range of *in vitro* and *in vivo* assays listed above.

Because no pharmacokinetic data or metabolism data were available in the mouse or rat, justification was presented by the Applicant as to why rat S-9 was used as a metabolic system in the *in vitro* genotoxicity studies as well as why the mouse was used in the micronuclei study.

The pharmacokinetic studies have shown that selamectin, with sesame oil as the vehicle (as also employed in the *in vivo* genotoxicity studies), is rapidly absorbed following oral administration in rats, cats and dogs. The highest oral dose level evaluated, 1000 mg/kg, is sufficiently in excess of the recommended topical dose of 6 mg/kg in the target species and the potential maximum topical dose of 12 mg/kg in a 20 kg child.

The pharmacokinetic studies in dogs indicate that absorption following topical administration of selamectin is approximately 4% of the dose. Excretion is predominantly via the faeces, indicating an involvement of the liver. The majority of the absorbed selamectin is excreted as unchanged parent drug. In the cat, absorption is approximately 83% (although grooming may have influenced this level), but the faeces are the main route of excretion, thereby involving the liver. Up to 43% of the absorbed selamectin is excreted as unchanged parent drug and the remainder is oxidised at C-24 to carboxylic acid.

The involvement of the liver in the metabolism of selamectin following topical administration justifies the use of the S-9 mix in the *in vitro* study. The results of the studies provide a sufficient safety margin with respect to user safety.

3.3.6 Carcinogenicity

The Applicant has not submitted the results of any *in vivo* carcinogenicity studies. The Applicant cites the lack of any *in vitro* or *in vivo* genotoxicity, the structural relationship with abamectin (non-tumorigenic in rats and mice) and low likelihood of chronic exposure of the user as justification for no requirement for carcinogenicity assays.

Evaluation of the potential for carcinogenicity was part of the human safety assessment. Because selamectin does not belong to a structural class of suspected potential carcinogenicity, lacks *in vitro* and *in vivo* genotoxicity, and is closely related to abamectin, which is not tumorigenic in rats and mice, there is no reason to suspect that this compound would be carcinogenic in humans.

The long half-life of selamectin results from the systemic circulation of drug within the treated animal. Pet owners clearly will not be exposed to the concentrations of drug in the blood of animals. Human exposure to the drug could result through contact with treated animals, but this exposure will be minimal and infrequent when the product is used as labelled.

The Applicant has justified the absence of carcinogenicity studies in terms of human safety by reference to the structure of selamectin and the satisfactory results of the mutagenicity studies. Comparison of the structure of selamectin with abamectin (shown not to be carcinogenic in *in vivo* studies), shows no structural alerts likely to suggest a carcinogenic potential for selamectin. The satisfactory results in the genotoxicity studies support the absence of long-term carcinogenicity studies.

3.4 STUDIES OF OTHER EFFECTS

3.4.1 Special studies (e.g. specific target organ toxicity)

Acute dermal irritation and corrosion

The potential of selamectin to produce irritation or corrosion to rabbit skin was assessed in line with OECD guideline 404. Very slight erythema was observed in 5/6 rabbits one hour after removal of the dressing. These reactions had cleared by the 24 hour reading. No oedema was observed at any of the reading times.

Dermal exposure to the user is stated as infrequent and intermittent, however, the animal will be treated regularly once a month and user exposure will reflect this treatment regimen. Based on a dermal absorption study in the dog, the daily exposure to selamectin by the user in the worst case

scenario was 0.0079 to 0.0347 mg/kg/day. The assumption is made that dermal absorption in the dog and man are identical based on *in vitro* absorption studies.

Acute ocular irritation

The potential ocular irritancy of selamectin was investigated in the rabbit eye according to OECD guideline 405, as required for a topically applied product.

There were mild reactions which had begun to resolve by the 48 hour reading and by day 7 reactions had totally cleared in 5/6 animals, with all reactions resolved by day 14.

The active substance selamectin was classified as slightly irritant to the rabbit eye.

Skin sensitisation

A Magnusson and Kligman maximisation test was performed on the active substance selamectin at concentrations of 16% for intradermal injections and 44% as a paste in water for injection for topical application. Under the conditions of this study, selamectin was shown not to induce sensitisation in the guinea-pig.

Selamectin has been classified as very slightly irritant to rabbit skin and slightly irritant to the rabbit eye and was negative in a skin sensitisation study. However, in response to the CVMP's concerns with respect to the final formulation of Stronghold and the high level of alcohol present, the Applicant believes that sufficient data on the alcohol existed in the literature to adequately assess the safety of the product. If accidental eye exposure occurs the eyes should be flushed with water immediately and medical attention sought. The Applicant has prepared monographs on all additional components of the commercial preparation.

Neurotoxicity

This class of compounds has been shown to induce neuropathies in use. Although the present application does not indicate neurotoxicity, the Applicant has addressed this issue.

Although species-specific neurological symptoms have been observed for other avermectins, no clinical signs of neurotoxicity or neuropathies were observed in any of the toxicology studies including those in which selamectin was daily administered orally in sesame oil at doses up to 80 mg/kg for 3 months. Likewise, no neurological clinical signs were observed in 18 ivermectin–sensitive collie dogs (6/dose level) following topical administration of the commercial formulation once every 28 days for 3 months at a minimum of 6, 18 or 30 mg/kg. Therefore, the evidence suggests that the use of selamectin will not induce neurological symptoms.

3.4.2 Observations in humans

Selamectin is being developed for use in veterinary medicine and no data concerning the potential effects in humans are available.

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

The Applicant has not submitted any information on microbiological studies on human gut flora and organisms used in food processing. However, the CVMP have agreed that such data are not needed for the avermectins.

3.5 USER SAFETY

In the event of accidental contact with the eye, the product label directs the user to flush eyes immediately with water and seek medical attention. This is a standard precaution for consumer products containing similar excipients.

The Applicant considered that because of the difficulty in assessing all permutations of possible exposure to users of the product (multiple pet households, over-dosing, etc.), a worst-case scenario should be considered assuming that the maximum dose is directly applied and not washed off. This approach overestimates the potential exposure to humans in all realistic conditions of actual use. For example, because of the rapid spreading and drying of the formulation, the exposure of the user to the medicinal product when stroking a recently (less than 2-hours) treated pet will be a very small fraction of the worst case dose. The exposure of the user would be even less from contact with pets beyond the 2 hour period after treatment. Even in a multiple pet household, the worst case scenario presented in the document overestimates the potential exposure. For example, assuming that 5% of the dose could be transferred to a user coming into contact with a recently treated dog, that person would have to experience that contact with 20 animals over a short time to approach the dose used in the worst case scenario. The monthly dosing interval also serves to minimize the opportunities for human exposure to the product.

The possibility of children stroking a treated pet (within two hours of dosing the pet) and then licking their fingers would also result in relatively low exposure still falling within the bounds of the worst case scenario. As the product rapidly spreads on the skin of the animal, a child stroking the pet could only be exposed to the fraction of the dose on the surface of the animals fur. Assuming that even as much as 5% of the dose (100 μL of a 2 ml dose) were transferred to the child's hand and then completely licked off, the resultant dose would still fall below the exposure for the worst case scenario. For a 20 kg child, the dose in this scenario is still 93-fold lower than the NOEL (5 mg/kg/day) in rats.

Based on the low inherent toxicity of selamectin combined with the low potential for exposure and systemic availability, the CVMP now considers the product is expected to be safe when used according to the advice given in the product literature.

The NOEL in rat was derived from a repeat dose study, whilst the potential exposure of a child via contact with a treated pet would be potentially a series of acute exposures. However, an appropriate warning to keep children away from treated animals for 30 minutes has been included in the Summary of Product Characteristics (SPC) and package literature.

Due to the high level of alcohol in the formulation, and its inflammability, an additional warning will be included. The sentence "Keep treated animals away from fires and other sources of ignition for at least 30 minutes or until the hair coat is dry." has been added to section 5.12 of the SPC, sections 9 of the labels and section 13 of the package insert.

The SPC and product literature have also been revised to include a warning against drinking whilst using the product.

3.6 ECOTOXICITY

Ecotoxicity Phase I

Stronghold topical endectocide for dogs and cats is a ready-to-use topical spot-on solution containing selamectin, an avermectin, in single use plastic tubes. The product is administered by application on the animal's back at the base of the neck in front of the shoulder blades. The recommended dose of selamectin provides approximately 6 to 12 mg/kg body weight per single application treatment, with repeat treatments possible every month year-round, depending upon the indication.

Selamectin was applied to dogs at approximately 12 mg/kg body weight. Over the 42 days of the study 18-20% of the dose was recovered in faeces, 1-3% in urine, 19-26% in shed hair, and an additional 15-17% in rinses of the cages and excreta collection pans. Selamectin accounted for 39-64% of the radioactivity in faeces and > 99% of the radioactivity in urine from treated dogs. Several metabolites were also detected in faeces, including O-desmethyl selamectin and oxidation products of this metabolite; none accounted for more than 10% of the total radioactivity. Similar findings were

obtained for cats, although a higher percentage of the dose was excreted in faeces and very little in shed hair. Parent selamectin accounted for 58-95% of the residues in urine and 26-43% of the faecal residues in cats.

The Committee also considered the likely contamination of soil after excretion of parent compound and major metabolites in faeces calculated from the "standard dog walk" model increasingly adopted for such calculations in some EU Member States. Based on metabolism data 50 mg active substance could be excreted during a 2 km walk on a 1 m lead. Resulting contamination of soil would be low and would be further reduced as most excreted material would be in faeces, which if deposited in gardens or parks would be placed in refuse. Shed hair was also not considered to be a significant route of exposure of the environment to the product.

A predicted environmental concentration (PEC) was estimated for a treated dog swimming in a pond. It was assumed that a 40 kg dog was treated at 6 mg active substance/kg body weight and then swims in a pond of 10 m x 10 m x 1 m deep (volume 100,000 litres).

It is likely that only a small percentage of the administered dose of selamectin would be available to wash off animals shortly after treatment. Bathing studies reported in the application demonstrate that efficacy is retained even when dogs are bathed with shampoo as early as two hours after product application. Dogs in that study were treated with the minimum recommended unit dose of 6 mg/kg. Although the company used a conservative approach and assumed that as much as 50% of the applied dose might wash off treated dogs, results of the bathing study demonstrate that this did not occur, as loss of 50% of this dose would, in fact, have adversely affected efficacy. On the basis of this result and data reported for partitioning and wash-off of other topically applied lipophilic agents such as doramectin and ivermectin, as well as data on the distribution of selamectin on dog skin, it is expected that no more than 5 to 10% of the applied dose of selamectin might be available for wash-off at 2 hours post-application.

Exposure of aquatic organisms to residues of selamectin would be intermittent and short term. Residues of selamectin would be rapidly depleted from the aqueous compartment by partitioning into sediment. Such intermittent exposures justify use of a 10-fold safety factor in estimating a predicted no-effect concentration (PNEC) value from acute toxicity data, as no compensation need be made for possible chronic exposure.

In a sediment/water toxicity study with *Daphnia* sp., 1.57 µg selamectin was found in sediment and 0.145 µg selamectin in water (72% recovery of amount added to system). Of the total recovered 8.45% of the active substance was in the water phase. If this is applied to the exposure assessment it can be estimated that of the 24 mg entering the pond, 8.45% or 2.028 mg would be in the water phase. This would give a PEC of 20.28 ng/l and a PEC/PNEC ratio of 0.845.

Using an alternative approach and assuming 10% of the dose is washed off into a 100 m^3 pond, the PEC for the sediment/water system is 240 ng/l. The sediment/water PNEC was previously estimated to be 300 ng/l. This gives a PEC/PNEC ratio of 0.8.

Therefore, if the data on partitioning from the *Daphnia* sediment/water study are used, both assessment approaches produce a PEC/PNEC ratio of approximately 0.8. This PEC/PNEC ratio is considered acceptable for intermittent exposures, although clearly it would vary with different pond sizes. However, it should be borne in mind that the highest dose would be used on large dogs and that for wash-off to occur they would need to be immersed in a reasonable depth of water.

It is concluded that the Company's proposed risk mitigation measure, ie to keep treated dogs out of surface waters for 2 hours after treatment, is acceptable. Instructions to this effect have been included on the SPC and on the product literature."

Ecotoxicity Phase II

Disposal of used containers or unused product was not expected to present a hazard to the environment. Used tubes would be discarded along with collected domestic refuse. If the entire contents of a tube delivering the maximum dose of 240 mg selamectin were emptied into a water body, only about 0.3 mg would be expected to partition into the aquatic compartment as in the PEC scenario above. The following disposal statement will be included on the product label:
Selamectin may adversely affect fish or certain water-borne organisms on which they feed. Containers and residual contents should be disposed of along with collected domestic refuse to avoid contamination of any water courses.

The Phase I and Phase II assessments were followed by one-page summaries of the following studies:

Data summary

One page summaries of the following studies were provided:-

- aqueous solubility
- octanol/water partition coefficient
- vapour pressure
- uv absorption spectrum
- melting temperature
- soil sorption/desorption
- sludge sorption/desorption
- aerobic biodegradation
- acute toxicity to earthworm
- effect on soil microbes
- acute toxicity to *Daphnia magna*
- acute toxicity to *Daphnia magna* in a sediment/water system
- effect on freshwater algae, *Selenastrum capricornutum*
- acute toxicity to rainbow trout
- radiotracer excretion with mass balance in beagle dogs
- metabolic profile in dog urine and faeces
- radiotracer excretion with mass balance in cats
- metabolic profile in cat urine and faeces
- confirmation of the efficacy of 6 mg/kg of selamectin against induced infestations of *Ctenocephalides felis* in dogs bathed after treatment
- confirmation of the efficacy of 6 mg/kg of selamectin against induced infestations of *Ctenocephalides felis* in cats bathed after treatment
- dose confirmation of selamectin against induced infestations of *Ctenocephalides felis* on dogs
- efficacy of selamectin against induced infections of *Dirofilaria immitis* larvae in dogs
- dose confirmation of selamectin against induced infections of *Dirofilaria immitis* in dogs
- dose confirmation of selamectin against induced infestations of *Ctenocephalides felis* on cats
- dose confirmation of selamectin against induced infections of *Dirofilaria immitis* in cats

The solubility of selamectin was found to be 435 µg/l.

The log Kow of selamectin was 3.15.

The vapour pressure was 2×10^{-8} Pascal.

The value of Kd, the Freundlich sorption coefficient, ranged from 894 to 5138 for the three soils. The corresponding Koc values ranged from 24,459 to 402,633. Desorption coefficients were correspondingly high, with desorption Kd values ranging from 2157 to 7076 and desorption Koc values ranging from 59,037 to 507,995.

In a soil biodegradation study mineralization of selamectin was not apparent during 120 days of incubation, with less than 2% of the applied activity recovered in the trapping solution. However, selamectin was biotransformed to multiple products which accounted for an average of 17 - 25% of the extractable residues after 120 days incubation. None of the products exceeded 5% of the total applied radioactivity and none were identified. About 50% of the radiolabel remained bound to the soil even after sequential extraction with several solvents following 120 days of incubation.

The acute toxicity of selamectin was determined against *Daphnia magna* under static renewal test conditions. [^{3}H]-selamectin was used in order to quantitate test article concentrations during the study. Nominal selamectin concentrations were 10, 18, 32, 58 and 110 ng/l. Mean measured concentrations of selamectin were 4.5, 7.1, 12, 23, and 35 ng/l. The calculated 48-hour EC_{50} was 26 (23 - 35) ng/l. Based on lethargy observed at 12 ng/l, the 48-hour no-observed effect concentration was considered to be 7.1 ng/l.

The acute toxicity of [^{3}H]-selamectin was determined against the water flea Daphnia magna under static test conditions in the presence of freshwater sediment. The test chambers were 600-ml beakers containing 50 ml of sediment and 500 ml of overlying dilution water. Selamectin was mixed with sediment at nominal concentrations of 3.1, 6.3, 13, 25 and 50 µg/kg dry weight sediment. Results were reported on the basis of mean measured concentrations of selamectin in the water. Overall mean measured concentrations of selamectin in the sediment were 2.2, 3.5, 7.2, 16, and 33 µg/kg; corresponding overall mean measured concentrations in centrifuged samples of overlying water were 0.044, 0.073, 0.098, 0.17 and 0.29 µg/l. The 48 hour EC_{50} value for *Daphnia magna* in the sediment:water system was 0.24 µg/l, the NOEC was 0.073 µg/l.

Forty dogs were allocated randomly to five treatments:
- negative control (T1)
- selamectin at 6 mg/kg (T2)
- selamectin at 6 mg/kg and bathed with water 2 hours after treatment (T3)
- selamectin at 6 mg/kg and bathed with shampoo 2 hours after treatment (T4)
- selamectin at 6 mg/kg and bathed with shampoo 6 hours after treatment (T5)

Percentage reductions in geometric mean flea comb counts for the selamectin treatments (T2, T3, T4 and T5) on days 7, 14, and 21 ranged from 99.8% to 100.0%. On day 30, the reduction in each treatment (T2, T3, T4, and T5) was 100%. Analysis of variance showed that on days 7, 14, 21, and 30 the log (flea comb count + 1) for the selamectin-treated animals (T2, T3, T4, and T5) was significantly lower (P=0.0001) than that for the placebo-treated animals (T1). Analysis of variance indicated that there were no significant differences (P>0.05) between the log (flea comb count + 1) of dogs that were not bathed (T2) versus those bathed with water 2 hours after treatment (T3), those bathed with shampoo 2 hours after treatment (T4), or those bathed with shampoo 6 hours after treatment (T5).

In the *Daphnia magna* study where sediment was present, test systems were made up by adding UK-124,144 to sediment rather than to water. Mean measured concentrations in sediment were 2.2, 3.5, 7.2, 16 and 33 µg/kg (55 - 71% of nominal) respectively. Mean measured concentrations in overlying water were 44, 73, 98, 170 and 290 ng/l respectively for centrifuged solutions.

The 48-hour EC_{50} was 240 ng/l, based on the measured concentrations in centrifuged solutions.

III.B. RESIDUE DOCUMENTATION

As the product is intended for use in companion animals only, there is no requirement for residues data to be submitted by the Applicant.

4. OVERVIEW OF PART IV OF THE DOSSIER: PRE-CLINICAL AND CLINICAL ASPECTS

IV.I. PRE-CLINICAL DATA

I.A 1 Pharmacodynamics

See also Part 3.
Selamectin is a semi-synthetic compound of the avermectin class. In common with other avermectins, selamectin is thought to induce neuromuscular paralysis of the parasite by interfering with chloride ion channel conductance. However, there is some controversy over the identity of the channel targeted by the avermectins i.e. GABA versus glutamate. One hypothesis is that avermectins activate or potentiate current through glutamate-gated chloride channels, which are located on pharyngeal muscle in nematodes. A study was submitted which demonstrated that selamectin potentially activated the glutamate-induced chloride channels and hence displayed agonistic properties on them. Avermectin sensitive glutamate-gated chloride channels have not been identified in man. Avermectins are known to interact with mammalian GABA receptors but binding affinity to mammalian brain is believed to be considerably lower than the affinity for binding sites in invertebrates (i.e. 100-fold lower in mammalian brain than in *Caenorhabditis elegans*).

The differences observed in the pharmacodynamic properties of selamectin between dogs and cats at intestinal sites reflect the greater concentrations of selamectin in plasma, leading to a greater exposure of blood feeding intestinal parasites to the molecule in cats compared with dogs.

I.A 2 Pharmacokinetics

See also Part 3.

Two target species pharmacokinetics studies are presented one in cats and one in dogs.

A GLP study was performed using a group of 6 male and 6 female cats. Each cat was dosed a total of 6 times as follows:

	Route	Dosage	Time	Comments
T1	Intravenous	0.05 mg/kg	Day 0	
T2	Intravenous	0.1 mg/kg	Day 42	
T3	Intravenous	0.2 mg/kg	Day 84	Formulation was outside specification
T3	Intravenous	0.2 mg/kg	Day 147	Re-treatment was necessary due to previous formulation problem
T4	Topical -80 mg/ml	24 mg/kg	Day 189	Preparation used contained a different concentration of active substance.
T5	Oral -24 mg/ml	24 mg/kg	Day 329	Sesame seed oil preparation used. Eleven of the original cats dosed plus one replacement cat

Plasma samples were taken at intervals up to 35 days post dosing (intravenous administration) and 43 days post dosing (oral and topical administration). The following results were obtained:

Intravenous Route

Dose (mg/kg)	C_0* (ng/ml)	Mean AUC_∞ (ng.h/ml)	Mean residence time	Mean systemic clearance	Mean $Vd_{(ss)}$	Mean terminal phase $t_{1/2}$
0.05	202	2045	85.2 hours	0.51 ml/min/kg	2.1 l/kg	58.9 hours
0.1	431	4756	110.0 hours	0.43 ml/min/kg	2.26 l/kg	78.2 hours
0.2	874	8526	93.7 hours	0.47 ml/min/kg	2.22 l/kg	74.4 hours

* maximum concentration at end of dosing

For the intravenous doses used in this study C_o and AUC were seen to be dose-related whereas mean systemic clearance, mean residence time and terminal half-life were independent of dose level.

Topical and oral route

Dose/route	Mean C_{max} (ng/ml)	T_{max} (hours)	AUC_∞ (ng.h/ml)	Mean terminal phase $t_{1/2}$
24 mg/kg topical	5513	15	767695	198.0 hours
24 mg/kg oral	11929	7	1116916	97.7 hours

The mean relative bioavailability of the topical dose compared to the oral dose was 68%. The mean systemic availability (compared to intravenous administration) was 109% for the oral route and 74% for the topical route.

A protocol similar to that of the previous study (using the same dosages and routes of administration) was used for a study which was performed in beagle dogs. The time points used for dosing and blood sample collection varied from the previous study. The topical preparation used in this study differed from that used in the cat study. An allergic reaction was seen in two of the dogs following intravenous administration. These two dogs were removed from the study and the intravenous preparation was reformulated and given at a slower infusion rate. Due to technical problems with the formulation specifications, one animal received two treatments during the intravenous phase of the study.

Intravenous Route

Dose mg/kg	Mean C_{inf}* ng/ml	Mean AUC_{∞} ng.h/ml	Mean residence time	Mean systemic clearance	Mean $Vd_{(ss)}$ L/kg	Mean terminal phase $t_{1/2}$
0.05	174	761	19.0 hours	1.189 ml/min/kg	1.24	13.8 hours
0.1	307	1534	18.9 hours	1.179 ml/min/kg	1.22	14.2 hours
0.2	636	3100	19.9 hours	1.167 ml/min/kg	1.26	14.7 hours

* maximum concentration at end of infusion

For the intravenous doses used in this study C_{inf} and AUC were seen to be dose-related whereas mean systemic clearance, $Vd_{(ss)}$, mean residence time and terminal half life were independent of dose level.

Topical and oral route

Dose/route	C_{max} (ng/ml)	T_{max}*(hours)	AUC_{∞} (ng.h/ml)	Mean terminal phase $t_{1/2}$
24 mg/kg topical	86.5	72	16104	267 hours**
24 mg/kg oral	7630	8	227985	45.7 hours

* A second lower peak concentration also occurred in plasma of each dog.

** Estimated value. Mean terminal half-life was calculated- after least squares regression. However, no estimate fulfilled all of the acceptance criteria.

The mean relative bioavailability of the topical dose compared to the oral dose was 7%. The mean systemic availability (compared to intravenous administration) was 62% for the oral route and 4.4% for the topical route.

The higher bioavailability in the cat compared with the dog following topical administration of selamectin appears to be attributable not only to grooming but also to greater transdermal flux rates and to differences in the rate of metabolism and excretion of selamectin.

In vitro studies carried out to evaluate the skin flux rates of selamectin in different species showed a flux rate in cat skin of 0.1 µg/cm^2.h. This value was at least three times greater than the measured flux rates in dog skin (0.03 µg/cm^2.h) and rat skin (0.04µg/cm^2.h) clearly suggesting that the rate and extent of selamectin skin absorption was greater in cats compared with dogs. The greater flux rate in cat skin is likely to have contributed significantly to a greater absorption from the site of topical administration and to the overall greater bioavailability in cats compared with dogs following topical administration.

In addition, the pharmacokinetic analysis following intravenous administration of selamectin at 0.05, 0.1 and 0.2 mg/kg in dogs and cats clearly showed that selamectin persists in cats for longer periods than it does in the dog. This was probably the result of differences in the rate of metabolism and excretion of the molecule as reflected in greater AUC values, longer half-lives and mean residence time (MRT) and slower clearance values of selamectin in cats compared with dogs.

In conclusion, the observation that selamectin penetrates cat skin at a faster rate than dog skin, and that following absorption it persists in the cat for a longer duration provide additional explanations for the differences in the pharmacokinetic profile following topical administration in the target species.

The Committee expressed its concerns that pharmacokinetic studies had been performed at a dose (24 mg/kg) largely superior to the one retained for clinical use without any demonstration of dose

linearity and with formulations different from those to be commercialised. Additional data using the recommended minimum dosage and the final formulation were therefore requested.

In data extracted from more recent studies, following a single topical administration of selamectin commercial formulation at a dose of 6 mg/kg, the plasma concentrations measured at the time of predicted Cmax were 27.8 ng/ml in dogs and 407 ng/ml in cats. The concentrations measured following topical administration of selamectin at the recommended commercial dose of 6 mg/kg are therefore included within the range of concentrations where the linearity of selamectin had been established.

In view of the aforementioned points it is considered that the linearity of selamectin was clearly established and the range of concentrations to be achieved during commercial use of selamectin are encompassed by the established linearity range for the active moiety.

With regards to the use of different formulations during the pharmacokinetic studies, the Applicant accepts that the topical formulation used in the pharmacokinetic studies was different from the final commercial formulation. However, it is important to note that the main objective of the pharmacokinetic studies was to support the design of the target animal reproductive safety studies and not to support the selection of a dosage regimen/interval for selamectin. The lack of sufficient correlation between selamectin plasma levels and efficacy against fleas severely restricts the value of pharmacokinetic data as the basis for the dose selection of selamectin. Instead, the commercial dose and regimen for selamectin were selected on the basis of dose selection studies using the dose limiting species, *Ctenocephalides felis*, in both dogs and cats to determine the final commercial dosage regimen of 6 mg/kg body weight, once monthly.

The Committee considered that adequate data on the pharmacokinetics of selamectin have now been provided.

The Applicant has provided further information and reassurances regarding the validation of the HPLC method, since concerns had existed regarding the variation in recovery, between consecutive samples, that was seen in the original validation report for the analytical methods. Modifications were made to the relevant section of the SPC to reflect this.

A topical dosage of 6 mg/kg and an interdose interval of one month was selected as the dosage regimen for dogs and cats based on definitive dose determination studies rather than on pharmacokinetic studies. In addition, the submitted series of dose confirmation studies and field studies against the parasite species of interest have clearly confirmed the efficacy of 6 mg/kg selamectin administered at monthly intervals in the dog and cat. This is regardless of the natural range of epidermal thickness in these animals suggesting their skin thickness does not affect absorption.

Metabolism Studies

The metabolism and excretion of a single topically administered dose of [^{3}H]selamectin were studied in cats and dogs. Four studies are presented, two in the cat and two in the dog. However, the formulation quoted in the cat study report is not identical to the formulation of Stronghold (selamectin 60 mg/ml topical solution) intended for commercialisation. The products differ in the relative proportions of the excipient components.

In dogs, following a single topical dose, selamectin was eliminated in the faeces (18-20% of the dose) and urine (1-2% of the dose). A large proportion of the radioactivity in the faeces (approximately 39% in female dogs and 64% in male dogs) and 99-100% in the urine was associated with the parent compound.

In cats, following a single topical dose, selamectin was eliminated in the faeces (48-60% of the dose) and urine (1-2% of the dose). A large proportion of the radioactivity in the faeces (approximately 43% in female cats and 26% in male cats) and in the urine (approximately 58% in female cats and 95% in male cats) was associated with the parent compound.

The major metabolite found in cat urine and faeces had undergone oxidation of the C-24 methyl group in the selamectin to carboxylic acid. In the dog several oxidative O-desmethyl metabolites of selamectin (constituting less than 10% of the total radioactivity) were found in the dog faeces samples. No metabolites were identified in dog urine.

IV.I.B Tolerance in the target species

The minimum recommended topical dose is 6 mg/kg. However, it appears from the dosing table in the SPC that the maximum dose for dogs would be almost 12 mg/kg (i.e. a 2.6 kg dog receiving a 30 mg dose) and the maximum dose for cats would be just over 17 mg/kg (i.e. a 2.6 kg cat receiving a 45 mg dose). The SPC also states that a dose of 15 mg should be administered to animals of less than <2.5 kg. Thus, there is the potential for much larger doses to be administered to 6 week old kittens/puppies.

CAT

Two tolerance studies are provided.

In a GLP study, the commercial formulation of selamectin (containing 60 mg/ml selamectin) was administered via the topical route to groups of 8 domestic short-hair cats from 6 weeks of age (4 males and 4 females) at a dose of either 6, 18, 30 or 60 mg/kg. An additional group of 8 cats were treated with a saline placebo. Further doses were administered at 28 day intervals until a total of 7 consecutive doses had been administered. The kittens were between 39-44 days of age and ranged from between 0.27-0.68 kg body weight at the time of the first dose. There were no unscheduled deaths during the study. No clinical signs attributable to treatment occurred and there were no treatment related effects on body weight. There were a small number of skin or coat abnormalities, including one cat that had a skin lesion at the site of jugular venipuncture, which was present for over two months. None of the lesions occurred at the treatment site and none were attributed to the treatment. Hair clumping and/or white powdery residue were frequently noted at the treatment site. A warning in the SPC at 5.4 *'Undesirable effects'* and product literature to this effect has been included.

No treatment related effects were seen in the haematology or in the urine/faecal analysis results. The clinical chemistry results showed some individual alanine aminotransferase (ALT) values to be above normal ranges. Some of these animals were those for which pale livers were seen at post-mortem (see below). Similar levels were, however, also seen for animals in the placebo group. One animal (number 36) in the highest dose group had very high ALT levels (up to 1523U/L), however, it is stated that the liver was normal on histopathological examination.

Some within-sex differences were also seen with respect to blood urea nitrogen (BUN) values, however in all cases the mean values were within the normal range and these findings were considered of no toxicological significance.

High creatinine phosphokinase (CPK) levels were also recorded for several animals in all dose groups. However, high levels were also recorded in some animals prior to treatment (days -7 to -5) and some of the placebo animals had levels above the normal range. From the data supplied the incidence of high post treatment CPK values does not appear to be dose related or related to the duration of treatment.

At the end of the study all animals were examined at post-mortem. In all groups, several gross pathological lesions were found (including animals in the highest dose group and in the placebo group that had pale livers). None were considered related to treatment. Histopathological examination was only carried out on the placebo and highest dose groups. Examination of the pale livers revealed various combinations of cytoplasmic vacuolation of the hepatocytes, mononuclear cell infiltrates and minimal hepatocyte necrosis and atrophy. Similar histopathological changes were seen in animals in both the placebo and highest dose group. Several other histopathological lesions were found in the placebo and highest dose group animals but none were considered related to treatment. It is unfortunate that more detailed clinical observations such as heart rates and respiratory rates were not

recorded in this study. Another possible criticism is that rectal temperature measurements were only taken on day -3 and day 196.

In a second GLP study the commercial formulation of selamectin (containing 60 mg/ml selamectin) was administered via the oral route to groups of 6 domestic shorthair cats (3 males and 3 females) at a dose of 6 mg/kg. (If the actual doses administered are calculated, using the day -5 bodyweights, it appears that the actual doses administered ranged from between 9.2-15 mg selamectin/kg). A second group of 6 cats received a placebo. The cats were aged between 0.5-3 years and weighed between 2.7-4.4 kg. Incomplete dosing was recorded for all six selamectin treated cats. It is stated that two cats lost between 0.25-0.5 ml. One of these cats salivated profusely after dosing. A third cat vomited food shortly after dosing and had to be repeat dosed. A small amount (≤50µL) was lost at this second dosing. The remaining three cats only lost a very small amount of the dose (≤50µL) due to gagging at the time of dosing.

With the exception of one cat, which vomited food and a hair ball 24 hours after dosing, no clinical signs were seen in the control group.

Abnormal clinical signs were recorded for 4 of the six cats in the treatment group. Two of these cats salivated (one profusely) after dosing. Another of the cats exhibited salivation and then vomited on five occasions (up to 10 hours post dosing). The fourth cat also vomited twice and was then re-dosed. This second dose also resulted in several episodes of vomiting (up to 24 hours post dosing). Both cats exhibited vomiting again on day 2 after dosing. A veterinarian's report was completed for these two cats.
No treatment-related changes were seen in the haematology, clinical chemistry, urinalysis or faecal analysis results.

In a summary it is stated that the vomiting was attributed to the alcohol excipient and that there were no signs of avermectin toxicosis. The Applicant argues that this will make voluntary ingestion unlikely and that vomiting and salivation will limit the systemic effects. However, cats are known to groom frequently and the Committee considered that it is likely that at least some of the product may be ingested.

Additional Information

Safety in heartworm positive cats.
In this GLP study 46 cats (aged between 8.9-9.9 months) were artificially infected (via the jugular veins) with six viable adult *Dirofilaria immitis* heartworms (at least two males and two females per cat) obtained from donor dogs. There had originally been 48 cats in the study but two cats died within 24 hours of inoculation from problems associated with heartworm disease. At least 20 days post-inoculation cats were treated with either selamectin at a dose of 24 mg/kg administered via the topical route or with a saline placebo. The selamectin product used in this study was a prototype formulation containing 8% selamectin. Each treatment was repeated six times at monthly intervals. The presence of *Dirofilaria immitis* was confirmed by the examination of blood samples (for microfilariae) taken at intervals during the study period and on the last day of the study prior to euthanasia.

There were no intolerance reactions at the treatment site. No adverse reactions or treatment related effects were recorded for any animal. A number of incidental clinical signs did occur such as abrasions around the surgical site on the ventral neck and one cat (EE3) which had a short period of pyrexia. One cat (D05) was suspected of having had a minor seizure, but, as this occurred 11 days after treatment with selamectin it is unlikely to be treatment related. Microfilaria examinations were positive for all cats 3 days prior to treatment with selamectin or the placebo. However, 3 control cats and one treatment cat were negative on the day of treatment. The number of cats with positive counts decreased over the course of the study and no positive counts were recorded after day 56 post-treatment. In a total of 6 of the treatment cats and 13 of the control cats, both male and female adult heartworms were found at post-mortem. A total of 10 treatment cats and 8 control cats were found to have adult heartworms of only one sex at post-mortem and 7 treatment cats and 2 control cats had no adult heartworms at post-mortem examination.

Reproductive safety - cat

It is stated that there were no adverse clinical signs relating to the treatment in a pharmacokinetic safety study in the cat. With the exception of one animal, which was euthanased due to urethral obstruction, no deaths occurred during the study. From the results of this study the predicted maximum, average and minimum plasma concentrations following the administration of a dose of 24 mg/kg at 14-day and 28-day intervals were calculated. These results were used to predict the level and frequency of overdosing required to ensure, on average, an exposure of the reproductive tract at key reproductive events to plasma concentrations that were equal to or greater than 3 times those obtained from administration of the product at its recommended minimum of 6 mg/kg once a month.

In a GLP-compliant study in female cats, 44 female domestic short-hair cats were treated topically with either saline (22 cats) or the proposed market formulation of selamectin (22 cats) at three times the recommended minimum unit dosage of 6 mg/kg administered every 28 days starting on day 0. The actual doses of selamectin administered on day 0 ranged from 24.6 to 55.1 mg/kg. After each cat had received at least two doses it was mated. The cats were then assigned to one of two treatment groups. One group received treatment every 28 days starting one day after mating (post-mating day 1) and the second group received treatment every 28 days starting 15 days after mating (post-mating day 15). This was intended to ensure that high plasma concentrations were present during folliculogenesis, ovulation, fertilisation, implantation, and development of the zygote. Treatment continued for each female until the kittens were weaned at 42 days old.

Two adult female cats (one control animal and one selamectin-treated animal) developed pyometra and were euthanased during the study. A third female (selamectin treated) failed to conceive. The only treatment-related clinical observations in cats treated with selamectin were local effects. These included licking and scratching at the treatment site following treatment (one cat) and stiffening and clustering of hair (with or without residue) at the treatment site, for 24 hours or less in all cats. There were no effects attributable to administration of selamectin in any of the clinical pathology variables.

Other effects noted included redness of the skin, hair loss, or scabbing of the skin on the dorsal neck caudal to the site of test material application, the ventral neck, and various parts of the head, or focal abscesses of the neck or face. These were observed in both saline- and selamectin-treated cats and were considered to be the result of aggressive, male breeding behaviour or rubbing against the cage by the oestrous female and were not related to the test product. Two saline-treated female cats developed mammary abscesses and one of these cats was euthanased 46 days after weaning her litter.

Congenital abnormalities were observed in two kittens. A kitten born to a saline-treated cat had a cleft palate. A kitten born to a selamectin-treated cat had failure of coelomic closure with prolapse of abdominal organs, absent or malformed sacral and caudal vertebrae, contracture of flexor tendons of the pelvic limbs, cartilaginous defect of the hind limbs, and hydrocephaly. There was no significant difference ($p>0.10$) in proportions of kittens with congenital abnormalities between saline-treated and selamectin-treated females. It should be noted, however, that the number of cats in each group was small (22) and this may affect the validity of conclusions drawn from the comparison, between treatment groups, of proportions of kittens with congenital abnormalities.

It is stated that the occurrence of congenital abnormalities in this study was comparable to the incidence of congenital abnormalities in the general cat population, which is 1.0 to 1.5 percent of all kittens born. The total number of kittens per litter at birth, the number of live kittens at birth for selamectin-treated females and weaning indices were not significantly different ($p>0.10$) from those of the control cats. Kitten body weights were similar for each treatment at each body weight determination. Eleven kittens (4 from control females and 7 from selamectin-treated females) were born dead or died between birth and 42 days of age. The deaths were due to enteritis, possible pneumonia, possible heart failure, urine retention and lack of maternal care. Two kittens were stillborn (one of which had a congenital defect). It is stated that these deaths were not related to the test product. Weaning indices (number of kittens at weaning /number of kittens born alive) for selamectin treated females were not significantly different ($p>0.10$) from saline treated females.

In a GLP-compliant reproductive safety study in male cats, 20 male domestic short-hair cats (aged between 2-4 years) were treated topically with either saline (negative control) or selamectin (commercial formulation) at three times the unit dosage of the recommended minimum of 6 mg/kg administered every 14 days for 16 to 17 treatments starting on day 0. After a total of at least seven treatments, each male cat was mated with two female cats. Treatment continued until all females in a batch had queened or had undergone 3 mating sequences. There were no effects attributable to administration of selamectin in any of the clinical pathology variables. One male cat (control) died prior to mating, despite veterinary treatment, as a result of probable uraemic poisoning due to an obstructed urethra. The only treatment-related clinical observations in cats treated with selamectin were local effects i.e. stiffening and clustering of hair (with or without residue) at the treatment site, for 24 hours or less.

All females conceived and reached parturition, yielding litters of 2 to 7 kittens each. One litter (sired by a selamectin-treated male) was delivered by caesarean section (both kittens from this female were stillborn). Only two kittens had congenital abnormalities. A kitten sired by a control male had a variant of the generalised congenital defect, conjoined twins i.e. incomplete duplication of the skull, bilateral elongation of the hindlimbs, spina bifida, and incomplete development of the reproductive tract. A kitten sired by a selamectin-treated male had a cleft palate and a cleft lip. It is stated that the occurrence of congenital abnormalities in this study was comparable to the incidence of congenital abnormalities in the general cat population, which is 1.0 to 1.5 percent of all kittens born. There were no statistically significant differences ($p>0.10$) between treatments for total number of kittens per litter and number of live kittens at birth per litter.

Additional Data

In a GLP-compliant study of margin of safety in cats, 24 domestic short-hair cats were treated topically four times at weekly intervals with either saline (negative control) or selamectin at dosages of 16, 48 and 80 mg/kg body weight in a prototype formulation of 8% selamectin.

There were no deaths, no intolerance at the site of treatment application and no treatment-related effects in any of the cats in the study. Plasma selamectin concentrations were found to be dose related and similar in both males and females.

Field Safety Overview - Cats

A total of 1886 cats (1847 cats in Europe and North America, and 39 cats in Australia) were treated with a total of 4357 topical doses of selamectin in the clinical efficacy and specific safety studies (excluding the oral tolerance study). In the non-clinical efficacy studies, dose confirmation studies, and specific safety studies, a total of 697 domestic shorthair cats received a total of 1431 topical doses of selamectin, (including some animals, which received multiples of the recommended usage dose in the specific safety studies). In the majority of these studies, the commercial formulation was used. In the field studies, a total of 1189 cats were treated topically with selamectin in Europe, North America and Australia (including approximately 2828 doses of 6% selamectin in the final commercial formulation). In the European and North American studies there were 114 pure-bred cats (of 18 different breeds) and 1036 mixed breed cats. Cats of both long and short hair types and a wide age range (6 weeks to 19 years) were included.

Adverse events possibly related to treatment with selamectin occurred in a total of 17 cats. In one study, a cat, which received 8% selamectin in a prototype formulation showed a localised transient alopecia and erythema at the site of treatment application. This lesion resolved in six days without medication. In one study a cat, which received the final commercial formulation, also showed alopecia and erythema. In this case the lesion resolved over a four-day period with symptomatic therapy.

In the field studies, a transient localised alopecia was recorded in eleven cats. Clinical signs in these cats also included crusting and thickening of the skin, localised pruritus and erythema. The incidence of pruritis is however low but a statement on the SPC advising of this possible occurrence will be

included. All of these cats were in the flea efficacy studies and the Applicant suggests that in these cases self-excoriation due to the flea infestation may have been a contributory factor. In all cases the clinical signs resolved satisfactorily with or without symptomatic therapy. The remaining adverse events seen in the field studies were other localised skin abnormalities (recorded in two cats) and vomiting and lethargy after each dose (one cat). There was one mortality in the field studies involving a kitten weighing 0.3 kg and five to six weeks of age at enrolment. The kitten, which appeared malnourished had been found abandoned (with a litter-mate) and its prior history was therefore unknown. The litter-mate died within a few days of being found. The kitten that was enrolled in the trial developed tonic spastic contractions and opisthotonus approximately 5.5 hours after treatment with selamectin and died approximately three hours later despite veterinary treatment. It is possible that this was a case of avermectin toxicosis, however, the report suggests that the kitten may have been metabolically abnormal at the time of treatment and that this may have been a contributing factor to any toxicosis. The following warning will be included in 5.4 *'Undesirable effects'* of the SPC and the product literature:
Use of the product in cats has occasionally been associated with a mild, transient alopecia at the site of application. In a small proportion of these cases transient focal irritation may also be observed. The alopecia and irritation are normally self-resolving, but symptomatic therapy may be applicable in some circumstances.

Selamectin was seen to cause hypersalivation and vomiting when given orally to cats. The natural grooming behaviour of cats and their natural tendency to ingest any topically applied product means that accidental ingestion of the product is likely to occur. It is also possible that in multi-cat households one cat may groom another recently treated animal and thus ingest the product. The following warning has been included in the SPC at 5.10 and product literature. "*It is important to apply the dose as indicated to minimise the quantity that the animal can lick off. If significant licking does occur, a brief period of hypersalivation may rarely be observed in cats*."

Other incidents, apparently unrelated to treatment with selamectin, were observed during the studies, and these are fully described in the individual study reports.

DOG

In a GLP-compliant study to evaluate the margin of safety in young dogs, the commercial formulation of selamectin was administered via the topical route at either 6, 18, 30 or 60 mg selamectin/kg to groups of eight beagle dogs, which were six weeks old at the start of the study. Doses were repeated every 28 days for a total of 7 doses. An additional group of 8 dogs were kept as controls and dosed on each occasion with a saline placebo. The actual doses administered on day 0 were greatly in excess of those stated in the study protocol i.e. one dog in the 60 mg/kg group received a dose of 114.0 mg/kg. Two dogs (one control and one in the 6 mg/kg dose group) died during the first 28 days of the study despite veterinary treatment. The deaths were attributed to parvovirus infection and not related to treatment. Some dogs were affected with coccidiosis. There were several observations of loose stools and several observations of respiratory disease in all groups but these were considered in the report to be unrelated to treatment. There were also other sporadic clinical signs but none were considered in the report to be related to treatment. No clinical signs attributable to treatment occurred and there were no treatment related effects on body weight or on the results of the faecal and urine analysis. Hair clumping and/or hair discolouration and/or white powdery residue were frequently noted at the treatment site. No treatment related effects were seen in the results for haematology, blood urea nitrogen and aspartate aminotransferase. Significant between-treatment differences were seen for creatinine and ALT. However, for both of these parameters the highest mean values occurred in the control group and the group mean values for both were within the normal reference ranges. No effects related to treatment were observed in any of the other serum chemistry variables. Histopathology was only carried out on the placebo group and the 60 mg/kg dose group. Gross pathological and histopathological lesions were seen in puppies from all groups but none were considered related to treatment.

There was a high incidence of disease in the group of young dogs used in this study (e.g. parvovirus, coccidiosis and respiratory disease). Two of the animals required antibiotic treatment for respiratory

disease and a number of dogs diagnosed with coccidiosis required fluid replacement therapy. The validity of this study was therefore questioned and the Applicant was asked to provide a further safety study in young dogs.

The Applicant declined to conduct a further safety study and instead re-analysed the data excluding animals with clinically significant disease. A statement was provided from the director of the laboratory where the study was conducted together with an expert opinion. Although the Committee agreed that the study was not strictly in compliance with EU guidelines adequate assurances had been provided.

Reproductive safety - dog

In an essentially pharmacokinetic study it was stated that there were no adverse clinical signs relating to the treatment in this study and no deaths occurred during the study. From the results of this study the predicted maximum, average and minimum plasma concentrations following the administration of a dose of 24 mg/kg at 14 day and 28-day intervals were calculated. These results were used to predict the level and frequency of overdosing required to ensure, on average, an exposure of the reproductive tract at key reproductive events to plasma concentrations that were equal to or greater than 3 times those obtained from administration of the product at its recommended minimum of 6 mg/kg once a month.

In a GLP-compliant reproductive safety study in female dogs, 44 female beagle dogs were treated topically with either saline (22 dogs) or the proposed market formulation of selamectin (22 dogs) at three times the recommended minimum unit dosage of 6 mg/kg administered every 28 days starting on day 0. After each dog had received at least two doses it was mated. The dogs were then assigned to one of two treatment groups. One group received treatment every 28 days starting one day after mating (post-mating day 1) and the second group received treatment every 28 days starting 15 days after mating (post-mating day 15). This was intended to ensure that high plasma concentrations were present during folliculogenesis, ovulation, fertilisation, implantation, and development of the zygote. Treatment continued for each female until the puppies were weaned at 42 days old.

There were no deaths of adult females and no effects attributable to selamectin in any of the clinical pathology variables. Hair clumping and/or hair discoloration and/or white powdery residue were frequently noted at the selamectin treatment site. A total of 11 control dogs and 11 selamectin treated dogs developed mastitis following parturition. Five different bacterial isolates were identified and all were considered in the report to be environmental pathogens. Due to the even distribution of between treated and control dogs it is considered unlikely that the mastitis was related to the product. Two female dogs (both treated with selamectin developed metritis following parturition but this was not considered in the report to be related to the product. There were no treatment related clinical observations amongst the puppies. Three females failed to conceive (one control and two selamectin treated animals). The only congenital abnormality in the puppies was kinked tails. This occurred in litters in all treatment groups and is stated in the report to be a common trait within this beagle colony. Sixteen puppies were born dead or died during the study. The causes of the deaths were listed. None of the deaths were attributed to the selamectin.

Weaning indices (number of puppies at weaning /number of puppies born alive) were not significantly different ($p>0.10$) than saline treated animals. For the post mating day 1 regime, the total number of puppies born alive and the total number of puppies per litter at birth for selamectin treated animals were not significantly different ($p>0.10$) to the saline treated animals (post mating day 1 regime). However, for the post mating day 15 regime, the total number of puppies born alive and the total number of puppies per litter at birth were significantly greater for the selamectin treated animals than for the controls. Puppy body weights were similar for each treatment at each interval.

The CVMP requested that in the light of the findings of the toxicity study in rats showing increased death rates of the offspring, the observation of increased death rate in target species should be re-analysed to combine both the treatment groups to ascertain whether the results from these two groups might raise concerns that possible differences between the groups could be concealed. However, the

Applicant has also performed statistical analyses using comparisons between groups (selamectin and control) which had been treated at the same stages of gestation. The death rate and the number of dead puppies from bitches treated with selamectin commencing on day 15 of gestation were not significantly different from the other selamectin treatment group or from either of the placebo treatment groups.

It is stated that the deaths of five of the eight puppies from selamectin treated bitches were attributable to causes (stillborn, chewed umbilicus, pulmonary oedema) associated with deaths from placebo-treated females and were in proportion with those seen in placebo treated groups. Two additional puppies from litters in the same group died from crushing injuries. The remaining dead puppy was unthrifty and died shortly after birth. Both of the selamectin treated litters in which puppies died from crushing injuries were large (nine and eleven puppies per litter respectively). If the two puppies that died from crushing injuries could be removed from the analysis then the number of dead puppies in the selamectin treated group would be 6/72 = 8.3%.

It is stated that the increased death rates in the rat toxicity study were associated with exposure some 340 times greater than exposure in dogs at the recommended dosage. The Applicant's arguments are considered to be acceptable and it is considered that the indication for administration to pregnant bitches should be retained.

In a GLP-compliant reproductive safety in male dogs, twenty male beagle dogs (aged between 14 months to 8.5 years) were treated topically with either saline (negative control) or selamectin (commercial formulation) at three times the unit dosage of the recommended minimum of 6 mg/kg. Treatments were administered every 14 days for 17 treatments starting on day 0. After a total of at least seven treatments, each male dog was mated with two female dogs. There were no deaths of adult males and no effects attributable to selamectin in any of the clinical pathology variables. Hair clumping and/or hair discolouration and/or white powdery residue were frequently noted at the selamectin treatment site. There were no significant differences ($p<0.10$) in semen volume, sperm motility, sperm count, number of primary abnormalities, number of secondary abnormalities and total abnormalities between the selamectin treated males and the control animals. Semen pH was significantly lower in the selamectin treated males but since mean values were within normal reference ranges this difference was not considered clinically important. Four female dogs (two mated to control males and two to selamectin treated males) required caesarean sections. No congenital abnormalities (except kinked tails) were observed in any of the puppies.

Additional Information - Special Safety Studies

Safety following oral administration.
In this GLP study the commercial formulation of selamectin (containing 60 mg/ml selamectin) was administered via the oral route to groups of 6 beagle dogs (3 males and 3 females aged between 6-7 months) at a dose of 6 mg/kg. A second group of 6 dogs received a placebo. There were no deaths and no treatment related effects in any of the dogs.

Safety in heartworm infected dogs.
In this GLP study 22 beagle dogs (aged between 6.3-7.2 months) were artificially infected (via the jugular veins) with 20 viable adult *Dirofilaria immitis* heartworms (10 males and 10 females per dog) obtained from donor dogs. Twenty-eight days post-inoculation (day 0) 20 of the dogs were treated with either selamectin at a dose of 18 mg/kg administered via the topical route or with a saline placebo. Each treatment was repeated three times at 28 day intervals. The presence of *Dirofilaria immitis* was confirmed by the examination of blood samples for microfilariae and antigen of adult *Dirofilaria immitis*. Hair clumping and/or white powdery residue were frequently noted at the selamectin treatment site. There were no other reactions at the treatment site. No deaths occurred and no adverse reactions or selamectin treatment related effects were recorded for any animal. Microfilaria counts for the control animals increased throughout the study. Counts for the selamectin treated animals peaked during days 7-14 and then declined. Both male and female adult heartworms were found at post-mortem in all of the dogs.

Safety in avermectin sensitive collies.
This GLP study (carried out in the USA) was performed using rough-coated collie dogs that had been tested for avermectin sensitivity by the subcutaneous administration of 200µg/kg ivermectin. Avermectin sensitivity was identified by the presence of neurological signs of avermectin toxicity i.e. depression, ataxia, mydriasis, salivation and muscle fasciculations. Sixty days after sensitivity testing groups of 6 animals were treated with the commercial formulation of selamectin by topical administration at doses of either 6, 18 or 30 mg selamectin/kg. A fourth group of 6 collies were treated with a placebo solution containing the excipients only. Treatments were repeated every 28 days for a total of 3 treatments. Observations were made post treatment for signs of avermectin toxicity and recorded according to a scoring system i.e. (0) normal, (1) mild, (2) moderate or (3) severe. A dog was considered sensitive to selamectin if any one or more of the following scores were seen during the 14 day post treatment period: depression ≥2, ataxia ≥1, mydriasis = 3, salivation ≥2 or muscle fasciculations ≥2.

There were no deaths, no treatment related effects and no clinically significant changes in body weight or clinical pathology variables during the study. No dogs were considered sensitive to selamectin. The only abnormal clinical observation noted during the study was salivation. Scores for individual observations were presented. Salivation with a score of 1 was noted in 2 placebo dogs, 4 dogs in the 6 mg/kg group, 1 dog in the 18 mg/kg group and 3 dogs in the 30 mg/kg group. It appears from this table that the animals which showed the maximum duration of mild salivation was number E66 (30 mg/kg group) which had a score of 1 at three time points (8, 10 and 12 hours) post treatment at the second dose. Only one dog (M53 in the placebo group) was recorded as having a salivation score of 2. Two animals (dog number M53 in the placebo group and dog number M94 in the 30 mg/kg group) had abnormal observations during the study. Dog number M94 had three areas of foamy yellow liquid (presumed to be vomit) on the pen floor on day 29 (one day after the second selamectin dose). This same dog had a small white foamy spot on the floor of the pen on day 36.

Safety in avermectin sensitive collies (related data).
In this cross-over GLP study (conducted 1994 in the USA) two groups of four known ivermectin-sensitive collie dogs (three males and five females) were treated with either a single topical administration of saline (negative control) or selamectin at 40 mg/kg body weight. The selamectin test product was a prototype containing 16% selamectin. Each treatment was administered to one of the two groups of dogs on day 0 and subsequently to the other group on day 62. The report does not state how the ivermectin sensitivity status of the dogs involved in this study was confirmed. A scoring system similar to that in the previous study was used. Clinical observations for the 14 days post treatment were all scored as zero. There were no deaths and no treatment-related effects in any of the dogs in the study.

Field Safety Overview - Dogs

A total of 2666 dogs (2570 dogs in Europe and North America, and 96 dogs in Australia) were treated with a total of 7799 topical doses of selamectin in the clinical efficacy and specific safety studies (excluding oral administration studies).

In the non-clinical efficacy studies, dose confirmation studies and specific safety studies, a total of 866 dogs received a total of 1704 topical doses of selamectin (including some studies where multiples of the recommended usage dose were given). In the majority of these studies, the commercial formulation was used. In these studies, the majority of the dogs were beagles, together with collies and some mixed breed dogs.

In the field studies, a total of 1800 dogs were treated topically with selamectin in Europe, North America and Australia. This represented approximately 5883 doses of 12% or 6% selamectin in the final commercial formulation, and 212 doses of 8% or 16% selamectin, in prototype formulations. In the European and North American studies there were 1187 pure bred dogs (of 149 different breeds) and 517 mixed breed dogs, and a wide age range (6 weeks to 17 years). Sixty-three dogs were included which were either pure bred or mixed breed collies.

Adverse events possibly related to treatment with selamectin occurred in a total of four dogs and there were no treatment-related mortalities.

In the laboratory-based studies one animal was recorded as showing transient mild mydriasis and salivation two hours after the first dose of selamectin. These signs had resolved six hours later and did not recur after subsequent monthly administrations. Although, the Applicant states that the absence of similar signs after the second and third doses suggests that this may not have been a case of avermectin intoxication, this cannot be excluded.

In the field studies three dogs showed suspected adverse reactions possibly related to treatment with selamectin, (two showed pruritus and one dog showed a discolouration of the coat after treatment). The Applicant states that since the two dogs that showed pruritus were in flea efficacy studies and that the flea infestation may have contributed to the pruritus.

Other incidents apparently unrelated to treatment with selamectin were observed during the studies, and these are fully described in the individual study reports.

IV.I.C Resistance

No documented resistance in the field to avermectins amongst parasites of dogs and cats has been reported to date. The development of side-resistance between different avermectins is a possibility and has been documented in some nematodes of livestock. However, as stated by the Applicant it is not likely to be a significant problem in the cat and dog.

A literature review was presented. Avermectins (including ivermectin and milbemycin) are frequently used for the prevention of heartworm in dogs. No resistance to avermectins has been reported despite widespread use at low dose rates. The Applicant suggests that this may be due largely to epidemiological factors (i.e. the presence of an insect vector) in the parasite life cycle.

Resistance amongst nematodes of cats and dogs to avermectins has also not been reported.

Resistance amongst arthropods to a range of insecticides/acaricides (including pyrethroids, organophosphates, organochlorines and formamidines) is well documented. However, the avermectins have a different mechanism of action and in laboratory studies avermectin cross-resistance with other classes of compounds was not found. Reports of resistance to avermectins amongst arthropods of any animal in the field was not detected in a 1998 literature search. The Applicant states that, although resistance has been reported in fleas to other classes of compounds, avermectin efficacy has not been tested in these resistant strains.

The development of resistance to selamectin in endoparasites and ectoparasites of cats and dogs appears unlikely.

IV 2 CLINICAL STUDIES

1. FLEAS

The laboratory studies appear to have been carried out to an acceptable standard. In all of the studies the dose of selamectin used was 6 mg/kg. This represents a minimum dose as the product was dosed according to a weight chart. This may have artificially increased the apparent efficacy of the product. It should be noted, however, that the weight charts used in the studies are consistent with those proposed in the SPC. Thus it is considered that the method of dosing used in the studies is consistent with that proposed for use in the field.

The dose confirmation studies showed that the product acts rapidly and in the cat study a % reduction in mean flea comb count (*C. felis*) of >98% was achieved by 24 hours post treatment. At all other subsequent time points (up to 30 days post treatment) the % reduction in mean flea comb count remained at or above 98%. In the dog study, a % reduction in mean flea comb count (*C. felis*) of >99% was achieved by 36 hours post treatment. At all other subsequent time points (up to 30 days post treatment) the % reduction in mean flea comb count remained at or above 99%.

Studies were performed to assess the effect of bathing on efficacy. The % reduction in mean flea comb counts (*Ctenocephalides felis*) remained above 97% in the cat and above 99% in the dog despite a shampoo bath being given 2 hours post treatment.

The claim proposed in the SPC is for *Ctenocephalides spp*. Efficacy against *C. canis* has been assessed in only one study. In this study the % reduction in mean flea comb count was 100% at the 7 and 14 days post treatment. However, at 30 days post treatment the % reduction in mean flea comb count was reduced to 91.8%. Two other studies were carried out to a similar protocol to assess the efficacy against *C. felis*. In these two studies the % reductions in mean flea comb count at 30 days post treatment were >99%. This may indicate a lower efficacy against *C. canis*. It appears from the field study reports that no differentiation was made between animals infested with *C. felis* and *C. canis*, however, as indicated by the Clinical Expert, the prevalence of *C. felis* in the field is much higher than that of *C. canis*. The Applicant was asked to demonstrate that the efficacy of selamectin against *C. canis* is equivalent to that against *C. felis*. Although no new data were provided, adequate reassurances and justification for retaining a claim against *C. spp.* were supplied.

The flea field studies included in the original dossier for selamectin were carried out in the UK, France, Germany and Italy. Whilst it was accepted that it would be possible that a proportion of the fleas found on the animals involved in the field trials may have been *C. canis*, this argument alone was not considered sufficient evidence to support the efficacy of Stronghold against this species of flea.

The guideline "Demonstration of Efficacy of Ectoparasiticides (Volume VII of The Rules Governing Medicinal Products in the European Community, 1994 p.116) states that "at least two controlled tests are recommended to demonstrate the efficacy of a new product against each ectoparasite species and stage of development as indicated on the label". The Applicant had only provided one study in which efficacy against *C. canis* was specifically studied.

The Applicant was requested to demonstrate that the efficacy of selamectin against *C. canis* is equivalent to that against *C. felis*. The Applicant's response was accepted and the indication for *C. canis* was therefore retained in the SPC and the product literature.

The duration of prevention of flea infestations was agreed at one month after a single topical application of the product at the recommended dose of 6 mg/kg.

The environmental control studies demonstrated that when cats and dogs are housed in an environment capable of supporting an established flea infestation the % reduction in mean flea comb counts following 2 or 3 monthly treatments with selamectin is >99%. It is considered that the data supports the SPC claim for the prevention of flea infestations.

Field studies performed in several sites throughout Europe have been presented. These included a wide range of breeds of dog and cat. The protocol for these field studies could be criticised in that fleas removed during flea comb counts were killed and this may have artificially increased the apparent efficacy results. However, this procedure was performed for both the positive control group and the selamectin group.

In the cat field trials the overall % reductions in mean flea comb counts were 92.8%, 92.7%, 97.7% and 98.4% at the 14 day, 30 day, 60 day and 90 day time points respectively.

In the dog field trials the overall % reductions in mean flea comb counts were 92.5%, 90.7%, 98.1% and 99.1% at the 14 day, 30 day, 60 day and 90 day time points respectively.

In both the dog and cat field studies the efficacy appeared to improve over time. This is not surprising as the level of the environmental challenge is also likely to reduce over time, due both to the low number of flea eggs being produced from selamectin treated animals and due to the treatment of 'supplementary' animals in the household. Advice is provided in the SPC that the product should be administered at monthly intervals throughout the flea season, starting on month before the fleas become active. This would seem sensible advice. It is considered that a claim for the treatment and prevention of *Ctenocephalides spp.* infestations in cats and dogs was approved.

The Committee considered that the data currently presented could support a modified claim for the use as part of a treatment strategy for flea allergy dermatitis.

The Applicant has applied for a Type II variation to add a claim for activity against flea eggs and larvae. A further study was presented to support the variation. This study examined the effect that debris, from dogs treated with selamectin, had on flea eggs and larvae, which had not been previously exposed to selamectin or any other insecticide. Percentage reductions in geometric mean hatched flea egg counts, on collection days 1, 7, 14, 21 & 30 were 100%, 96.6%, 96.7%, 98.3% & 70.1% respectively. Percentage reductions in geometric mean viable larval counts, on collection days 1, 7, 14, 21 & 30, were 100%, 100%, 98.5%, 99.6% & 98.4% respectively. Percentage reductions in geometric mean counts of viable adult fleas developing from larvae left undisturbed to develop into adults, on collection days 1, 7, 14, 21 & 30, were 100%, 99%, 100%, 100 & 100% respectively. Percentage reductions in geometric mean viable adult flea counts, on collection days 1 & 7, were 70.7% & 20.2% respectively.

Debris collected from dogs treated with a single topical dose of 6mg/kg selamectin was highly effective over a 30 day period in preventing the hatching of normal flea eggs, in killing normal flea larvae and in preventing the development of normal flea larvae to the adult stage. The data show that the ovicidal and larvicidal activity of selamectin effectively breaks the flea life cycle through exposure of flea eggs (both on the host and in the environment) and flea larvae (only found in the environment) to debris deposited in the environment from selamectin treated dogs. It was noted that the new study was conducted in dogs and the indication (for fleas) is for both dogs and cats. This was considered to be irrelevant, as it was an experimental study using *Ctenocephalides felis*, which affects both dogs and cats, and the life cycle of the flea is the same in both dogs and cats.

Several studies reported in the original dossier are highly relevant to this application and they generated very similar results for both dogs and cats. The data from the new study can, therefore, be applied to both species. The study has adequately confirmed that selamectin has ovicidal and larvicidal activity and does, therefore, break the flea life cycle.

The Opinion on this variation was adopted by the CVMP on 19 July 2000.

The Applicant has applied for a Type II variation to add a claim for the protection of the litter from flea infestations (*Ctenocephalides felis*) and adult roundworm infections (*Toxocara cati and canis*) in cats and dogs respectively by treatment of the queen or bitch. Two studies were submitted in support of this claim.

First study:
This was a controlled and blinded study conducted to evaluate the safety and efficacy of selamectin administered to pregnant and lactating bitches (not their puppies), in the prevention of *T. canis* infections and *C. felis* infestations in their suckling puppies. The study was conducted according to the EU guideline on Good Clinical Practice (GCP).
A total of 21 bitches showed signs of pro-oestrus, were mated and finally selected having > 30 fleas each and with patent *T. canis* infections. They were 2-7 years old, 11.9-22.9kg at first weighing (within 3 days prior to the first treatment). They were randomly allocated to one of two groups – selamectin or placebo (negative control) treated. The commercial formulation of selamectin was administered topically to give at least 6mg/kg (the minimum recommended dose). Treatments were administered at approx. 6 and 2 weeks before delivery (i.e. approx. 1 month apart) and on post-partum Days 10 & 38 (i.e. approx. 1 month apart). Puppies were not treated.
There were no treatment related mortalities and no adverse drug effects. Of the 21 bitches which entered the study, 19 whelped and 17 (9 placebo treated and 8 selamectin treated) completed the study with their litters. Of the 120 pups which were born (63 from placebo treated bitches and 57 from the selamectin treated bitches), 78 completed the study (37 from placebo treated bitches and 41 from the selamectin treated bitches).
As typically observed in mature bitches with endemic *T. canis* infection, faecal egg excretion was very low in the pre-partum and early post-partum periods. It then increased rapidly from approx. 3 weeks post-partum. Analysis of variance showed that the differences between selamectin and placebo treated bitches were significant on post-partum Day 45 ($p=0.0001$). Analysis of variance showed that the differences between puppies from selamectin and placebo treated bitches were significant on both days ($p=0.0001$). Worms were present in at least one puppy in each of the nine litters from placebo treated bitches which completed the study. Analysis of variance showed that the differences between puppies from selamectin and placebo treated bitches were significant ($p=0.0018$). No fleas were found on any selamectin treated bitch at any time post-partum. Analysis of variance showed that the differences between selamectin and placebo treated bitches were significant on all days after treatment was administered ($p=0.0001$). No fleas were found on any puppy born to selamectin treated bitches at any time during the study. Analysis of variance showed that the differences between the puppies from selamectin and placebo treated bitches were significant on all days ($p=0.0001$).

Second study:
This was a controlled and blinded study conducted to evaluate the safety and efficacy of selamectin administered to pregnant and lactating queens (not their kittens), in the prevention of *T. cati* infections and *C. felis* infestations in their suckling kittens. The study was conducted according to the EU guideline on Good Clinical Practice (GCP).
Thirty eight queens were selected. They were 1-5 years old, 2.4-5.3kg at first weighing (within 3 days prior to the first treatment) and were randomly allocated to one of two groups – selamectin or placebo (negative control) treated, with 19 queens in each. The commercial formulation of selamectin was administered topically to give at least 6mg/kg (the minimum recommended dose). Kittens were not treated.
There were no treatment related mortalities and no adverse drug effects. Of the 38 queens which were enrolled, 16 were diagnosed pregnant and completed the study on post-partum Day 49 (10 placebo treated and 6 selamectin treated). Fourteen completed the study with their litters (8 placebo treated and 6 selamectin treated). Of the 73 kittens which were born (51 from placebo treated queens and 22 from the selamectin treated queens), 53 completed the study (36 from placebo treated queens and 17 from the selamectin treated queens).
Although the faecal output of *T. cati* eggs was greatly reduced in the selamectin compared to the placebo treated queens, analysis of variance showed that the differences were not statistically significant. Larvae and adult *T. cati* were found in the intestinal contents of kittens born to all 8 placebo treated queens that completed the study and in 33/36 of their kittens. No larvae or adults were found in any of the kittens born to selamectin treated queens. Analysis of variance showed that the differences between kittens from selamectin and placebo treated queens were significant ($p=0.01$ & 0.0002 for L4 and adults resp.). No fleas were found on any selamectin treated queen at any time post-partum. Analysis of variance showed that the differences between selamectin and placebo treated queens were significant on all days ($p=0.0001$). No fleas were found on any kitten born to selamectin treated queens at any time during the study. Analysis of variance showed that the differences between

the kittens from selamectin and placebo treated queens were significant on all days (p=0.0001). None of the selamectin treated queens or their kittens needed any additional flea control. Of the 10 placebo treated queens which completed the study, 7 of them, and/or their kittens, required additional flea control on welfare grounds due to large flea infestations after Day 0.

The CVMP, during its meeting from 12 – 14 September 2000, decided that it was not possible to adopt an Opinion at this stage, but instead agreed to a list of questions. The main questions related to the fact that in cats maternal transmission of *Toxocara cati* is via the milk only and no *in utero* transmission occurs. Treatment of queens before parturition is not necessary and the Committee could therefore not accept the claim for the treatment of pregnant and lactating queens to prevent roundworm infection in kittens. In dogs, it is not necessary to administer the product twice before parturition and consequently only one application is required. Because the claim for the protection of litter against roundworms was accepted in dogs and not in cats this should be reflected under Section 5.1 Indications for use of the SPC.

The Applicant responded to the list of questions sent to them, acknowledged that transmission of *T. cati* is not known to occur *in utero* and proposed the application of selamectin to pregnant bitches two weeks before parturition.

The CVMP, during its meeting from 7 – 9 November 2000, concluded that the Applicant's response was considered to be satisfactory and recommended that:

- A claim for the protection of puppies and kittens against fleas, by treating the bitch or queen should be accepted.
- A claim for protection of kittens from roundworm infection by treatment of the queen should not be granted.
- The Applicant's proposal for application of selamectin to pregnant bitches approximately two weeks before parturition should be accepted.

The Opinion on this variation was adopted by the CVMP on 8 November 2000.

The Applicant applied for a Type I variation for deletion of the claim for treatment of pregnant and lactating bitches to prevent roundworm infection in puppies. The original claim was based on data derived from a laboratory study. Preliminary data from ongoing studies with Stronghold indicated that the claim may not be supported in all circumstances and the Applicant has presented a justification for its withdrawal.

This justification is based on the preliminary results of a multicentre clinical trial undertaken in Belgium, France and Germany. Bitches were first treated with either selamectin or placebo approximately 45 days after last mating, and on days 12 and 42 post partum. Faecal samples were collected from the pups on approximately days 21 and 35 post partum, and examined for *Toxocara canis*. Clinical signs of *Toxocara canis* infection did not appear to be significantly lower in pups from selamectin treated bitches than in pups from placebo treated bitches. Signs of clinical *Toxocara canis* infection were observed in one or more pups from 4 litters out of the total of 21 where the bitch was treated with selamectin. One pup from a selamectin treated bitch died as a result of *Toxocara canis* infection, indicating that the administration of selamectin to bitches during pregnancy and lactation may not provide pups with adequate protection.

The proposed change to delete the claim for the treatment of pregnant and lactating bitches to prevent roundworm infection in puppies has been satisfactorily explained.

The EMEA therefore accepted the variation on 22 November 2001.

2. Heartworm

Dogs

A number of laboratory studies have been presented which demonstrate the efficacy of selamectin in preventing *D. filaria* infection in dogs. The percentage prevention rate in these studies was 100% at doses of both 3 mg/kg and 6 mg/kg. The studies also demonstrate that efficacy was not reduced following a shampoo bath at 2 hours post treatment.

Only one field study in dogs is presented. This was performed in Italy. It is stated in the Guidelines that field trials should normally be performed in at least two geographical areas. However, the distribution of *D. filaria* is localised to the Southern parts of Europe i.e. Italy, Spain, Portugal, South of France and Greece. In addition, the field trial was carried out in 5 veterinary practices and it is stated that dogs were recruited from a wide geographical region in the North-West of Italy. The location of the field trial is therefore considered appropriate.

Cats

A number of laboratory studies are presented which demonstrate the efficacy of selamectin in preventing *D. filaria* infection in cats. The percentage prevention rate in these studies was 100% at doses of both 3 mg/kg and 6 mg/kg. The studies also demonstrate that efficacy was not reduced following a shampoo bath at 24 hours post treatment. It is unfortunate that the animals in these studies were not bathed at 2 hours post treatment. The Applicant argues that the higher systemic exposure (e.g. C_{max}) and the more rapid T_{max} in cats indicates that the efficacy in cats would not be decreased by bathing 2 hours after treatment. It should also be noted that the prevention rate was 100% at a dose of 3 mg/kg. This implies that if 50% of the recommended dose (i.e. 6 mg/kg) were removed during bathing then no reduction would be seen in efficacy.

In a further study carried out using cats, the effect of bathing 2 and 6 hours after treatment on the efficacy of selamectin against an artificially induced infestation of *C. felis* was examined. It was concluded that there were no significant differences between the log (flea comb count +1) of cats bathed after treatment and those not bathed after treatment.

The justification provided by the Applicant and the above data is considered adequate to support the claim that shampooing or soaking the animal 2 or more hours after treatment will not reduce the efficacy of Stronghold in cats.

No field studies in cats have been presented. The Applicant argues that this is justified because cats are less susceptible than dogs to natural heartworm infection and that it is very difficult to ascertain the infection status of them before they are entered into trials. The Committee noted that in the laboratory studies cats were tested for blood microfilariae and heartworm antigen prior to inclusion in the studies.

The justification that microfilaraemia is infrequent and transient in cats and as most infections were due to only a few worms (very often only one male or one female) adult heartworm antigen tests have an insufficient sensitivity is not acceptable and in the absence of field study data this claim shall be deleted from the SPC. The approved anti-*D. filaria* antibody tests for the diagnosis of feline heartworm infection was not available when the studies were carried out in 1997.

The Applicant cites The World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs *et al.*, 1994), which state that when the same genus or species of parasite is common to both dogs and cats it is possible to show efficacy in one species using efficacy studies performed in the other, provided that the pharmacokinetic profile of the drug supports the claim, as follows; “if efficacy has been convincingly demonstrated in the dog, it may be possible to confine the number and scale of cat studies with such parasites to the minimum needed to demonstrate bioequivalence”. The example cited in the reference is that of *D. immitis*: “For example, as adult *D. immitis* are constantly immersed in blood it is probable that treatments that produce similar plasma profiles of a drug or its metabolites in dogs and cats will be equally efficacious (but not necessarily equally safe) in both hosts”. It is important to note that it is exactly the same species of *dirofilaria* (*D. immitis*) that infects both dogs and cats world-wide in all regions where the parasite occurs (Bandi *et al*., 1998).

The Applicant believes that the efficacy of selamectin against *D. immitis* larvae has been clearly demonstrated in the cat in well controlled laboratory-based studies and in the dog in both laboratory and field (veterinary patient) studies. Furthermore pharmacokinetic studies have shown that the bioavailability of the drug is at least equivalent and in fact substantially superior in cats compared with dogs. Consequently, the Applicant originally stated that field studies in cats are not essential under the guidelines, however new field studies have been provided as further support for the claim.

A series of four veterinary patient studies in cats in university clinics and veterinary practices were conducted in support of the efficacy and safety of selamectin in the prevention of adult heartworm disease in cats. A total of 94 cats were treated with selamectin at the recommended minimum dose of 6 mg/kg once monthly for 6 or 7 months (as epidemiologically appropriate). These studies demonstrated that selamectin was safe and 100% effective in the prevention of heartworm disease in cats. A large battery of high quality studies including dose confirmation, field (veterinary patient) and pharmacokinetic studies in both dogs and cats substantiate the claim for prevention of heartworm in cats.

3. Ear Mites

It should be noted that a single administration of selamectin is recommended for the treatment of ear mites in cats.

DOSE CONFIRMATION - EAR MITES

CATS

A single unit dose of selamectin providing the recommended minimum of 6 mg/kg was 100% effective in the treatment of natural aural *O. cynotis* infestations in cats. The selection of this dosage for cats was therefore the logical outcome of these studies. No evidence was provided to justify the claim that monthly use of the product would prevent subsequent infestations.

FIELD STUDIES - EAR MITES

CATS

The Committee agrees that a single dose of selamectin administered topically at the recommended minimum dose of 6 mg/kg was effective in the control of clinical signs of otodectic mange in naturally infested cats In the data presented, it is debatable, whether preventive efficacy was sufficiently distinguishable from therapeutic efficacy to justify separate claims and consequently only a therapeutic claim is now agreed.

4 Sarcoptic Mange

The Applicant comments on the number of treatments with selamectin which were required for the treatment of dogs with sarcoptic mange. In the dose confirmation studies complete efficacy was achieved after two treatments, 30 days apart. The results of the field trials also suggested that two treatments 30 days apart were required to completely eliminate mites in all dogs.

In the SPC the Applicant has recommended treatment on two consecutive months. This is considered appropriate.

It is noted that none of the sarcoptic mange laboratory studies investigated the effect of bathing on efficacy. The SPC states that dogs can be bathed within 2 hours with no effect on efficacy. In the dog heartworm studies and in the dog flea studies bathing at 2 hours after treatment did not interfere with efficacy. In some of the sarcoptic mange field trials dogs were bathed but details were limited. It is considered that this claim can be extended to include *Sarcoptes scabei*.

The claim on the SPC is for the treatment and prevention of sarcoptic mange in dogs. No data have been presented to support the claim for "the prevention of sarcoptic mange." The claim has therefore been restricted to the **treatment** of sarcoptic mange only.

The effects of climate and length of hair have been addressed through the large and extensive series of field (veterinary patient) studies for selamectin which were conducted in a wide range of climates with numerous breeds of widely varying coat length. There were no effects on efficacy or safety in dogs and cats. Selamectin is considered safe and efficacious when used under varying climatic conditions in dogs and cats with varying haircoat lengths and types.

5. Nematodes

DOSE CONFIRMATION

CATS - ASCARIDS AND HOOKWORMS

The Applicant has conformed with the guideline on 'Anthelmintics: General Requirements' in the Guidelines for the Testing of Veterinary Medicinal Products, July 1994, in terms of the provision of at least two controlled dose confirmation trials, using the Controlled Test to demonstrate efficacy using a mixture of natural and artificial infections.

It is generally accepted that a new anthelmintic should be more than 90% efficacious against the target parasites (90% of the worm population removed) although products with a lower percentage efficacy may be considered where justified. Efficacy was greater than 90% in all the studies except one artificial infection study in which the efficacy against *A. tubaeforme* was 84.7%.

Appropriate studies have not been presented to support a claim that the product will prevent hookworm and roundworm infections in cats. The Applicant has suggested that the data provided in the field trials may support a claim for the "control" of hookworm and roundworm infections in cats. It is proposed that, in the case of hookworms and roundworms, the environmental challenge is likely to have been such that cats in the field trials will have been constantly exposed to infection. It has been demonstrated in the field trials that cats treated with selamectin did not become re-infected with hookworms or roundworms during the 30 days following treatment.

The response is, however, not considered to be acceptable. There is, at present, no clear definition of the term "control" and this term has not been retained by the CVMP. The words "and control" have been deleted from the claim for the treatment and control of adult intestinal hookworm *(Ancylostoma tubaeforme)* infections and adult intestinal roundworm (*Toxacara cati*) infections in cats.

DOGS - ASCARIDS

Two laboratory studies are presented, which were placebo-controlled, randomised studies using the commercial formulation, designed to investigate the efficacy of the product against the nematode, *Toxocara canis* (adult) in the dog. The first study also investigated efficacy against *Ancylostoma caninum* (adult).

For *T. canis*, in all studies, the differences between the placebo and treatment groups were significantly different.

For *A. caninum*, there were no significant differences between the treatment and placebo groups, in either mean worm egg counts or mean faecal egg counts at any time point. Correctly, no claim against *A. caninum* is made in the SPC.

No abnormal health observations that could be attributed to treatment were observed.

FIELD STUDIES

CATS - ASCARIDS

Three studies using the commercial formulation are presented, in which the efficacy of selamectin against ascarids was evaluated after each of two doses applied at an interval of one month.

Significant decreases in ascarid faecal egg counts were obtained with selamectin, which were at least comparable with the reference products and the treatment claim is supported.

There was one suspected adverse reaction in selamectin treated animals. This involved the death of one kitten approximately 3 hours after the development of tonic spastic contractions and opisthotonus, which developed approximately 5.5 hours after treatment with selamectin. The kitten had been abandoned, was malnourished and its litter-mate had already died. It weighed 0.3 kg and was estimated to be 5-6 weeks old. Although it is possible this was a case of avermectin toxicity, it is possible that its nutritional and metabolic status was a contributing or predisposing factor. The SPC states that the product is contra-indicated in cats less than 6 weeks of age.

CATS - HOOKWORMS

Nine studies using the commercial formulation are presented, in which the efficacy of selamectin against hookworms was evaluated after each of two doses applied at an interval of one month.

Compared with day 0, significant decreases in ascarid faecal egg counts were obtained with selamectin. There were no significant differences between selamectin and the reference products and a treatment claim is supported.

There were no clinically significant adverse events in the selamectin treated cats. One selamectin treated cat salivated excessively for 2-3 minutes after the application of the second dose. It was noted that this may have been related to the stress of handling. It is of note that a number of selamectin treated cats were observed to experience ear mite infestations.

The low number of cats presented with hookworms infections in Europe (8 out at least 990 cats screened by faecal examination) reflects the relatively low prevalence of infection, particularly in Northern Europe. The Applicant, nevertheless, comments that feline hookworm infection is spreading in Southern Europe. It is considered that the data presented support an indication in cats for the treatment of adult *Ancylostoma tubaeforme* and adult *T. cati* infections.

DOGS - ASCARIDS

A multi-centre field study is presented. Dogs were treated twice with selamectin at an interval of one month (days 0 and 30) and observed after 30 and 60 days.

It is noted that in the field trial the efficacy of selamectin against *T.canis* was just less than 90% at the 14 day time point (89.5%) however, by the 30 day time point efficacy was well above 90% (95.5%) and remained above 90% for the duration of the trial (i.e. 94.0% at day 44 and 94.7% at day 60).

It is also noted that efficacy against *T. canis* appears to have been slightly higher in the dose confirmation studies nevertheless the response is considered acceptable and the claim for treatment of adult intestinal roundworm infections (*Toxacara canis*) in dogs is supported.

Appropriate studies have not been presented to support a claim that the product will prevent roundworm infections in dogs. However the Applicant proposes that the data provided in the field trials may be sufficient to support a “control” claim for roundworm infections in dogs. The Applicant suggests that the environmental challenge is likely to have been such that dogs in the above field trial were constantly exposed to infection. It has been demonstrated in the field trial that the efficacy of selamectin against *T.canis* remained above 90% for the 30-day period after second selamectin treatment (i.e. 94.0% at day 44 and 94.7% at day 60).

The response is, however, not considered to be acceptable. There is, at present, no clear definition of the term "control" and this term has not been retained by the CVMP. The words "and control" will therefore need to be deleted from the claim for the treatment and control of adult intestinal roundworm (*Toxacara canis*) infections in dogs.

6. Lice

The Applicant has applied for a Type II variation to add a claim for the treatment of lice in dogs (*Trichodectes canis*) and cats (*Felicola subrostratus*). Two studies were submitted in support of this claim.

First study:

Twenty dogs, 8 males and 12 females, between 4 months and 3 years of age, were randomly allocated, on the basis of similar numbers of lice, to one of two treatments – selamectin or placebo. The day of treatment was Day 0. The commercial formulation of selamectin was administered topically to give at least 6mg/kg (the minimum recommended dose). This study was in two phases with 10 dogs in each. Dogs were not enrolled if they had been treated with avermectins or ectoparasiticides in the 30 days before the trial treatment day (Day 0). Clinical assessments of lice infestation were performed on Days –3, 7, 14, 21, 28, 35 and 42, i.e. at weekly intervals, after treatment. The lice (live adults and nymphs) were then counted using a standardised coat parting technique. On Day 43 the lice (live adults and nymphs) on each dog were counted using a whole body combing technique. Continuing until Day 43 ensured that no larvae from newly hatched eggs developed. There were no treatment related mortalities and no adverse drug effects. For the selamectin treated dogs, on all post-treatment days lice counts were significantly lower than on pre-treatment Day –3 ($p=0.0001$). Analysis of variance showed that the differences between selamectin and placebo treated dogs were significant on all post-treatment days.

Second study:

Eighteen cats, 9 males and 9 females, between 3 months and 4 years of age, were randomly allocated, on the basis of similar numbers of lice, to one of two treatments – selamectin or placebo. The day of treatment was Day 0. The commercial formulation of selamectin was administered topically to give at least 6mg/kg (the minimum recommended dose). Cats were not enrolled if they had been treated with avermectins or ectoparasiticides in the 30 days before the trial treatment day (Day 0). Clinical assessments of lice infestation were performed on Days –1, 7, 21, 35 and 42. The lice (live adults and nymphs) were then counted using a standardised coat parting technique. Continuing until Day 42 ensured that no larvae from newly hatched eggs developed. There were no treatment related mortalities and no adverse drug effects. No lice were found on cats treated with selamectin on any post-treatment assessment days whereas lice were found on all placebo treated cats on all post-treatment assessment days. For the selamectin treated cats, on all post-treatment days lice counts were significantly lower than on pre-treatment Day –1 ($p=0.0001$). Analysis of variance showed that the differences between selamectin and placebo treated cats were significant on post-treatment days.

The CVMP, during its meeting from 12 – 14 September 2000 decided that it was not possible to adopt an Opinion at this stage, but instead agreed to a list of questions. The main points in this list of questions were that the claim should be changed to the treatment of biting lice in dogs (*Trichodectes canis*) and cats (*Felicola subrostratus*), that the Applicant should comment on the use of log transformation and geometric means, instead of arithmetic means, to calculate the percentage reduction in live louse count.

In the response to the list of questions the Applicant stated that logarithmic transformation is conventionally used to transform parasite count data prior to statistical analysis. The Applicant stated that, for skewed distributions, the geometric mean is generally closer than the arithmetic mean to the median of the data. In addition, the Applicant pointed out that the draft VICH guidelines (GL07 – Efficacy requirements for anthelmintics: overall guidelines) recommended the use of geometric means.

The CVMP, during its meeting from 7 – 9 November 2000, concluded that the response from the Applicant supported the requested variation.

The Opinion on this variation was adopted by the CVMP on 8 November 2000.

CONCLUSIONS ON EFFICACY

It is considered that the following claims are supported by the data presented in the dossier:

1. Treatment and prevention of *Ctenocephalides spp.* infestations in cats and dogs.
2. Use as part of a treatment strategy for flea allergy dermatitis in cats and dogs.
3. Prevention of heartworm disease caused by *Dirofilaria immitis* in cats and dogs.
4. Treatment of ear mites (*Otodectes cynotis*) in cats.
5. Treatment of sarcoptic mange (*Sarcoptes scabei* infection) in dogs.
6. Treatment of adult intestinal hookworms (*Ancylostoma tubaeforme*) in cats.
7. Treatment of adult intestinal roundworms (*Toxocara cati* and *Toxocara canis*) in cats and dogs.
8. Shampooing or soaking the animal 2 or more hours after treatment will not reduce the efficacy of Stronghold.
9. Treatment of biting lice in dogs (*Trichodectes canis*) and cats (*Felicola subrostratus*).

5. RISK-BENEFIT ASSESSMENT AND CONCLUSION

The dossier covers two different strengths of solution, presented in 6 sizes of unit dose container. The composition is appropriate and the active ingredient in bulk and in the finished product is extremely stable. However, the application of a single, unified Finished Product Specification to all the presentations has generated problems. The ingress of small quantities of moisture or loss of small quantities of the volatile solvent Isopropyl Alcohol produces disproportionately high percentage changes in the smallest pack size, 0.25 ml. Consequently barrier specifications for packaging have had to be increased during development. New stability tests in the enhanced packaging are of short duration. Finalising the Release and Check Specifications for the packs has therefore presented difficulties. Nevertheless, there appears only minimal risk in accepting the extrapolations proposed for the 24 month shelf-life of the product. The overall chemistry and pharmacy dossier is satisfactory.

The Applicant has submitted a comprehensive dossier relating to the toxicity profile of selamectin. The acute toxicity is relatively low. The adverse effects of selamectin at high acute doses (800 – 1600mg/kg) are related to avermectin-like CNS toxicosis, whilst repeat dose major effects were liver related. In reproduction/teratogenicity studies in rats, the oral no-effect level was 10mg/kg/day. The Applicant has not submitted data on carcinogenicity due to lack of *in vitro and in vivo* genotoxicity, the stated close relationship to abamectin and the low likelihood of exposure of users to the product. Selamectin was satisfactory in *in vitro* and *in vivo* mutagenicity studies. It is considered that the data submitted in the dossier, supported by satisfactory responses to questions, are sufficient to demonstrate that use of the product as recommended would not constitute a hazard to the user.

The ecotoxicity assessment is based on a single treatment, but the product could be used every 30 days. A significant amount of the dose is absorbed and subsequently excreted, but this has not been considered in terms of soil exposure, nor as a factor that would reduce the amounts entering water from the dog's fur. However, the risk to the environment from the excretion route of exposure is expected to be low. The main issue is exposure of the aquatic environment through dogs swimming in ponds and streams. PECwater values based on sediment:water systems would have been more appropriate to the assessment than clean water PEC's, and could be compared with the data in the *Daphnia* study where sediment was present. Using the Applicant's assumption that 50% of the dose is washed off the dog into a pond, the PEC would be 4x the short-term PNEC. Based on this assessment, risk mitigation measures have been included on the product literature. However, data from bathing studies indicate that efficacy was not reduced when animals were bathed 2 - 24 hours after treatment.

The product is well tolerated when given by the oral route in the dog but the product was seen to cause hypersalivation and vomiting when given orally to cats. It is considered that there are tolerance issues regarding the potential ingestion of this product in cats. A warning has been included in the SPC and product literature.

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 81/852/EEC and supported the claim for the treatment and prevention of flea infestations caused by *Ctenocephalides* spp. in dogs and cats; use as part of a treatment strategy for flea allergy dermatitis in cats and dogs; prevention of heartworm disease caused by *Dirofilaria immitis* in cats and dogs; treatment of ear mites (*Otodectes cyanotis*) in cats; treatment of sarcoptic mange (*Sarcoptes scabiei*) in dogs, treatment of adult intestinal hookworms (*Ancylostoma tubaeformae*) and adult roundworms (*Toxocara cati*) in cats; treatment of adult roundworms (*Toxocara canis*) in dogs; treatment of biting lice in dogs (*Trichodectes canis*) and cats (*Felicola subrostratus*).

