

## **SCIENTIFIC DISCUSSION**

### **1. SUMMARY OF THE DOSSIER**

PRILACTONE 10 mg, 40 mg & 80 mg Tablets contain spironolactone and are intended for the treatment of congestive heart failure caused by valvular regurgitation in dogs, in combination with standard therapy. The Applicant for this veterinary medicinal product is CEVA SANTE ANIMALS.

The active substance in PRILACTONE Tablets, spironolactone, is an aldosterone antagonist (ATCvet Code QC03DA01). Spironolactone inhibits aldosterone-induced sodium retention in the kidney, leading to an increase in sodium, and subsequently water, excretion, thereby decreasing cardiac preload. Laboratory studies demonstrate that in the cardiovascular system spironolactone inhibits aldosterone induced fibrosis and improves endothelial function. PRILACTONE Tablets are administered at a dose of 2 mg of spironolactone per kg body weight once daily. Since bioavailability of the product is better in fed dogs, it is recommended to administer the product together with food.

The benefits of PRILACTONE Tablets are that dogs treated with spironolactone in addition to standard therapy demonstrated a reduction in cardiovascular disease deterioration compared to dogs treated with standard therapy alone. The most common side effect demonstrated in safety studies is that a reversible prostatic atrophy is often observed in entire male dogs.

The approved indication is: "For use in combination with standard therapy (including diuretic support, where necessary) for the treatment of congestive heart failure caused by valvular regurgitation in dogs."

### **2. QUALITY ASSESSMENT**

#### **Composition**

PRILACTONE Tablets are presented as uncoated oval/oblong tablets in three different strengths, containing 10 mg, 40 mg and 80 mg spironolactone per tablet. All the excipients have been previously used in the manufacture of authorised oral veterinary medicines. Beef flavouring is used to improve palatability to the target species.

The lower strength tablet has a single break-line whilst the other two tablet strengths have three parallel break-lines.

#### **Container**

The tablets are packed in polyamide (25 µm)/aluminium (45 µm) /polyvinyl chloride (60 µm) blisters, with an aluminium foil (25 µm) lidding. Appropriate specifications are provided.

#### **Development Pharmaceutics**

The formulation, method of manufacture (conventional wet granulation) and dissolution test have been carefully selected and justified. The tablets are produced from a common blend.

Spironolactone is practically insoluble in water (0.28 µg/ml). The use of a micronised form of the active substance and the formulation design aid solubility. Certain of the excipients, their level and the phase in which they are included in the product have been selected to mask the bitter taste of the active substance.

An aqueous granulation method was selected as the micronised active substance is not suitable for direct compression.

Blister packs were selected to provide the necessary protection to the product.

The use of part tablets (halves and quarters) has been justified by the necessity to administer the correct dose to animals in certain ranges of bodyweights. The division of tablets, particularly into quarters, has however been avoided as far as possible through the careful construction of the dose banding table. Studies confirm that each of the tablet strengths can be accurately divided.

### **Method of manufacture**

A wet granulation process is employed, using water as the granulating solvent. Conventional and unremarkable processes and equipment are used for drying the granules, blending the granules with other components and then compressing them into tablets.

### **Control of Starting Materials**

#### ***Active substance***

A current Certificate of Suitability is presented in support of micronised spironolactone from the proposed manufacturer. The micronised active substance is required to comply with the relevant monograph of the European Pharmacopoeia as well as with the additional tests defined in the Certificate of Suitability.

The particle size and polymorphic form of spironolactone are relevant to its bioavailability. Particle size is controlled, with the active substance being micronised to increase bioavailability. In relation to polymorphism, a specific single polymorphic form has been chosen for the manufacture of PRILACTONE tablets. The polymorphic form is determined by the conditions of crystallisation. X-ray diffraction pattern data have been generated for three batches of micronised spironolactone demonstrating that the desired form is consistently produced, nevertheless, the specification for spironolactone includes a non-routine test designed to confirm its polymorphic form. This test is performed on at least one batch per year.

The dosage form manufacturer relies upon the results of the supplier for the tests for residual solvents and particle size.

Three production batches of micronised spironolactone have been stored under VICH real time and accelerated conditions for up to 60 months. Relevant parameters, including polymorphic form, have been monitored and information on the methods applied during the stability studies provided.

Apart from one batch where a minor colour change occurred following 60 months storage under real time conditions, no significant changes are observed under either of the storage conditions.

A photostability study has been conducted on one batch of the active substance. The VICH guideline was followed. No degradation was observed in any of the samples examined suggesting that the active substance is not light sensitive. Whilst light protection seems to be unnecessary, there is no objection to the inclusion of this particular storage precaution, particularly since the European Pharmacopoeia monograph recommends storage protected from light.

In the absence of full validation data for the analytical methods used in the stability study, the applicant has agreed to retest the active substance immediately prior to use in the manufacture of PRILACTONE tablets.

#### ***Excipients***

The excipients in PRILACTONE tablets are: mannitol; sodium lauryl sulphate; microcrystalline cellulose; povidone K30; sorbitol; beef dry flavouring; talc; and magnesium stearate. Purified water is added during the wet granulation process.

Apart from the beef dry flavouring, all the excipients comply with their respective monographs in the European Pharmacopoeia.

A specification and test methods are presented for the Beef Dry Flavouring. These include tests and limits for appearance, identity by GC, sodium chloride content, water content and microbial purity. The specification is considered to be acceptable.

The supplier has also confirmed that the flavour complies with the EEC Council Directive 86/388 concerning the use of flavouring in foodstuffs. Certificates of Analysis are presented for three batches of the beef flavour that demonstrate compliance with the proposed specification. It is indicated that the beef dry flavouring can be stored in airtight containers for at least 12 months.

### ***Packaging***

Specifications are presented for the polyamide (25 µm)/aluminium (45 µm)/polyvinyl chloride (60 µm) blister material and for the aluminium foil (25 µm) used for the lid. These control appearance, identity of the contact material, width and weight/m<sup>2</sup> and are considered to be acceptable. Certificates of analysis are presented for batches of the blister and lidding materials which demonstrate compliance with the proposed specifications. Statements on the suitability of these materials for food contact applications are presented.

### **Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies**

None of the formulation ingredients are of animal origin. There is also a declaration from the Applicant confirming that the tablets and their components do not fall within the scope of the guideline on TSE. Further supporting documentation are provided in the dossier including confirmation that Sodium Lauryl Sulphate and Magnesium Stearate are of vegetable origin. Dry Beef Flavouring, despite the name, is not of animal origin.

The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE. Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

### **Control tests on intermediate products**

Not applicable.

### **Control Tests on the Finished Product**

The description of the methods used for the control of the finished product (appearance, dimensions, average weight and weight uniformity of the tablets, weight uniformity of half and quarter (not 10mg) tablets, identity and assay (HPLC) for spironolactone, moisture content, dissolution and microbial purity) and the specifications were provided. Appropriate method validation data have also been provided which demonstrate the suitability of the methods for their intended purpose. The specifications proposed at release and at the end of shelf-life are appropriate to control the quality of the finished product and are justified by data from batch analyses and stability studies.

The absence of a test and limits for hardness is justified as hardness testing is carried out as an in-process control and stability studies show no deterioration in hardness on storage. Likewise the absence of a friability test is justified by the process validation studies and the blister packing of the tablets.

The analytical methods have been appropriately validated.

Batch analysis data are presented for three batches, one of each tablet strength. The proposed manufacturer produced the batches in 2002 at a scale of 65 kg. The batches demonstrate full compliance with the proposed specifications.

## Stability

The proposed shelf-life specifications are identical to those proposed for release purposes with the addition of limits for impurities which are justified by stability data. The analytical methods used for the shelf-life specifications are identical to those used for release purposes with the exception of a different wavelength for the detection of one of the impurities. The method has been appropriately validated.

Nine batches of tablets, three of each tablet strength, were placed on a controlled stability test programme using the proposed commercial pack under VICH accelerated and real time conditions. Stability results from 36 months storage of the tablets are presented. Under both real time and accelerated conditions there is no evidence of any time related reduction in active substance content. Under real time conditions no increase in degradation products is observed although under accelerated conditions a minor increase is observed. The appearance of the tablets does not present any change during the stability study. The other physico-chemical characteristics studied are within their defined specifications, particularly dissolution of the active substance from the tablets, which remains rapid. The only other notable changes are that under real time conditions, in some cases, a slight softening is observed. Despite this the tablets comply with the test for uniformity of weight. Moisture levels reach a maximum of 2.0% in the real time and accelerated studies, with all results complying with the limit in the specification.

A photostability has been conducted on one batch of 40 mg tablets and one batch of 80 mg tablets in accordance with the VICH guideline. No changes were observed even for the tablets stored outside their primary packaging and hence the tablets are not considered to be light sensitive. It is not considered necessary to perform a separate photostability study on the 10 mg tablets.

In conclusion, the stability results, from both the real time storage over 36 months at 25°C and the data obtained from accelerated conditions of 6 months storage at 40°C / 75% RH, show that there is no significant change in the characteristics studied and confirm the 36 months shelf-life without a specific condition of storage. Thus, they justify the following statements in the SPC and other product information:

Section 6.3 of the SPC:

“3 years as packaged for sale.”

Section 6.4 of the SPC:

“This veterinary medicinal product does not require any special storage conditions.”

A stability study has been performed on part tablets. The appearance, water content, spironolactone content and the content of degradation products were tested, on half-tablets from two batches of the 10 mg dosage form, and on quarter tablets from two batches of the 80 mg dosage form, stored 7 days in ambient conditions after opening of the blister. The stability results presented demonstrate that, after first opening of the blister, part tablets are stable for 7 days, even though it is unlikely that part tablets will be stored for more than 3 days because of the daily dosing schedule. Therefore the study performed is considered sufficient and it is not considered necessary to define an in-use shelf-life for part tablets on the SPC, but it is prudent to include the following statement in the SPC and other product information:

Section 6.4 of the SPC:

“Partially used tablets should be stored in the original blister pack.”

## GMO

Not applicable.

## OVERALL CONCLUSIONS ON QUALITY

The supporting data are well presented and for the most part comprehensive.

The tablets are soundly formulated and are prepared from a common tablet blend. All of the excipients, including the beef flavouring (which is of non-animal origin) appear to have been used previously in the manufacture of tablets.

The 10 mg tablets are scored so that they may be divided into halves, whilst the 40 mg and 80 mg tablets may be divided into halves and quarters. The Applicant has demonstrated that fresh and aged batches of these tablets, and tablets produced at the extremes of hardness, can be accurately divided and quartered, where appropriate.

The manufacturing process is a standard one using an aqueous granulation, blending and then compression. The process has been validated at the smallest proposed batch size.

The active substance is the subject of a Ph. Eur. Certificate of Suitability. This incorporates additional tests, including those for particle size which are critical in the case of these tablet formulations in view of the very low aqueous solubility of spironolactone. Spironolactone has two polymorphic forms. The desired form has been identified and steps taken to ensure that the correct polymorphic form is always used in the manufacture of the product. The polymorphic form is also monitored on stability studies performed on the active substance.

Comprehensive release and shelf-life finished product specifications are in place. Analytical control methods are presented and for each of these very good validation packages are provided.

Stability data are presented for the micronised active substance and for the tablets. The data indicate good stability for both the active substance and the tablets in the proposed commercial packaging. However, the validation of the analytical methods used for the active substance is incomplete so the Applicant has undertaken to retest the active substance immediately prior to its use in the manufacture of these tablets.

The stability of part tablets, removed from their protective packaging, has also been shown.

### 3. SAFETY ASSESSMENT

#### Pharmacokinetics

Literature data are provided, as well as trials demonstrating the pharmacokinetics of spironolactone in rats, dogs, monkeys and man. Three of the studies were conducted in dogs in 2003 and are in accordance with GLP. The other studies are over 25 years old and are non-GLP, however, they are of good scientific standard and the conclusions are valid.

Following oral administration, spironolactone is well absorbed in all species and demonstrates effective hepatic clearance with increased bioavailability after food. Plasma disappearance occurs in 2 phases, the first very rapid and the second with a half-life in the dog of 26.2 hours. Linear pharmacokinetics was shown in a dose range of 2 to 4 mg/kg following oral administration. No accumulation is observed after multiple oral doses of 2 mg/kg spironolactone for 10 consecutive days. The mean  $C_{max}$  of the primary metabolites, 7 $\alpha$ -thio-spirolactone (TMS) and canrenone, are achieved after 2 and 4 hours respectively. Steady state concentrations are reached after 2 days.

A study investigating bioavailability and food interaction following a single oral and intravenous administration of spironolactone in beagle dogs demonstrated that the absolute bioavailability for 3 metabolites of spironolactone was 32% to 49%. The relative increase in bioavailability was between 1.6-fold and 2.4-fold for the metabolites when administered with food. Since spironolactone is highly lipophilic and feeding stimulates biliary secretion, this facilitates the absorption. The following advice was therefore included in the SPC (section 4.9) "The product should be administered with food. The tablet can either be mixed with a small amount of food offered prior to the main meal, or administered directly into the mouth after feeding." with an explanation of the reason in section 5.2 of the SPC.

After administration (by either the oral or intravenous routes), spironolactone distributes preferentially into the gastro-intestinal tract, kidneys, liver and adrenals. In humans, both spironolactone and canrenone (the main metabolite) were found to be extensively plasma protein bound (> 89%), in dogs the mean volume of distribution ( $V_{ss}$ ) of TMS and canrenone are 153L and 177L respectively. The MRT ranges from 9 to 14 hours according to the metabolite. In rats, concentrations in the testes, liver, kidneys and adrenals were 2 to 9 times higher than in the blood.

The biotransformation of spironolactone is complex and species-dependent. There are two categories of metabolite. In the first pathway, the sulphur in the parent molecule is retained. Metabolites include 7 $\alpha$ -thio-spirolactone and 7 $\alpha$ -thiomethyl-spirolactone. In the second pathway, the sulphur is removed and the most important metabolite is canrenone, which then undergoes further transformation. Spironolactone undergoes rapid biotransformation in the liver, the predominant, but less active, metabolites being canrenone and TMS. These metabolites are, together with spironolactone, responsible for the therapeutic effects of the substance.

In female dogs, the half-life of spironolactone after intravenous administration was less than 10 minutes. Studies using  $^{14}C$ -labelled spironolactone showed that radioactivity disappeared in two phases. The second phase half-life was 26.2 h in the dog. The major extractable constituent in the 4 hours after oral administration was canrenone.

The main metabolites exhibit anti-mineralocorticoid activity similar to spironolactone, but to a lesser extent. Potency ratios of approximately 5/1 were found for the renal anti-mineralocorticoid effect for spironolactone/canrenone in dogs.

Spironolactone is extensively metabolised by the liver and literature data suggests that spironolactone may irreversibly inhibit cytochrome P-450. An *in vitro* study was conducted to evaluate the effect of furosemide and meloxicam on the biotransformation of spironolactone. The study involved the use of canine hepatocytes and demonstrated no evidence for the induction or inhibition of cytochrome P450 enzymes by spironolactone, nor any interactions between spironolactone and either meloxicam or furosemide. The results of the study indicate that the co-administration of spironolactone with furosemide or meloxicam does not change the intrinsic clearance of spironolactone. However, the

CVMP considered that the veterinarian may not be alerted to the extensive hepatic metabolism of spironolactone. In the absence of evidence from the clinical studies proving the safety of spironolactone in dogs with hepatic dysfunction, a warning was included in section 4.5 of the SPC: "As spironolactone undergoes extensive hepatic biotransformation, care should be taken when using the product to treat dogs with hepatic dysfunction."

Species differences are observed in the elimination pathways, however, the excretion profiles after oral or intravenous administration are similar in each species. The primary route of excretion in dogs (and rats) is faecal due to the extensive biliary excretion of the metabolites. Spironolactone is excreted mainly as metabolites in the faeces (70%) and urine (20%). The mean clearance for TMS for beagle dogs was  $10.2 \pm 4.1$  L/h, and for canrenone was  $20.4 \pm 11.6$  L/h.

## Toxicology

### Single dose toxicity

Published data on the effects in laboratory animals of a single dose of spironolactone administered by the intraperitoneal or intragastric routes were provided. In rats, mice and rabbits, the LD<sub>50</sub> following oral (intragastric) route was greater than 1000 mg/kg. Although the data were not compliant with GLP and showed some deficiencies, it was concluded that following single oral administration, spironolactone is of low acute toxicity.

### Repeated dose toxicity

Published reports of studies conducted in rats, dogs and monkeys were provided; however, the data submitted were considered insufficient to make any significant conclusions and no NOELs could be established but changes were slight and were shown to be reversible. The overall conclusions are that the changes observed are consistent with stimulation of liver metabolism and this often results in secondary effects occurring.

Only one study in dogs, conducted in 2004 with oral doses of up to 20 mg/kg for 91 days, was in accordance with GLP with satisfactory data.

Increases in liver weight occurred at 20 mg/kg/day in male dogs. Anti-androgen or oestrogenic effects (i.e. increase of progesterone levels, decrease of testosterone levels and atrophy of the prostate) were observed in dogs even at the therapeutic dose (2 mg/kg/day).

### Reproductive toxicity, including teratogenicity

A number of published reports of non-GLP compliant studies conducted in rats, mice, rabbits and monkeys were provided. The studies indicated effects of spironolactone on reproductive hormones, thus influencing reproduction. In female rats and mice, spironolactone caused infertility by preventing ovulation and decreasing the number of embryos. In male rats, spironolactone decreased sperm concentration. The studies did not establish NOELs.

In rats, developmental toxicity was observed in the pups, which in some cases persist into adulthood. In male pups, doses that did not cause feminisation were capable of disrupting hormonal levels in the F1 generation. *In utero* exposure that did not cause feminisation of male external genitalia led to endocrine dysfunctions in both males and females and showed signs of male feminisation and inhibition of prostate development.

Due to the absence of conventional studies, the data submitted were considered insufficient to demonstrate clearly the effects on reproduction or developmental effects and or to draw any significant conclusions. The CVMP acknowledged that the product is contra-indicated for pregnant and lactating female dogs and dogs intended or used for breeding and agreed that no further studies would be required. Appropriate warnings for the target species have been included in the product literature.

## Mutagenicity

Two studies conducted in 2003 were described, an *in vitro* bacterial reverse mutation test in *Salmonella typhimurium* and an *in vivo* study (bone marrow micronucleus test in mice). The submitted tests were GLP-compliant and in accordance with VICH guidance.

Spironolactone did not show mutagenic activity in the bacterial reverse mutation test under the experimental conditions of the test. In the second experiment with and without metabolic activation, moderate to strong toxicity was noted but there was no significant increase in revertants in any of the 5 strains. There was no evidence of an increase of micronuclei in the micronucleus test and spironolactone did not induce damage to the chromosomes or mitotic apparatus of mice bone marrow cells.

The CVMP noted that the two studies indicated that spironolactone had no mutagenic activity; however, a third study was required to conclusively assess clastogenicity. In addition, a further *in vivo* genotoxicity test using a target tissue other than bone marrow was required to confirm whether spironolactone is genotoxic or not.

Data from two new GLP-compliant studies conducted in 2006 were provided; an *in vitro* mouse lymphoma assay and *in vivo/in vitro* liver UDS assay.

The results show that spironolactone does not show any mutagenic or genotoxicity activity.

## Carcinogenicity

A study in rats, published in 1978, was provided, in addition to a review by the International Agency for Research on Cancer (IARC). The study results indicated an increase in thyroid adenomas and interstitial cell tumours of the testes; while the IARC review of spironolactone concluded that there is inadequate evidence for carcinogenicity in humans and limited evidence in experimental animals, thus giving an overall evaluation that spironolactone is not classified as to its carcinogenicity to humans (IARC Group 3). The carcinogenic potential of spironolactone would be most likely associated with hormonal disturbances (anti-androgenic activity) and secondary to induction of liver metabolising ability.

The CVMP expressed concern that only limited data were available. However, the Committee took note of the results of the four genotoxicity tests performed both *in vitro* and *in vivo*, using different target tissues, which were all negative. Also, spironolactone has no structural analogy with known carcinogens.

The CVMP also considered that effects on the liver function (hepatocellular proliferation in rats) were only seen after oral administration of very high doses of 250 mg/kg (toxicity studies) and that chemically induced cell proliferation does not necessarily correlate qualitatively with the development of tumours. In addition, no product-related adverse effects on the liver were observed in dogs during the clinical trials and no liver tumours have been reported in humans although spironolactone has been widely used in human medicines for more than 20 years.

Taking these considerations into account, the CVMP concluded that there is low carcinogenic risk and that no additional studies would be required.

## Studies of other effects

### *Immunotoxicity*

There were no effects of toxicity of the immune system observed during the repeat dose toxicity studies and therefore no special immunotoxicity study was conducted. However, the literature was reviewed for the effects of spironolactone on blood cells. In summary, spironolactone did not



significantly alter macrophage Fcγ receptors or produce any significant changes in immunological pattern. However, it did suppress the release of inflammatory cytokines and is a potential inhibitor of leukocyte migration

#### *Effects on special organs*

The effects on liver and kidney function in rabbits was studied and a slight reduction in serum cholesterol and urea was observed but these effects were not seen in the repeat dose studies in other species. The effects on adrenal function in guinea pigs was studied and a variety of non-specific effects at high doses were observed but are likely to be a consequence of drug toxicity. The effect on lipids on the renal cortex might be associated with the inhibition of aldosterone.

#### *Irritation and sensitisation studies*

Studies on skin and eye irritation and skin sensitisation, conducted in accordance with the relevant OECD guidelines on the active ingredient spironolactone, were submitted. No studies have been submitted on the final formulation.

There were no cutaneous reactions, erythema or oedema observed in the skin irritation study and it was concluded that spironolactone was a non-skin irritant.

There was very slight chemosis, redness of the conjunctiva and iritis observed after day 1 and a very slight redness of the conjunctiva persisted to day 2 and 3; all other effects were reversible after day 1. No corneal opacity was observed and it was concluded that spironolactone was a non-eye irritant.

The skin sensitisation studies gave conflicting results. In the maximisation test of Magnusson & Kligman, at a concentration of 25%, cutaneous reactions were observed after the challenge application in the treated group at 24 and 48 hours with dryness of skin in almost all the animals at 48 hours. It was concluded that spironolactone induces delayed contact hypersensitivity in guinea pigs. However, in the Buehler test, at a concentration of 10%, no cutaneous reactions were observed at the challenge application and it was concluded that spironolactone does not induce delayed contact hypersensitivity in guinea pigs. The Magnusson & Kligman method is considered to be the more sensitive method and indicates potential to cause sensitisation whereas the Buehler method reflects the risk of sensitisation. The overall assessment of the data concludes that there is a potential for skin sensitisation.

#### *Studies on metabolites, impurities, other substances and formulation*

No studies were specifically conducted on the metabolites of spironolactone or on the excipients in the final formulation. Considering the ADME data of spironolactone, in particular the extensive metabolism, it is considered that the toxicity studies conducted have already addressed any potential toxicity of metabolites. All the excipients are used in veterinary medicines and are not expected to present any additional hazard to the use of the product and therefore specific studies are not considered necessary. No studies have been submitted on the final formulation.

#### **User safety**

Several literature publications provided show that spironolactone has been used in human medicine for more than 40 years, especially for heart failure. It is indicated for several conditions, namely primary hyperaldosteronism (daily dosage of 400mg for 4 weeks); oedematous conditions linked to congestive heart failure, cirrhosis of the liver accompanied by oedema and/or ascites and nephritic syndrome (daily dosage from 25 – 200 mg for 5 days); essential hypertension (daily dosage from 50-100 mg for 2 weeks) and hypokalaemia (daily dosage from 25 – 100mg). An important dose-related effect of spironolactone, on both the high and low pressure systems, appears to play a major role in the drug vascular and antihypertensive effects. Several publications provided on absorption, metabolism and elimination in humans show that, in summary, spironolactone is well absorbed, especially when administered with food, and it is rapidly and extensively metabolised. 90% of the dose (as metabolites) is recovered in excreta, with urinary and faecal excretion of equal importance.

The adverse effects observed in humans after the oral administration of spironolactone are mainly related to the anti-androgenic activity of the drug and several publications provided report such adverse effects. The studies indicate that the effects can occur at oral doses of 25mg/day. The studies did not establish NOELs but they did indicate that the effects were not life threatening and were reversible.

The acute toxicity of spironolactone is low and repeat dose toxicity data show that in rats, liver metabolism is stimulated giving rise to thyroid hormone deficits and thyroid tumours, but this is after prolonged and high doses of spironolactone. The changes observed are consistent with stimulation of liver metabolism and this often results in secondary effects occurring. Other changes are related to its anti-androgen or oestrogenic effects. Some effects could be observed at low doses, such as prostate atrophy in dogs receiving 2mg/kg/day and liver enlargement in rats receiving 10 mg/kg. However, there was evidence of reversibility of many effects following cessation of treatment.

The reproductive toxicity data were not adequate to draw conclusions as to the risk to users and it is difficult to make an assessment of the effects on reproduction or developmental effects, but there are unlikely to be significant risks for the person administering the product. The mutagenicity data show that spironolactone does not show any mutagenic or genotoxicity activity. The carcinogenicity data are barely adequate but the IARC evaluation concludes that there is inadequate evidence for carcinogenicity in humans and limited evidence in experimental animals.

Spironolactone is a non skin irritant and a non eye irritant, but studies show that it has the potential for skin sensitisation. The product is presented as uncoated oval/oblong tablets in 3 strengths of tablet, 10 mg, 40 mg and 80 mg. The tablets are packed in blister strips and supplied in boxes containing 3 blisters of 10 tablets or 18 blisters of 10 tablets. The product is presented as uncoated tablets and exposure to the user will, therefore, primarily be by the dermal route, but possibly also by accidental transfer to eyes from hands.

*Dermal absorption and skin sensitisation:*

There is negligible absorption after dermal administration of spironolactone, but there are data which show evidence of the potential for spironolactone to cause sensitisation, although it is acknowledged to be a rare occurrence. However, because the tablets are presented as uncoated tablets, the dermal exposure is considered to provide a risk to a user and therefore appropriate user warnings are included in the product literature.

*Accidental ingestion by young children:*

The other route of exposure that must be considered is the accidental oral ingestion by young children. The tablets are presented in blister packs that are themselves a deterrent because it is difficult for young children to get the tablets out of the blister. The tablets have a very bitter taste, so it is unlikely that a child will ingest more than one tablet because of the taste. If a young child of approximately 20 kg was to ingest one of the highest strength tablets it would receive a dose of 4 mg/kg. It is unlikely that this will cause serious effects to the child, but medical attention should be sought if such an event occurred.

*Exposure to pregnant women or women trying to conceive:*

The demonstration of negligible absorption is significant in the risk assessment for pregnant women or women trying to conceive. Although it is acknowledged that the reproductive toxicity data are not fully acceptable, it is considered that because of the negligible absorption exposure, exposure from handling the tablets is unlikely to present an unacceptable risk for pregnant women or women trying to conceive. Therefore, CVMP concluded it was acceptable that no specific warning is included for pregnant women or women trying to conceive.

*Conclusions on user safety, including risk management proposals:*

Spironolactone is not a skin or eye irritant, therefore there are no risks of irritation, but there is a risk of skin sensitisation from dermal contact and persons who have a known sensitisation should not

handle the tablets. It is recommended that the user should wash hands after use. Appropriate warnings have been included in the product literature:

- May cause skin sensitisation: Persons known to be allergic to spironolactone should not handle the product.
- Wash hands after use.
- In the event of accidental ingestion, seek medical advice immediately.

The CVMP concluded it was acceptable that no specific warning is included for pregnant women or women trying to conceive.

### **Resistance development in human medicine**

Not applicable. Spironolactone does not have microbiological activity and the product is indicated for non-food producing species.

### **Environmental safety**

An environmental risk assessment is provided and concludes that no significant impact on the environment is expected from the use of the product.

The product is intended for the treatment of congestive heart failure in the dog. Dogs will receive a dose of 2 mg spironolactone/kg bodyweight daily. Treatment can last for several weeks. Following oral administration to dogs, 80-90% of the dose is recovered in excreta. The major route of excretion is via the faeces with 70% of the dose excreted by this route. A large number of metabolites, but no parent compound, are present in excreta.

A Phase I assessment, carried out with reference to current guidance (CVMP/VICH/592/98-FINAL and EMEA/CVMP/055/096-FINAL), concludes that the environmental risk assessment of PRILACTONE Tablets does not need to proceed beyond Phase I because:

- the product is only used in non-food animals;
- the product will only be used to treat a specific group of the target species, i.e. those dogs suffering from heart failure;
- the active substance spironolactone is extensively metabolised and is not present in excreta of treated animals;
- a large proportion of excreta will be deposited in urban areas which may be considered less environmentally sensitive;
- urine and faeces from treated animals may be removed from the environment in domestic refuse or other waste disposal systems.

The CVMP concluded that the use of the product poses no unacceptable risk to the environment and that no further assessment in Phase II is required.

### **OVERALL CONCLUSIONS ON SAFETY**

The pharmacodynamic and pharmacokinetic data are satisfactory. The toxicology data are mostly published papers which are poorly reported and generally follow inadequate study designs, however, the data are sufficient to draw conclusions.

Spironolactone is of low acute toxicity. The main target organs in dogs following repeated administration are the liver (very high doses only) such as increases in liver weight and anti-androgen or oestrogenic effects (therapeutic doses already) such as increase of progesterone levels, decrease of testosterone levels and atrophy of the prostate.

Data on reprotoxicology are limited and not conclusive, although there are indications that spironolactone might have some developmental toxicity. Since the product is contra-indicated in

pregnant and lactating female dogs and dogs intended or used for breeding, no further data were required.

Spironolactone does not show any mutagenic or genotoxicity activity and there is a low carcinogenic risk.

There were no effects of toxicity of the immune system.

Spironolactone is not eye or skin irritant but has a potential for skin sensitisation.

Potential routes of exposure to the user will primarily be by dermal route and accidental oral ingestion. The potential risk of the uncoated tablets to cause skin sensitisation following dermal contact was addressed and appropriate user warnings are included in the SPC and product literature. Accidental oral ingestion e.g. by young children was considered to be a rare event; however, a warning was included to seek medical advice immediately in case of accidental ingestion.

The following user warnings are included in the product literature:

- Wash hands after use
- May cause skin sensitisation: Persons known to be allergic to spironolactone should not handle the product.
- In the event of accidental ingestion, seek medical advice immediately

The environmental risk assessment (Phase I) has demonstrated that exposure of the environment to spironolactone following the use of PRILACTONE Tablets will not be extensive. The environmental safety of PRILACTONE Tablets is considered to be acceptable.

## 4. EFFICACY ASSESSMENT

Spirolactone acts as an antagonist of aldosterone by binding competitively to the mineralocorticoid receptor located in the kidney, heart and blood vessels. Extensive literature references and some studies describe the role of the Renin-Angiotensin-Aldosterone system (RAAS) in water/electrolyte balance and its effects on the cardio-vascular system in heart failure.

### Pharmacodynamics

#### Action of spironolactone on water and electrolyte balance:

*In vitro* studies have shown the effect of aldosterone in the kidney, culminating in re-absorption of Na<sup>+</sup> and increased secretion of K<sup>+</sup> and H<sup>+</sup> in the late distal tubule and collecting duct of the nephron. *In vivo* studies in the dog showed that spironolactone antagonised this effect in a dose-dependent manner.

In addition to the literature references, results were provided from a GLP compliant pharmacodynamic study conducted in 2003-2004 to determine the lowest dose of spironolactone effective in blocking the renal electrolyte imbalance resulting from an intramuscular injection of aldosterone in beagle dogs. The trial was conducted in two phases, a preliminary trial established an ED<sub>80</sub> of 3 µg/kg for the anti-natriuretic effect of aldosterone. In the main trial, animals received either a placebo or 3 µg/kg aldosterone (= ED<sub>80</sub>) together with spironolactone at 0.75 mg/kg, 2 mg/kg or 8 mg/kg. Statistically significant changes were noted at a dose of 2 mg/kg and above. This study provided evidence of the dose-dependent antagonism of aldosterone by spironolactone and demonstrated a significant inhibitory effect of spironolactone at a dose of 2 mg/kg under the given experimental conditions.

The CVMP noted that the plasma aldosterone concentrations (PAC) achieved following the 3 µg/kg dose in the preliminary study were at least equivalent to PAC values of dogs with congestive heart failure at inclusion in the clinical studies. The Committee, therefore, supported the use of this model in the dose determination studies.

#### Action of aldosterone on the cardio-vascular system :

##### *Anti-fibrotic effect of spironolactone (cardiac remodelling):*

*In vitro* studies showed that collagen synthesis by fibroblasts increased when they were incubated with either angiotensin II or aldosterone. Studies in hypertensive rats demonstrated that spironolactone had a beneficial effect on myocardial fibrosis, which may be independent of its hypertensive effect since it occurred at a dose below that required to abolish hypertension. In a model of congestive heart failure in dogs, long term treatment with eplerenone reduced cardiac muscle fibrosis. In the Randomized Aldactone Evaluation Study (RALES) in man, treatment with spironolactone was associated with a decrease in circulating levels of markers of fibrosis, suggesting that the beneficial effect may have been associated with the anti-fibrotic effect of spironolactone. These findings support the hypothesis that aldosterone induces cardiac fibrosis in heart failure, and this effect may be dependent on activation of the RAAS locally within the heart. Spirolactone appeared to block the pro-fibrotic effect, independent of any antihypertensive effect of the drug.

##### *Effects on the vasculature:*

It was demonstrated *in vitro* that angiotensin II induced proliferation of rat aortic smooth muscle cells was inhibited by spironolactone, suggesting that this effect is mediated by locally produced aldosterone. Aldosterone causes increased generation of oxygen free radicals which inactivate nitric oxide, an important endogenous vasodilator, and cause local vascular damage in end-organs.

In human patients and rats, spironolactone improved endothelial dysfunction through increased nitric oxide bioactivity.

## Other effects

In dogs, high doses (24 – 37.5 mg/kg) of spironolactone prevented the effects of aldosterone on baroreflex depression. Evidence of the parasympatholytic effect of aldosterone was also demonstrated in human congestive heart failure patients where treatment with spironolactone reduced heart rate and increased heart rate variability.

*In vitro* studies showed that high doses of spironolactone may exert a direct effect on slow calcium channels in vascular smooth muscle cells in a similar manner to calcium blockers.

At therapeutic doses, an anti-androgen effect may be observed in dogs through competitive antagonism of spironolactone at the dihydrotestosterone receptor in the prostate.

During a clinical study in dogs receiving long term treatment with an ACE inhibitor and furosemide, increased aldosterone values were noted. When used in combination with ACE-inhibitors, spironolactone may counteract these effects of “aldosterone escape”. Appropriate reference to this effect is made in section 5.1 of the SPC.

## **Pharmacokinetics**

After the oral administration of spironolactone to dogs, the absolute bioavailability for 3 metabolites was 32% to 49% of the administered dose. Food increases the bioavailability to 80 to 90 %. The metabolites showed linear kinetics over the dose range 1-4 mg/kg and the clearance of the two main metabolites also remained constant, suggesting first-order kinetics over the dose range tested. After multiple oral doses of 2 mg spironolactone per kg for 10 consecutive days, no accumulation is observed. Steady-state conditions are reached by day 2. Mean  $C_{max}$  of 382 µg/l and 94 µg/l are achieved for the primary metabolites, 7 $\alpha$ -thiomethyl-spironolactone (TMS) and canrenone, after 2 and 4 hours respectively. In dogs, the mean volumes of distribution ( $V_{ss}$ ) of TMS and canrenone are 153L and 177L, respectively. Spironolactone undergoes rapid biotransformation in the liver, the predominant metabolites being canrenone and TMS, which are both pharmacologically active (although to a lesser degree than the parent compound). Plasma clearance of the metabolites ranges from 10 to 20 L/h in Beagles. Spironolactone is excreted mainly as metabolites in the faeces (70%) and urine (20%).

## **Target animal safety**

The applicant provided several bibliographic references indicating that spironolactone was in general well tolerated following repeated administration to dogs and that it did not result in significant changes to haematology or clinical chemistry, in particular serum electrolyte levels. However, minor changes were noted in males, i.e., decrease in weight / atrophy of the prostate and/or testes.

In addition, a GLP-compliant target animal tolerance study was conducted in 9-10 month Beagle dogs. Spironolactone (in the final formulation) was administered at 0, 2, 10 and 20 mg/kg (0, x1, x5 and x10 recommended dose) for 91 days. Additional groups of dogs treated with 0 and 20 mg/kg spironolactone were recruited to a reversibility study which ended 21 days after treatment was withdrawn.

At the recommended treatment dose of 2 mg/kg, the main findings were reversible atrophy of the glandular epithelium of the prostate, a moderate increase in the weight of the liver in male dogs and elevated aldosterone levels. The changes observed in the prostate may be related to interaction of spironolactone at the androgen receptor site. The effects of spironolactone on oestrogen levels throughout the reproductive cycle was not monitored in this study; but as the product is not recommended for use in pregnant and lactating females, this was considered acceptable.

In male dogs, the increase of progesterone levels and decrease of testosterone levels seen at 10 times RTD, and prostate atrophy, were associated with the oestrogenic effects of spironolactone (inhibition of the biosynthesis of testosterone and competitive interaction with dihydrotestosterone at the

androgen receptor site). The Committee recommended that the use of spironolactone in dogs used or intended to be used for breeding should be avoided (section 4.3 of the SPC). A warning is also included in the SPC (section 4.6) that “a reversible prostatic atrophy is often detected in entire male dogs”. Taking into account that the study was conducted in healthy, young animals, while the target group for treatment with PRILACTONE Tablets is more likely to be old dogs which tend to develop a prostatic hypertrophy (BPH), the impact of spironolactone on the prostate was not considered problematic.

The CVMP also noted that spironolactone might induce a decrease in plasma cortisol. However, in the tolerance study (using doses of up to 10 x RTD) there was no evidence of an increased incidence of gastro-intestinal signs or histological changes in the cortisol producing areas of the adrenal gland, so the Committee concluded that doses of spironolactone far in excess of those recommended for therapeutic use would be needed to induce a decrease in plasma cortisol.

In addition to these studies, a report summarises the adverse reactions reported in dogs during the clinical studies. The clinical studies confirmed that, in general, PRILACTONE was well tolerated. Most adverse events were due to well-diagnosed concomitant pathologies, e.g. related or non-specific signs. All deaths were explained and the frequency was not higher than the reference group.

Renal and urinary tracts appear to be most sensitive organs regarding adverse reactions, however, the frequency of severe renal adverse reactions was no different from that generally observed in dogs suffering from heart failure. The CVMP considered that adequate warnings are included in the SPC and product literature.

#### **Laboratory and dose titration studies:**

Several experimental models /results from pre-clinical studies for dose determination were provided.

An allometric study was provided, i.e. an empirical examination of the relationships between bodyweight and pharmacokinetic parameters. The study showed that a dose of 1.743 mg/kg spironolactone in dogs was equivalent to a dose of 50 mg in man – a moderate dose for causing natriuresis.

PK/PD modelling was used to build an  $E_{max}$  model between plasma canrenone concentrations and log urinary  $Na^+/K^+$ , as a surrogate, to evaluate the anti-aldosterone effect of spironolactone on electrolyte balance. From this model, a dose of 1.836 mg/kg spironolactone was derived as being effective in restoring the urinary  $Na^+/K^+$  ratio to normal.

A GLP-compliant dose titration study demonstrated that 2 and 8 mg/kg doses of spironolactone completely inhibited the mineralocorticoid effect of aldosterone in healthy dogs. The inhibitory effect lasted for at least 12 hours for both doses and there were still detectable metabolites in the plasma after 24 hours.

The CVMP noted the difficulties in producing a model in healthy dogs which would be representative of the physiological levels of aldosterone in diseased dogs with congestive heart failure. However, the Committee acknowledged that this model would be adequate for the purpose of dose determination and accepted the dose of 2 mg spironolactone / kg bodyweight.

#### Interactions

Literature references discussing the potential for interactions between spironolactone and digoxin, furosemide, pimobendan, NSAIDs and glucocorticoids show that appropriate warnings have been included in the SPC and product literature:

- Spironolactone decreases **digoxin** elimination and hence raises digoxin plasma concentration. As the therapeutic index for digoxin is very narrow, it is advisable to monitor closely dogs receiving both digoxin and spironolactone.

- Do not use in conjunction with non-steroidal anti-inflammatory drugs (**NSAIDs**) in dogs with renal insufficiency (kidney impairment/dysfunction). Dogs treated concomitantly with spironolactone and NSAIDs should be correctly hydrated. Monitoring of their renal function and plasma potassium levels is recommended before initiation and during treatment with combined therapy.
- The administration of either **deoxycorticosterone** or **NSAIDs** with spironolactone may lead to a moderate reduction of the natriuretic effects (reduction of urinary sodium excretion) of spironolactone.
- Kidney function and serum potassium levels should be evaluated before initiating combined treatment with spironolactone and **ACE inhibitors**. Unlike in humans, an increased incidence of hyperkalaemia (raised blood potassium levels) was not observed in clinical trials performed in dogs with this combination. However, in dogs with renal impairment there may be an increased risk of hyperkalaemia and regular monitoring of renal function and serum potassium levels is therefore recommended.
- Concomitant administration of spironolactone with ACE-inhibitors and other potassium-sparing drugs (as angiotensin receptor blockers,  $\beta$ -blockers, calcium channels blockers, etc) may potentially lead to hyperkalaemia.

In addition, it was demonstrated that co-administration of the product with **furosemide** and **pimobendan** did not cause adverse reactions and reference to this is made in the product literature (section 4.8 of the SPC): “In clinical studies, PRILACTONE was co-administered with furosemide and pimobendan without evidence of associated adverse reaction.”

### Field trials

One dose confirmation study and four field studies demonstrated the efficacy of PRILACTONE at a dose of 2 mg/kg.

#### Dose Confirmation study:

In the dose confirmation study, the objective was to demonstrate the efficacy of PRILACTONE at a dose of 2 mg/kg by measuring its natriuretic effect via the change of urinary  $\text{Na}^+/\text{K}^+$  ratio in ‘spot’ samples taken on Day 1 and Day 10. Dogs from 12 different sites in Belgium, France and Germany, with mild to moderate heart failure, were either treated with 2 mg/kg/day spironolactone or with a negative control (placebo). The dogs included were mainly small breeds (average weight 8.5 kg) and of an average age of 10 years. The tablets were administered orally, with food, for 10 days. The dogs did not receive concurrent therapy for their condition. The parameters included clinical signs, urine samples ( $\text{Na}^+$ ,  $\text{K}^+$ , and creatinine), blood samples and radiography (heart size and pulmonary oedema).

There was no significant difference between the groups at Day 10 in relation to the primary efficacy criteria, sodium excretion; and treated dogs showed a significant improvement in only one secondary criterion, “activity” compared to dogs in the placebo group.

The study failed to demonstrate efficacy according to the natriuretic effect of PRILACTONE. This may have been because of the method of urine sampling and limited severity of the disease of the recruits. Because of the lack of other appropriate models and measurements to evaluate the efficacy of spironolactone, the CVMP accepted that the study, in conjunction with the dose titration study and the PK/PD model would provide sufficient evidence for the efficacy of the proposed spironolactone dose of 2 mg/kg bodyweight.

#### 3 month field trial:

A multicentre, randomised and placebo controlled field study, conducted under double blind conditions was provided to demonstrate the efficacy of PRILACTONE at the dose of 2 mg/kg daily in improving the quality of life in dogs with congestive heart failure, treated concurrently with standard therapy (which included an ACE inhibitor).



The study included dogs mainly with valvular disease. Only a small number of animals enrolled were diagnosed with dilated cardiomyopathy (DCM). Exclusion criteria were: treatment with cardiovascular drugs other than ACE inhibitors or digoxin, treatment with NSAIDs or corticoids within the previous 2 weeks, dogs with acute pulmonary oedema, severe renal or hepatic dysfunction, or with other chronic diseases, e.g. diabetes, Cushing's syndrome. Dogs were treated orally either with 2 mg/kg spironolactone or with placebo. The tablets were administered daily with food for 84 days. Each dog also received an ACE inhibitor authorised in the country of recruitment.

The main efficacy parameters were the improvement of "quality of life" and sodium excretion in the urine, which was assessed by log urinary ( $\text{Na}^+ \times 10/\text{K}^+$ ). The "quality of life" was assessed on the basis of 6 clinical signs: cough, dyspnoea, syncope, mobility, activity and demeanour.

As regards improvement in clinical signs, the test group appeared to show a consistent, although very modest, and not statistically significant advantage over the negative control group. However, the study failed to demonstrate the natriuretic effect of spironolactone, possibly due to methodological difficulties.

#### 2 month field trial:

A multicentre, randomised, placebo controlled, double-blind field study was conducted in Belgium, France, Germany and Italy between August 2003 and April 2005. The dogs involved had either valvular disease or dilated cardiomyopathy and presented with cardiac enlargement and three clinical signs, with at least one in each group (cough, dyspnoea and syncope, and mobility, activity and demeanour) and at least one month prior treatment with an ACE inhibitor, and one day prior treatment with furosemide before inclusion. The mean age of the dogs was 11.5 years. Exclusion criteria were treatment with calcium channel antagonists,  $\beta$ -blockers or any other cardiac drugs (other than those above) or NSAIDs or corticoids within the preceding 2 weeks, and also dogs with acute pulmonary oedema, severe chronic pathology, or severe renal or hepatic dysfunction.

Dogs were treated orally either with 2 mg/kg/day spironolactone or with placebo. The tablets were administered daily with food for 56 days. An ACE inhibitor was mandatory throughout the study. Furosemide was mandatory at the time of inclusion. Digoxin and carnitine were allowed if needed.

The main efficacy parameters were the improvement of "quality of life" and sodium excretion in the urine, which was assessed by log urinary ( $\text{Na}^+ \times 10/\text{K}^+$ ). The "quality of life" was assessed on the basis of the 6 clinical signs above. Statistical tests were performed with the unilateral hypothesis.

The mean values for the measure of sodium excretion in both groups were close to normal throughout the study. The sampling method (one shot sampling) was not relevant enough to characterise sodium excretion which is very variable over the day. However, the "quality of life" showed a large difference between the groups on day 8 (73.2% improvement in the test group compared to 51.9% improvement in the placebo group), but improvement in the 2 groups was similar by the end of the 2 month period. Improvement of clinical signs was observed in both treatment groups and no statistical difference was observed between groups.

The CVMP again considered that assessment of the main efficacy criteria failed to show a significant difference between the test and placebo groups, although there was a very modest trend in favour of the spironolactone group in the improvement of quality of life.

#### 12 month field trial:

Results from a long-term field study investigating the long term efficacy and safety of spironolactone versus placebo in dogs with congestive heart failure and treated with standard cardiac therapy were provided. This study was conducted in accordance with GCP, and was multi-centre, randomised, double blind, and conducted between October 2003 – September 2006, in Belgium, France, Germany and Italy.

The study included dogs of various (mainly small) breeds with an average age of 11 years, which had participated in the previous studies (either 2-month or 3-month) and were at least on ACE inhibitor treatment. Exclusion criteria were the same as for the previous two studies. In addition, dogs requiring more than 10 mg/kg/day furosemide were also excluded.

All dogs received standard cardiac therapy, i.e. ACE inhibitor (mandatory) and digoxin, carnitine and/or furosemide, if necessary. Dogs were treated orally either with 2 mg/kg spironolactone or with placebo. The tablets were administered daily with food for 12 months.

All dogs were examined at the start of the study (Day 1; = final visit of previous study) and subsequently on Days 84, 168, 252 and 336. Clinical examinations, blood and urine sampling and thoracic radiography were conducted at each visit. Echocardiographic examinations were conducted on Days 1, 168 and 336. The main efficacy criterion was the “survival”; i.e. the percentage of dogs remaining in the study. “Events” were recorded as (i) dogs removed from the study for lack of improvement/deterioration of the heart conditions, (ii) euthanasia due to deterioration of the heart conditions, and (iii) death due to heart failure. Secondary criteria were “quality of life”, sodium excretion, clinical signs, etc, as for the previous studies. Statistical tests were performed with unilateral hypothesis.

In the PRILACTONE group, the survival at the end of 1 year in the per-protocol (PP) population was 92%, compared to 77% in the placebo group. There was no significant difference between groups in the survival probability in the PP population ( $p=0.081$ ), but in the intention-to-treat (ITT) population the difference was significant ( $p=0.036$ ). In the PRILACTONE group, the risk of cardiovascular morbidity/mortality was reduced by 66% compared to the reference group. Regarding quality of life, sodium excretion and biochemical parameters, there was no statistical difference between the groups; however, a slight trend was observed for the test product to reduce the rate at which clinical signs deteriorated.

In order to answer to the outstanding issues raised by the CMVP, the Applicant provided additional survival analysis performed on the global period of 14 to 15-months including the two short-term studies and the 12-month study. The analysis included dogs with mild to severe signs of heart failure. For each population (Safety, ITT and PP population), the survival analysis evidenced a significant difference ( $p=0,017$ ,  $0,024$  &  $0,019$  respectively) between the group receiving PRILACTONE as compared to the one receiving standard therapy alone. The Applicant concluded in this analysis a significantly better survival rate with PRILACTONE (84% vs 67% - ITT population). The efficacy on clinical signs was also assessed on this 14 to 15 months period and there was a trend towards a better performance in clinical signs for PRILACTONE group, although statistical significance was only reached for 4 of the 14 comparisons made.

The CVMP concluded that, in dogs with congestive heart failure treated with standard cardiac therapy, the administration of PRILACTONE Tablets improved their 1-year “survival” rate compared to the placebo group. The Committee considered the data sufficient to support the claim for the treatment of congestive heart failure in combination with standard therapy. However, the pathophysiology and clinical progression of dilated cardiomyopathy (DCM) and valvular disease are quite different. Since the number of dogs with DCM included in the field studies was insufficient for the demonstration of efficacy, the CVMP restricted the indications to “.....congestive heart failure caused by valvular regurgitation. ....”.

#### **Meta-analysis:**

A meta-analysis of all the clinical field studies (including a “life span study”, up to the death or withdrawal of the remaining followed cases) was provided in order to demonstrate the efficacy of PRILACTONE in reducing the risk of morbidity/mortality over a long treatment period (3 years). The analysis included dogs with mild to severe signs of heart failure. The applicant concluded in this analysis a significantly ( $p=0.047$ ) better survival rate (68%) with PRILACTONE as compared to the placebo treatment (53%). In order to answer to the outstanding issues raised by the CMVP, the Applicant provided additional survival analysis considering only death and euthanasia as “events”

(and not premature withdrawal due too severe cardiovascular deterioration). In the PRILACTONE group, the survival was 80%, compared to 64% in the placebo group. There was a significant difference between groups ( $p=0,017$ ) and the risk of cardiovascular mortality was reduced by 59% in the PRILACTONE group. However, the CVMP considered that the results obtained from the different clinical trials were not robust enough and the clinically meaningful benefit had not been sufficiently demonstrated to accept this indication. The CVMP did however acknowledge a reduction in cardiovascular disease morbidity compared to dogs treated with standard therapy alone. A statement was therefore included in section 5.1 of the SPC (pharmacodynamic properties) instead of section 4.2 (indications).

#### **Palatability:**

Spironolactone has a bitter taste and bitterness of the tablet may have contributed to a slightly increased incidence of gastrointestinal adverse reactions in dogs treated with the product. However, a beef flavouring was added to the formulation to increase its palatability, and, in the clinical trials, more than 70% of dogs ingested the tablets spontaneously. There was no difference between the palatability of the tablets containing the active substance and the placebo tablets.

### **OVERALL CONCLUSIONS ON EFFICACY**

Spironolactone acts in a dose dependant manner as a specific antagonist of aldosterone by binding competitively to the mineralocorticoid receptor located in the kidneys, heart and blood vessels. The results from a study in dogs demonstrate the dose-dependent antagonism of aldosterone by spironolactone and a significant inhibitory effect of spironolactone at a dose of 2 mg/kg under the given experimental conditions.

The study showed the natriuretic effect of spironolactone at the renal level on water and electrolyte balance. In the kidney, spironolactone inhibits the aldosterone-induced sodium retention which leads to an increase in sodium and subsequently water excretion, and potassium retention, resulting in a decrease in extracellular volume and consequently in a decrease of cardiac preload and left atrial pressure.

Laboratory studies demonstrate that, in the cardiovascular system, spironolactone also expresses an anti-fibrotic effect by inhibiting aldosterone induced cardiac fibrosis and that improves endothelial dysfunction.

At therapeutic doses, an anti-androgenic effect may be observed in dogs through competitive antagonism of spironolactone at the dihydrotestosterone receptor in the prostate.

The CVMP agreed that the mode of action as a mineralocorticoid receptor antagonist suggests that spironolactone is effective in the treatment of heart failure in dogs with hyperaldosteronaemia.

After the oral administration of spironolactone to dogs, the absolute bioavailability for 3 metabolites was 32% to 49% of the administered dose. Food increases the bioavailability to 80 to 90 %. The metabolites showed linear kinetics over the dose range 1-4 mg/kg and the clearance of the two main metabolites also remained constant, suggesting first-order kinetics over the dose range tested. After multiple oral doses of 2 mg spironolactone per kg for 10 consecutive days, no accumulation is observed. Steady-state conditions are reached by day 2. Mean  $C_{max}$  of 382  $\mu\text{g/l}$  and 94  $\mu\text{g/l}$  are achieved for the primary metabolites, 7 $\alpha$ -thiomethyl-spironolactone (TMS) and canrenone, after 2 and 4 hours respectively. In dogs, the mean volumes of distribution ( $V_{ss}$ ) of TMS and canrenone are 153L and 177L, respectively. Spironolactone undergoes rapid biotransformation in the liver, the predominant metabolites being canrenone and TMS, which are both pharmacologically active (although to a lesser degree than the parent compound). Plasma clearance of the metabolites ranges from 10 to 20 L/h in Beagles. Spironolactone is excreted mainly as metabolites in the faeces (70%) and urine (20%).

The applicant provided an experimental model based on PK/PD calculations, which were supported by results from the pre-clinical studies for dose determination, suggesting an effective dose of 2 mg spironolactone / kg bodyweight. The CVMP noted the difficulties in reproducing a model in healthy dogs, which could be representative of physiological levels of aldosterone in diseased dogs with congestive heart failure. However, the Committee acknowledged that this model would be adequate for the purpose of dose determination.

Interactions with other medicinal products are sufficiently addressed, and relevant warnings for a number of substances have been included in the SPC (and product literature).

Evidence is provided that PRILACTONE Tablets, administered at the proposed therapeutic dose rate of 2 mg/kg for 3 months, was well tolerated in healthy dogs, although some (reversible) adverse effects were observed: prostatic epithelial atrophy, a moderate increase in the weight of the liver in male dogs, and elevated aldosterone levels. As it is not recommended to administer the product to breeding males, it is not considered that the prostatic atrophy will be of clinical significance. Data from the clinical studies show that renal and urinary tract appear to be most sensitive, and appropriate warnings are included in the SPC (and product literature).

Neither hyperkalaemia nor renal dysfunction were observed in healthy dogs at overdose. However, it is acknowledged that dogs with renal impairment are less able to regulate K<sup>+</sup> levels, and, following observations in man, it is therefore recommended in the SPC (section 4.5) that renal function and plasma K<sup>+</sup> should be monitored in such patients when spironolactone is co-administered with an ACE inhibitor.

In current veterinary practice, spironolactone is considered to be a relatively weak diuretic. More potent loop diuretics, such as furosemide, which also do not carry the risk of inducing hyperkalaemia, would be used as first line diuretic therapy. Spironolactone however may be used for its potassium-sparing properties where chronic use of loop diuretics has induced hypokalaemia.

In the pre-clinical studies the applicant has demonstrated the natriuretic effect of the proposed dose of spironolactone when it is administered to healthy dogs which have been treated with aldosterone.

Owing to the lack of other appropriate laboratory models and measurements to evaluate the efficacy of spironolactone under field conditions, the CVMP agreed that the dose confirmation study, in conjunction with the dose titration study and the PK/PD model, provided sufficient evidence for the efficacy of the proposed dose of 2 mg/kg bodyweight/day.

The four multicentre, randomised and placebo controlled field studies investigated the efficacy of 2-months, 3-months, 12-months and “life span” treatment of dogs with congestive heart failure with spironolactone in addition to standard therapy, which included an ACE inhibitor.

The main efficacy parameters in the 2 and 3-months trials were the improvement of “quality of life” (defined by a combination of 6 clinical signs) and sodium excretion. Due to the difficulty in demonstrating a natriuretic effect under field conditions, “survival” was used as the main efficacy parameter in the long-term study. This was also considered to better reflect all the proposed pharmacodynamic effects of spironolactone on the cardiovascular system.

Concerning the improvement in the “quality of life” in the short term-trials, the PRILACTONE group showed a consistent, although very modest and statistically insignificant, advantage over the negative control group. The administration of PRILACTONE during a 14 to 15-months period significantly improved the survival rate compared to the placebo group. There was a significant difference between groups in the survival probability ( $p=0.024$  in the ITT population) with a 53% reduction of the risk of morbidity/mortality (84% survival rate with PRILACTONE vs 67% with standard therapy alone). The assessment (meta-analysis) on a longer period of time, up to 3 years, demonstrated a significant reduction ( $p=0.017$ ) of the risk of mortality (59% reduction with a 80% survival rate with PRILACTONE vs 64% without). Taking into account all the data available, the Committee agreed that the data would be sufficient to support the claim for the treatment of congestive heart failure (in

combination with standard therapy). However, the pathophysiology and clinical progression of dilated cardiomyopathy (DCM) and valvular disease are quite different. Since the number of dogs with DCM included in the field studies was insufficient for the demonstration of efficacy, the CVMP restricted the indication to “...congestive heart failure caused by valvular regurgitation.....”.

The Committee noted that the studies were conducted in dogs that had all been treated concurrently with ACE inhibitors, and also, in 59% of cases, with furosemide. Concern was raised that as a consequence of the historical use of spironolactone for its diuretic effect, the product might be incorrectly used as a first line diuretic, unless the indication and product literature gave full information, so the complete indication approved was: “For use in combination with standard therapy (including diuretic support, where necessary) for the treatment of congestive heart failure caused by valvular regurgitation in dogs.”

Medicinal product no longer authorised

## 5. BENEFIT RISK ASSESSMENT

The Quality part of this application for PRILACTONE Tablets is generally very good and takes into account the relevant current European and VICH guidance.

The active substance, spironolactone, has been licensed for many decades for human use, and has been the subject of a European Pharmacopoeia monograph for a long time. Adequate information on its manufacture is provided, and the routine tests and specifications are considered sufficient to assure constant quality of the active substance.

Development pharmacetics of the formulation is satisfactorily explained, the manufacturing process for the product is described in detail and is suited to produce tablets of constant quality.

Spironolactone is of low acute toxicity. Data on reprotoxicology are limited, but it appears that spironolactone might have some developmental toxicity. Since the product is contra-indicated in pregnant and lactating female dogs and breeding male dogs, no further data were required.

Spironolactone does not show any mutagenic or genotoxicity activity and there is a low carcinogenic risk. There were no effects of toxicity of the immune system.

Spironolactone is neither eye nor skin irritant but has a potential for skin sensitisation. Potential routes of exposure to the user will primarily be by the dermal route and accidental oral ingestion. The potential risk of the uncoated tablets to cause skin sensitisation following dermal contact was addressed and appropriate user warnings are included in the SPC and product literature. Accidental oral ingestion e.g. by young children was considered to be a rare event; however, a warning was included to seek medical advice immediately in case of accidental ingestion.

Since the product is only used in companion animals, a Phase I Environmental Impact assessment was performed following current guidelines. The CVMP agreed that environmental safety of PRILACTONE Tablets is considered to be acceptable.

Spironolactone acts in a dose dependant manner as a specific antagonist of aldosterone by binding competitively to the mineralocorticoid receptor located in the kidneys, heart and blood vessels. A study showed the natriuretic effect of spironolactone at the renal level on water and electrolyte balance. In the kidney, spironolactone inhibits the aldosterone-induced sodium retention leading to increase in sodium and subsequently water excretion, and potassium retention, resulting in a decrease in extracellular volume and consequently in a decrease of cardiac preload and left atrial pressure. Laboratory studies demonstrate that in the cardiovascular system, spironolactone also expresses an anti-fibrotic effect by inhibiting aldosterone induced cardiac fibrosis and improves endothelial dysfunction. The CVMP agreed that the mode of action as a mineralocorticoid receptor antagonist suggests that spironolactone is effective in the treatment of heart failure in dogs with hyperaldosteronaemia.

After oral administration of spironolactone to dogs, the absolute bioavailability for 3 metabolites was 32% to 49% of the administered dose. Food increases the bioavailability to 80 to 90 % and it is therefore recommended to administer the product with food. After repeated administration, no accumulation is observed, steady-state conditions are reached by day 2. Spironolactone undergoes rapid biotransformation in the liver into two main metabolites, which are both pharmacologically active (although to a lesser degree than the parent compound). Spironolactone is excreted mainly as metabolites in the faeces (70%) and urine (20%).

Dose determination was made on an experimental model based on PK/PD calculations, which were supported by results from pre-clinical studies, suggesting an effective dose of 2 mg spironolactone / kg bodyweight. The CVMP noted the difficulties in reproducing a model in healthy dogs, which could be representative of physiological levels of aldosterone in diseased dogs with congestive heart failure. However, the Committee acknowledged that this model would be adequate for the purpose of dose determination to be later confirmed by clinical studies.

The issue of interactions applicant was sufficiently addressed and relevant warnings for a number of substances have been included in the SPC and product literature.

PRILACTONE tablets were well tolerated when administered at the proposed therapeutic dose in healthy dogs, although some adverse effects (reversible) were observed: prostatic epithelial atrophy, a moderate increase in the weight of the liver in male dogs and elevated aldosterone levels. As it is not recommended to administer the product to breeding males, it is not considered that the prostatic atrophy will be of clinical significance. Data from the clinical studies show that renal and urinary tract appear to be most sensitive and appropriate warnings are included in the SPC and product literature.

Results were provided from four multicentre, randomised and placebo controlled field studies investigating the efficacy of 2-months, 3-months, 12-months and life span treatment with spironolactone in dogs with congestive heart failure, treated concurrently with standard therapy, which included an ACE inhibitor.

The main efficacy parameters in the 2 and 3-months trials were the improvement of “quality of life” (dependent on clinical signs) and sodium excretion, while the main efficacy parameter in the long-term studies was “survival”.

Concerning the improvement in the clinical signs in the short term trials, the PRILACTONE group showed a consistent, although very modest, and not statistically significant advantage over the negative control group. The administration of PRILACTONE improved significantly the long term survival time as compared the placebo group. However, taking into account all the data available, the Committee agreed that the data would be sufficient to support the claim for the treatment of congestive heart failure in combination with standard therapy (including diuretic support, where necessary). However, the pathophysiology and clinical progression of dilated cardiomyopathy and valvular disease are quite different. Since the number of dogs with dilated cardiomyopathy included in the field studies was insufficient for the demonstration of efficacy, the CVMP restrict the indications to “congestive heart failure caused by valvular regurgitation”.

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