SCIENTIFIC DISCUSSION

This module reflects the scientific discussion for the assessment of Aivlosin and was last updated in November 2009. For information on all changes to the marketing authorisation, please refer to module 8.

1. SUMMARY OF THE DOSSIER

Aivlosin is a veterinary medicinal product containing the macrolide antibiotic tylvalosin (previous name: acetylisovaleryltylosin) as active substance. The target species are pigs, chickens and pheasants.

The product is presented for pigs as a premix for medicated feedingstuff to be incorporated into meal feed or pelleted feed or as an oral powder to be added to meal feed. The oral powder is to be used in individual pigs on farms where only a small number of pigs are to receive the medicine while large groups of animals would be medicated with medicated feedingstuff containing the premix. In pigs, chickens and pheasants, the product is available as granules for use in drinking water. The granules are either mixed directly into the drinking water system or first mixed as a stock solution into a smaller amount of water, which is then added into the drinking water system.

In pigs, Aivlosin is indicated for the treatment and prevention of Swine Enzootic Pneumonia at a dosage of 2.125 mg tylvalosin per kg bodyweight per day in-feed for 7 consecutive days. The product has also been authorised for the treatment of Porcine Proliferative Enteropathy and for the treatment and prevention of Swine Dysentery at a dosage of 4.25 mg tylvalosin per kg bodyweight per day in-feed for 10 consecutive days. The product is also authorised for the treatment and prevention of Porcine Proliferative Enteropathy at a dose rate of 5 mg tylvalosin per kg bodyweight per day in drinking water for 5 consecutive days.

In chickens, Aivlosin is indicated for the treatment and prevention of respiratory disease associated with *Mycoplasma gallisepticum* at a dosage of 25 mg tylvalosin per kg bodyweight per day in drinking water for 3 consecutive days. When used as an aid in the prevention strategy (where infection *in ovum* with *Mycoplasma gallisepticum* is likely), chicks are to be medicated in their first three days of life and this is to be repeated at the period of risk, i.e. at times of management stress such as administration of vaccines (typically when birds are 2-3 weeks old).

In pheasants, Aivlosin is indicated for the treatment of respiratory disease associated with *Mycoplasma gallisepticum* at a dosage of 25 mg tylvalosin per kg bodyweight per day in drinking water for 3 consecutive days.

The withdrawal period is 2 days for meat and offal (pigs, chickens and pheasants); however, for the granules for use in drinking water for pigs, the withdrawal period for meat and offal is one (1) day. Aivlosin is not authorised for birds laying eggs for human consumption and should, therefore, not be used in laying birds or in the two weeks before birds are likely to start laying eggs for human consumption.

No side effects in pigs, chickens or pheasants have been reported during the clinical trials.

Due to the skin-sensitising potential of tylvalosin in laboratory animals, the product literature includes a user warning for people with known hypersensitivity to tylvalosin tartrate.

2. QUALITY ASSESSMENT

Composition of the veterinary medicinal product

Aivlosin as a premix for medicated feeding stuff contains 42.5 mg/g or 8.5 mg/g of tylvalosin (as tylvalosin tartrate) as the active substance, in a carrier of wheat feed flour and magnesium trisilicate along with other conventional pharmaceutical excipients. The oral powder presentations are identical to the 8.5 mg/g and 42.5 mg/g premix for medicated feeding stuff presentations, containing 8.5 mg/g and 42.5 mg/g of tylvalosin, respectively.

The granules for use in drinking water for pigs, chickens and pheasants are identical and contain 625 mg/g tylvalosin (as tylvalosin tartrate) as the active substance and lactose monohydrate as excipient.

Clinical Trial Formula

Clinical trials in pigs have been conducted using the premix formulation containing 42.5 mg/g tylvalosin. For chickens, pigs and pheasants, clinical trials have been conducted using granules for use in drinking water, containing 625 mg/g tylvalosin.

Development pharmaceutics

Data on the development of the intermediate product (which is used to prepare the 8.5 mg/g premix and oral powder presentations) have been presented. The selection of the excipients and the granulation method are explained. Intra-bag homogeneity of the intermediate product has been examined following transportation. The results indicate that there is no tendency for segregation to occur.

For the premix, inert carriers, wheat feed flour and magnesium trisilicate are used as the diluents. Intra-bag homogeneity of the premix has also been examined following transportation. The results indicate that no segregation occurred in the bags. Dust studies demonstrated that the 42.5 mg/g premix and 8.5 mg/g premix were both classified as being of medium dustiness.

The 8.5 mg/g oral powder formulation is identical to the 8.5 mg/g premix formulation. No special development studies were originally performed but in response to questions the bulk density of the powder: tapped/un-tapped and pre/post transport have been examined. The differences observed were acceptable.

For the granules for use in drinking water, the selection of the excipient, the granulation method and the need for moisture resistant packaging are explained.

The dustiness and friability of the product was not evaluated, but since the complete sachet of the granules for use in drinking water will be used at once without measuring out, the absence of such data is acceptable.

Inclusion rate / homogeneity of the premix / oral powder

An HPLC method is used for the analysis of medicated feed. A comprehensive validation report was presented demonstrating the suitability of this method. The concentration of active substance in the 42.5 mg/g premix is such that the inclusion rate of the premix in feed will be 0.1%, whereas the European Pharmacopoeia monograph for medicated premixes indicates that inclusion rates should not usually be less than 0.5%. The Committee noted that an inclusion rate of less than 0.5% is not permitted in some EU Member States and included appropriate recommendations in the SPC and the product information. In addition, as a post-authorisation commitment, a lower strength premix (8.5 mg/g) was submitted and authorised in February 2006 resulting in an inclusion rate of 0.5%. This inclusion rate corresponds with the recommendations given in the European Pharmacopoeia monograph for premixes for medicated feeding stuffs.

Meal feed: The premix is to be incorporated into feed at a rate of 1 kg or 2 kg and 5 kg or 10 kg per tonne of feed for the 42.5 and 8.5 mg/g presentations, respectively. The SPC recommends that an initial dilution step is used and that a horizontal ribbon mixer is used. It was demonstrated that the premix could be homogenously incorporated into a number of different types of animal feeding stuffs. The oral powder should be added to the estimated quantity of daily ration for each pig, in a bucket or similar receptacle, and thoroughly mixed, taking into account a possible reduced appetite in sick pigs.

Pellets: The batches of medicated feed prepared have also been pelleted, during which the medicated feeding stuff was pre-conditioned with steam for 5 minutes and then pelleted at 70°C. The recommended pelleting conditions are included in the SPC. The oral powder cannot be mixed thoroughly into pelleted feed; therefore, the use of the oral powder is restricted to dry, non-pelleted feeds.

In May 2006, the Marketing Authorisation Holder **extended the indications** for two further claims (Porcine Proliferative Enteropathy (ileitis) and Swine Dysentery). Since the new indications use a dose rate of 4.25 mg tylvalosin/kg bodyweight (i.e. 2 x the dose rate used for the initial indication), the inclusion level of the product in feeds for these dosages increased accordingly. The applicant provided homogeneity and stability data to support the higher inclusion rate and the CVMP considered these to be satisfactory.

In a study with the premix, one type of pig feed was medicated at both the lower and higher inclusion rate at a mill using a ribbon blender. The medicated meal was then pelleted. The results obtained were satisfactory with average assay results being within ± 2% of nominal. However, low recoveries in another trial were attributed to the double pelleting process used by the feed mill, which involved non-standard (high temperature) pelleting conditions. The CVMP, therefore, modified the wording of the recommendations for pelleting: "Pelleting conditions involve a single pre-conditioning step with steam for 5 minutes and pelleting, at not more than 70ºC under normal conditions."

Since in some clinical studies the range of dose rates actually eaten by the pigs was consistently lower than the (nominal) dose expected to be consumed, a new formula was introduced in the product literature to facilitate the calculation of the correct inclusion rate in feed.

With regard to the **8.5 mg/g oral powder** presentation, the Committee noted that animals of more than 97 kg bodyweight would require more than 10 x 10 ml scoops of oral powder per animal. The Committee considered that there is a danger of miscounting when such large multiples are required causing potential problems of over- or underdosing. Therefore, a 25 ml scoop was added to the oral powder presentation.

In 2009, the marketing authorisation was extended to include a new 42.5 mg/g oral powder for pigs. In order to ensure that the product can be sufficiently well mixed into the daily ration to achieve the desired pharmacokinetic profile, the SPC includes an instruction to thoroughly mix Aivlosin 42.5 mg/g Oral Powder into approximately 200-500 g of feed and then to thoroughly mix this pre-mixture into the remainder of the daily ration. Dosing scoops of capacity 1 ml and 5 ml have been provided to ensure accurate dosing.

Solubility of the granules in drinking water

The solubility of the granules was investigated according to current requirements, i.e. in concentrations up to the most concentrated stock solutions at different temperatures (20°C, 8°C and 4°C), to cover the range of temperatures likely to be encountered throughout Europe. At low concentrations, the granules dissolve rapidly (within 1 minute) to give a clear solution in hard and soft water and at all temperatures. However, higher product concentrations take longer to dissolve and, even when fully dissolved, some cloudiness remained at 20°C. However, chemical analysis of these cloudy solutions confirmed that the active ingredient was fully available and therefore the efficacy of the product would not be affected. Information is provided in the SPC to explain the mixing procedure needed under different circumstances and to explain that stock solutions may be cloudy even after the active substance is fully dissolved.

Manufacturing Process

The 8.5 mg/g premix and oral powder formulations are manufactured via the intermediate product Aivlosin 17% Granules. Manufacturing formulae for the intermediate product and the final premix and oral powder are presented. The intermediate product is produced using a roller compaction granulation process. A conventional blending procedure is then employed to dilute the intermediate product. The 42.5 mg/g premix is manufactured by blending all of the formulation ingredients and then granulating the blend by roller compaction to produce the final product.

For the granules for use in drinking water, tylvalosin tartrate and lactose monohydrate are blended together and then transferred to a granulation system which uses a roller compaction process.

Satisfactory flow diagrams and detailed descriptions of the method of manufacture, including in-process controls, are presented for the all presentations of Aivlosin.

All of the above manufacturing processes have been validated.

Active substance

Tylvalosin tartrate is a white to light yellow powder and is not described in any pharmacopoeia. The specification for tylvalosin tartrate is based upon the monograph for this substance as set out in "The minimum requirements for pharmaceutical products not requiring approval for veterinary use in Japan". Manufacture is via fermentation, using a genetically modified strain of *Streptomyces thermotolerans*. Tylvalosin is isolated from the fermentation broth and the tartrate salt is formed with the final material being spray dried. No organic solvents are employed during the manufacturing process.

In-process controls, their limits and methods, as performed during the fermentation, purification and spray drying stages are fully documented. Comprehensive specifications and linked test methods, and an indication of which tests are conducted on receipt, are presented for each of the materials used during production.

Satisfactory process validation data of the active substance are provided. The in-process results at various stages of manufacture of the active substance show consistency between batches. The final batch results demonstrate compliance with the authorised specification.

Stability data are presented according to CVMP-VICH guidelines on a number of batches of the active substance. A 9-month re-test period for the active substance was accepted.

Excipients

Excipients that appear in a pharmacopoeia comply with the relevant monograph. Detailed specifications for the other excipients are presented. Certificates of analysis have been provided for all excipients.

Packaging

The intermediate product is packed in four-layer aluminium laminated bags with an innermost layer of polyethylene. The 42.5 mg/g premix is packed in laminated bags (laminate composed of polyester, aluminium and low density polyethylene) containing 5 or 20 kg. The 8.5 mg/g premix and oral powder presentations are packed in polyethylene lined paper bags containing 5 or 20 kg for premix and 1 kg or 3 kg bags for the oral powder. Full specifications have been provided for the packaging.

For the oral powder presentation, polystyrene measuring scoops of 5 ml, 10 ml and 25 ml (for 8.5 mg/g) and 1 ml and 5 ml (for 42.5 mg/g) are supplied with each pack. The suitability of the scoops for food contact applications has been confirmed. Scoop accuracy and precision were satisfactory.

The granules for use in drinking water are packed in single, laminated sachets (laminate composed of polyester, aluminium and low density polyethylene).

For chicken, pack sizes of 40 g and 400 g are available, which would allow the treatment of a total of 1,000 kg or 10,000 kg bodyweight of chickens per sachet, respectively (e.g. 20,000 birds with average bodyweight of 50 g or 500 g, respectively). For pheasants, pack sizes of 16 g and 40 g are available, which would allow the treatment of a total of 400 kg or 1,000 kg bodyweight of pheasants per sachet, respectively (e.g. 1000 birds with an average bodyweight of 400 g or 20,000 birds with a bodyweight of 50 g, respectively). For pigs, pack sizes of 40 g and 160 g are available, which would allow the treatment of a total of 5,000 or 20,000 kg bodyweight of pigs per sachet, respectively (e.g. 250 pigs with an average bodyweight of 20 kg or 400 pigs with an average bodyweight of 50 kg, respectively). For treatment of smaller flocks or animal numbers, the preparation of a stock solution is required.

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

For the oral powder and premix presentations, no materials of animal origin are contained or used in the manufacture of the product.

The granules for use in drinking water contain lactose monohydrate as excipient, which is derived from milk sourced from healthy cows under the same conditions as milk collected for human consumption. No ruminant material other than calf rennet is used in the manufacture of the lactose. The product therefore complies with the CVMP TSE guideline (EMEA/410/01 rev 2).

Finished product specifications

Regarding the active substance, with the exception of one impurity, all impurities present routinely at levels > 0.2% have been identified. A comprehensive discussion of the impurities and degradation products has been provided. The qualification of the limits for the individual impurities is detailed and the defined limits for impurities, including the limit for total impurities, are justified. Satisfactory analytical methods for the determination of the active substance and the impurities and degradation products are provided.

The possibility of carry over of viable organisms from the fermentation process, host cell proteins and DNA into the active substance has been investigated using four batches of the active substance. Batch analysis data demonstrate full compliance with the proposed specification.

The specification for the intermediate product used in the manufacture of the 8.5 mg/g premix and oral powder controls relevant parameters using suitably validated methods. The parameters controlled include: description, identity, particle size, moisture, and assay.

The Finished Product Specification for the 42.5 mg/g and 8.5 mg/g premixes control relevant parameters. They include tests for appearance, identity, particle size, moisture content, assay, limits for impurities and microbial purity. Limits set are appropriate and justified. Batch analysis data provided on 3 batches of the premix formulations, demonstrate that the defined limits can be consistently met. Satisfactory validation data for the analytical methods presented are included in the dossier.

The specification for the 8.5 mg/g oral powder presentation is identical to the 8.5 mg/g premix apart from the addition of limits for bulk density.

The specifications for the 42.5 mg/g oral powder presentation are identical to those applied to the 42.5 mg/g premix presentations, apart from the addition of limits for bulk density.

The Finished Product Specification for the granules for use in drinking water controls relevant parameters. It includes tests for appearance, identity, particle size, moisture content, assay, limits for impurities and microbial purity. It also includes a dissolution test at different concentrations and temperatures in hard and soft waters and a test for uniformity of dosage units as the complete contents of one sachet may be used to medicate drinking water. Limits set are appropriate and justified. Batch analysis data provided, demonstrate that the defined limits can be consistently met. Satisfactory validation data for the analytical methods presented are included in the dossier.

Stability tests on the finished product

The shelf-life specification for the intermediate product used in the manufacture of the 8.5 mg/g premix and oral powder is identical to the release specification. The shelf-life Finished Product Specifications differ from the release Finished Product Specifications in respect of higher limits for certain impurities and a lower limit for tylvalosin assay.

Intermediate product
Stability data in accordance with current CVMP-VICH guidelines are presented for 3 full-scale batches, which have been stored in the commercial packaging. No significant changes were observed following 24 months at 25°C/60%RH. The absence of accumulation of degradation products indicates that degradation is not occurring. A two year shelf-life is supported for the intermediate product.

Premix and oral powder

Stability studies in accordance with current VICH guidelines have been conducted on production scale batches. It was demonstrated that following storage for 24 months at 25 C/60%RH the 42.5 mg/g premix did not comply with the shelf-life specification and the shelf life was restricted to 18 months.
Data are available after 36 months storage for the 8.5 mg/g premix and oral powder presentations and support the 3 year shelf life assigned by the CVMP.

The stability data presented for the 42.5 mg/g oral powder presentation are those previously assessed and considered suitable to support an 18 month shelf life for Aivlosin 42.5 mg/g premix. The same shelf life was therefore set for the oral powder.

Granules for use in drinking water:

Stability studies in accordance with current VICH guidelines have been conducted on full-scale pilot batches. Samples have been stored in the aluminium foil laminated sachets under VICH real time and accelerated storage conditions up to 36 months at 25°C/60 %RH. The data demonstrated that the shelf-life limits are justified and a 3 year shelf-life is supported for the product when stored at temperatures up to 30°C.

No stability data are provided for open sachets and a warning not to store opened sachets is included in the SPC.

In-use Stability Tests

In feed-use
The stability of different batches of medicated meal and of medicated pellets has been studied over a period of one month following storage under controlled conditions involving varying temperatures, humidity, light and open/closed bags, and in different feeds. An in-feed shelf-life of 1 month for medicated meal and pellets for the 42.5 mg/g premix is supported by the data presented. For the 8.5 mg/g premix, a 1 month shelf-life for medicated meal was also accepted. However, based on the data submitted, a shelf-life of only 2 weeks was accepted for medicated pellets.
Feed containing the oral powder should be replaced if not consumed within 24 hours. Therefore, in-feed stability data are not considered necessary for the oral powder.

Use in drinking water
Stability data of the medicated drinking water were provided with samples stored in polyethylene buckets and exposed to light, tested according to current requirements (CVMP Guideline on The Quality Aspects of Pharmaceutical Veterinary Medicines for Administration via Drinking Water). The stability of tylvalosin in both, hard and soft water was satisfactory after 48 hours storage. Since the majority of drinking water systems are composed of plastic components, it was also considered that the data provided are sufficient to demonstrate the stability of the diluted product in typical drinking water systems.

OVERALL CONCLUSION ON QUALITY

The active substance is suitably controlled using validated methods. The specification limits for individual impurities have been justified in terms of batch data, stability data and safety.

The method of manufacture for the **42.5 mg/g premix** is well defined. The premix is suitably controlled using validated methods. The shelf-life Finished Product Specification includes limits for four named degradation products and for total impurities and these limits have also been suitably justified in terms of batch data, stability data and safety.

Stability data are presented which confirm: the shelf-life for the intermediate product (24 months), the shelf-life for the 42.5 mg/g premix (18 months) and the in-feed shelf-life (1 month).

It has been demonstrated that the premix can be homogeneously incorporated into pig feed to give an in-feed concentration of 42.5 mg tylvalosin/kg feed. A specification was proposed for feed medicated with the premix.

The inclusion rate of the 42.5 mg premix in feed will be 0.1%. Since inclusion rates of less than 0.5% are not permitted in some EU Member States, an appropriate recommendation was included in the SPC and the product information: “Consideration should be given to official guidance on the incorporation of medicated premixes in final feeds.” In addition, the applicant confirmed to submit an application for a new presentation with a lower strength resulting in an inclusion rate of 0.5%.

The data presented subsequently for the **lower strength premix** (8.5 mg/g) premix are comprehensive. The 8.5 mg/g premix is prepared using an intermediate product unlike the 42.5 mg/g premix, which is prepared directly from its constituent ingredients. The intermediate product is simply subjected to a dilution step in order to produce the 8.5 mg/g premix. The data submitted for the 8.5 mg/g premix results in higher inclusion rate for the 8.5 mg/g premix in feed is in accordance with recommendations in the European Pharmacopoeia and national legislation in the EU concerning the medication of feed.

The **8.5 mg/g oral powder** presentation is identical in formulation to the 8.5 mg/g premix presentation. However, as the oral powder is to be added to the feed of individual animals, the pack size is smaller and measuring scoops are supplied with each pack. Scoop accuracy and precision have been demonstrated. In view of the volumetric measurement of the dose, the bulk density of the oral powder is controlled.

An 18 month shelf life was initially agreed for the 8.5 mg/g premix and the oral powder when stored below 25ºC; this was later extended to 3 years. The oral powder cannot be mixed thoroughly into pelleted feed; therefore, the use of the oral powder is restricted to dry, non-pelleted feed.

In 2006, the Marketing Authorisation Holder extended the indications to two further claims (Porcine Proliferative Enteropathy (ileitis) and Swine Dysentery) with a higher dosage resulting in higher inclusion rates for the premix in feed (85 mg/g tylvalosin/kg feed). Satisfactorily results from homogeneity and stability data were provided. However, low recoveries of the feed medicated with the **premix** which were attributed to the double pelleting process used by the feed mill resulted in slight changes in the wording of the SPC regarding the recommended pelleting conditions. Also, a

new formula was introduced in the product literature to calculate the correct inclusion rate in feed. With regard to the **oral powder** presentation, an additional larger size scoop was requested.

In 2008, the marketing authorisation was extended to include a new pharmaceutical form, **granules for use in drinking water for chickens**. Satisfactory data were provided to demonstrate that the product is suitably formulated and quality-controlled. The solubility of the product has been investigated according to the relevant CVMP Guideline. In higher concentrations, presence of cloudiness was noted, which was demonstrated to be a very fine secondary precipitate without impact on the efficacy of the product. Appropriate instructions have therefore been included in the SPC. Stability studies have been presented confirming the proposed shelf-life of 3 years for the finished product. No stability data are provided for open sachets and opened sachets should not be stored. Sufficient data have been presented to demonstrate the stability of the diluted product in typical drinking water systems. In 2009, the marketing authorisation was further extended to allow the use of **granules for use in drinking water for pigs and pheasants.**

In 2009, the marketing authorisation was extended to include a **42.5 mg/g oral powder for pigs** that is identical to 42.5 mg/g Premix containing 42.5 mg/g. No additional development studies were performed. However, as the oral powder is to be added to the feed of individual animals, the pack size is small and measuring scoops are supplied with each pack. Scoop accuracy and precision have been demonstrated. In view of the volumetric measurement of the dose, the bulk density of the oral powder is controlled.

3. SAFETY AND RESIDUE ASSESSMENT

A. SAFETY ASSESSMENT

Pharmacokinetics

Absorption
Tylvalosin is rapidly absorbed after oral administration.
In pigs, plasma concentrations are below the limit of quantification after administration of the recommended dose.
In chickens, mean peak plasma concentration after a single oral dose of 30 mg/kg bodyweight (bw) was achieved at approximately 1 hour.

Distribution
Tylvalosin is rapidly distributed to the major organs.
In pigs, highest concentrations were found in bile, spleen, lung, kidney and liver. Tylvalosin concentrations in the lung were observed up to 12 hours after the last administration. Although at the therapeutic dose, relatively low lung concentrations of tylvalosin in the range of the MIC_{90} values were recorded, the concentration of the main metabolite 3-AT might be higher than that of the parent compound. Therefore, a significant part of the overall efficacy of the product is assumed to be due to the activity of the metabolites rather than to tylvalosin alone.

Metabolism
Tylvalosin is rapidly metabolised with 50% being metabolised within 30 minutes. The main metabolite is 3-O-acetyltylosin (3-AT), which possesses equivalent microbiological activity to the parent compound. It is not known to what extent other metabolites contribute to the overall effect of the drug. In rats and pigs, residues of 3-AT were higher than residues of the unchanged substance within 2 hours of administration.

A study in pigs receiving 2.5 mg tylvalosin/kg bodyweight for 7 consecutive days showed extensive metabolism of tylvalosin with the unchanged substance accounting for less than 7% of the radioactivity in both urine and faeces. 3-AT and at least 8 other metabolites were observed in pooled urine and faeces samples. The metabolite profile in urine and faeces was similar following the first dose and last dose indicating that the metabolism of tylvalosin did not change over the 7-day dosing period. There were no gender-related differences in excretion kinetics, tissue distribution of residues or metabolism.

In chickens, following oral administration via drinking water, tissue concentrations of tylvalosin and 3-AT were very low or absent within 12 hours of the last administration. The highest concentrations of total residues were found in the liver and kidneys, the major organs of elimination. The elimination half-life of total radioactivity over the period 3-7 days post last dose for liver and kidneys was about 3 days.

Excretion
In pigs, excretion was rapid, with the major route of elimination being via the faeces. Less than 10% of the administered dose is detectable (using HPLC studies) in urine and faeces collected up to 72 hours after dosing. However, since high concentrations of microbiological activity are detectable in bile, it is assumed that excreted products are broken down in the gastrointestinal tract.

Toxicology

Single dose toxicity
The acute oral toxicity of tylvalosin is low to moderate with acute oral LD_{50} values of 758 mg/kg bodyweight in male mice and more than 3016 mg/kg bodyweight in rats. The main metabolite 3-0-acetyltylosin (3-AT) and three other by-products of the manufacture of the substance were also of low acute oral toxicity in mice (values greater than 1000 mg/kg bodyweight).

Repeated dose toxicity
In rats, a repeated-dose toxicity study was carried out with a diet containing up to 50 g tylvalosin/kg feed for 4 weeks. A lowest-observed-effect-level (LOEL) of 400 mg/kg feed was identified for the study. In another study in rats, using the same dose levels with the medicated diets administered for 13 weeks, a no-observed-effect-level (NOEL) was determined of 2000 mg/kg feed, equivalent to 135.9 and 159.1 mg/kg bodyweight/day.

In mice, fed for 13 weeks with diets containing up to 25 g tylvalosin tartrate/kg feed, a LOEL was determined of 250 mg/kg feed, equivalent to approximately 46 and 55 mg/kg bodyweight/day in male and female mice, respectively.

Reproductive toxicity
In a 2-generation reproduction study in rats fed on diets containing up to 10 g tylvalosin tartrate/kg feed for 10 weeks, a NOEL of 400 mg/kg feed was identified, based on reduction in body weight gain in parents and reductions in litter and pup weights. This corresponded to approximately 22 - 65 and 32 -67 mg/kg bodyweight/day in males and females, respectively.

Embryotoxicity/foetotoxicity, including teratogenicity
Mice were given daily oral doses of up to 700 mg tylvalosin/kg bodyweight/day by gavage from days 6 to 15 of gestation. Toxicity in dams and reduced mean foetal litter weight were observed at dose rates of 400 and more mg/kg bodyweight/day. The overall NOEL for the study was 200 mg/kg bodyweight/day.

A teratogenicity study was carried out in which Sprague-Dawley rats were given daily oral doses of up to 2000 mg tylvalosin/kg bodyweight/day from day 7 to 17 of pregnancy. Maternal toxicity was evident at 500 and 2000 mg/kg bodyweight with a dose-related increase in salivation and water intake and reduced food consumption during the treatment period. There was no evidence of foetotoxicity or teratogenicity at any dose level.

Studies in laboratory animals have not produced any evidence of teratogenicity and maternal toxicity has only been observed in rodents at high overdoses. However, since no studies have been performed in the target animal species, an appropriate warning has been included in the product literature (under "Use during pregnancy and lactation" of the SPC and under "Special warnings" in the package leaflet).

Mutagenicity.
Tylvalosin tartrate gave negative results *in vitro* in gene mutation assays in prokaryotic systems and mouse lymphoma cells and *in vivo* in a micronucleus test in the bone marrow of mice.

Positive results were obtained in an *in vitro* chromosomal aberration assay in Chinese hamster ovary cells with a stronger positive response in the presence of metabolic activation. However, the test showed negative results for the main metabolite 3-AT. Since tylvalosin is rapidly metabolised, it was concluded that the substance is not mutagenic.

Carcinogenicity
No carcinogenicity studies were carried out. However, the chemical structure of the substance did not possess any alerting features and it was concluded that carcinogenicity studies were not required.

Studies of other effects – Acceptable Daily Intake (ADI)

The previously established microbiological ADI of 1.02 µg/kg bw (i.e. 61 µg/person) for tylvalosin was modified as described below:

The MIC_{calc} values were calculated for both tylvalosin and 3-AT and found to be 0.335 µg/ml and 0.226 µg/ml, respectively. The fraction of the oral dose available for microorganisms was found to be 40%. The microbiological ADI was based on the MIC_{calc} for the metabolite (3-AT), which is lower than that of the parent tylvalosin. The microbiological ADI was confirmed as the overall ADI and existing MRLs for tylvalosin remain unchanged.

The CVMP concluded that the microbiological ADI for tylvalosin is modified to 2.07 µg/kg bodyweight (124.2 µg per person). As the previously established toxicological ADI of 220 µg/kg bodyweight is higher, the microbiological ADI remained as the overall ADI for tylvalosin.

User safety

Tylvalosin is not used in human medicine and no studies have been provided on human safety. Humans may be exposed to tylvalosin via inhalation, by accidental ingestion or by skin/eye contact.

Inhalation risk
Aivlosin premix is moderately dusty but most particles are not in the respirable range and the product is therefore considered a relatively low inhalation risk. In addition, an appropriate warning recommending the use of protective clothing is included in the product literature (under "Special precautions to be taken by the person administering the medicinal product to animals" of the SPC and under "Special warnings" in the package leaflet).

A new dusting study was provided for the 8.5 mg/g premix. Although a moderate degree of dustiness was found, which was higher than previously found for the 42.5 mg/g Premix, the dust collected contained a lower concentration of active substance. Since overall the new dusting study showed similar results to those for the previously approved product (42.5 mg/g Premix), the CVMP considered that the current warnings in the product literature would be sufficient.

Accidental ingestion
The acute oral toxicity of tylvalosin is low to moderate and accidental ingestion of large amounts of a product for incorporation into feed would be unlikely to occur. However, instructions as to the appropriate action to take in the event of accidental ingestion were included in the product literature (under "Special precautions to be taken by the person administering the medicinal product to animals" of the SPC and under "Special warnings" in the package leaflet).

Eye and skin irritation
In rabbits a 10% solution of tylvalosin tartrate was slightly irritating to skin and eyes but the extent of this irritation was insufficient to warrant classification as an irritant.

A skin sensitising study in guinea pigs showed mild reactions (discrete or patchy erythema) in 60% of the animals 1 – 2 days after challenge. It was concluded that tylvalosin tartrate is capable of causing sensitisation and a warning was, therefore, included in the product literature (under "Special precautions to be taken by the person administering the medicinal product to animals" of the SPC and under "Special warnings" in the package leaflet).

In 2006, the Marketing Authorisation Holder extended the indications for two further claims (Porcine Proliferative Enteropathy (ileitis) and Swine Dysentery) with a higher dose rate of 4.25 mg tylvalosin/kg bodyweight (i.e. 2 x the dose rate used for the initial indication) over 10 days (i.e. 3 days longer than the initial indication). The CVMP concluded that the approved user warnings would remain appropriate because they address the most likely routes of exposure.
Likewise, the CVMP agreed that the warnings as expressed for the existing pig applications were also relevant for the granules for use in drinking water (for chickens, pigs and pheasants), and also for the oral powders.

Resistance development in macrolides used in human medicines

The main bacterial species of concern as zoonotic organisms are *E. coli*, *Salmonella* spp, *Campylobacter* spp and *Enterococci* spp. Tylvalosin has no antibacterial activity against Enterobacteriaceae including *E. coli* and *Salmonella* spp (they are naturally resistant). *Campylobacter* spp and *Enterococcus* spp are inherently susceptible to macrolides.

The Committee acknowledged that macrolides are important to human medicine. The exposure of human intestinal flora to this class of drugs is likely to be high and the resistance genes that may be amplified by use in pigs and chickens are the same as those of importance in human medicine. The available data relating to antimicrobial resistance for the proposed use of tylvalosin for chicken were partly non-conclusive. Based on general knowledge on macrolides and considering the high concentrations in the gastrointestinal tract, the risk for transfer of zoonotic bacteria (such as *Campylobacter* spp) or resistance genes might be substantial but would, however, not differ from that from other macrolides.

Studies in chickens conducted by the applicant showed no evidence of selection of resistant *Campylobacter* or *Enterococcus* as a result of treatment with Aivlosin at the recommended dose rate. Furthermore, it was noted that macrolides are widely used to treat *Mycoplasma* infections in human medicine but strains with acquired resistance to macrolides have been rarely described despite being obtained *in vitro*.

Limited knowledge is available on possible cross-resistance of tylvalosin to other macrolides.

The CVMP, therefore, concluded that the impact of the use of tylvalosin in relation to development of resistance in human medicine was considered aceptable.

Environmental safety

Aivlosin **premix for medicated feedingstuff for pigs** might be used in a large number of animals. Predicted Environmental Concentration (PEC) calculations were produced based on a worst-case scenario of a 100% excretion rate of the administered dose via faeces using soil depths of 25 cm and 5 cm and taking into account different manure management practices in Northern and Southern Europe.

The PEC_{soil} values based on 5 cm soil depth exceeded the trigger value of 100 µg/kg for piglets and fattening pigs, but not for adult pigs. A Phase II assessment was therefore provided for piglets and for fattening pigs in accordance with the CVMP guidance for Phase II, i.e. soil degradation, soil adsorption/desorption, earthworm toxicity, phytotoxicity and acute toxicity to aquatic invertebrates (*Daphnia magna)*.

Tylvalosin was of low toxicity to earthworms, terrestrial plants and water fleas. Tylvalosin was not considered persistent in soil. There was a wide margin of safety in the PEC/effect ratios for soil and groundwater. Although the Applicant had not calculated $PEC_{surfacewater}$ values, this was acceptable considering that exposure of surface water was unlikely because of the high Koc. It was considered that even if exposure of surface waters was to occur it was unlikely that there would be an adverse risk to aquatic species. This conclusion was made on the basis of the assessment for groundwater, where there was a very large margin between the PEC and toxicity to *Daphnia*.
It was concluded that Aivlosin 42.5 and 8.5 mg/g Premixes - if used according to the recommended posology - are expected to be of low risk to the environment.

In 2006, the Marketing Authorisation Holder extended the indications for **two further claims in pigs** (Porcine Proliferative Enteropathy (ileitis) and Swine Dysentery). Since the new indications use a dose rate of 4.25 mg tylvalosin/kg bodyweight (i.e. 2 x the dose rate used for the initial indication) over 10 days (i.e. 3 days longer than the initial indication), a new environmental risk assessment was submitted. PEC_{soil} calculations exceeded the value of 100 µg/kg and a Phase II assessment in accordance with the CVMP Phase II guidance was carried out. The risk assessment used appropriate

assessment factors and the assessment was complete at Phase II Tier A. The CVMP concluded that the risk to the environment from the use of Aivlosin using the higher dosage over 10 days was acceptable.

In 2008, the marketing authorisation was extended to a new target species (**chickens**) to be administered via **drinking water**. A new environmental impact assessment (Phase II) in accordance with VICH guidance was carried out using the PEC_{soil} value determined for broiler chickens which represented the worst case in terms of exposure of the environment. In order to update the dossier to comply with VICH guidance acceptable studies on the effects of tylvalosin on nitrogen transformation in soil micro-organisms, earthworm reproduction, blue-green algae and fish were submitted. Tylvalosin had no effect on soil micro-organisms or earthworm reproduction. It was of low toxicity to fish. Tylvalosin could be considered toxic to blue-green algae. Risk quotients for the terrestrial and aquatic environments were all less than 1 indicating an acceptable risk to the environment. The CVMP concluded that the risk to the environment from the use of the product in chicken was acceptable when used as recommended. No risk mitigation measures were considered necessary.

In 2009, the marketing authorisation was extended to allow the use of granules **for use in drinking water in pigs** and an updated environmental risk assessment, in compliance with VICH and CVMP guidelines, was provided which included several new studies. The PEC_{soil} values for total residue exceeded the 100 µg/kg value for both weaner and fattening pigs and as a consequence further assessment in Phase II was undertaken. For the soil environment there was no adverse effect on nitrogen production by soil micro-organisms at soil concentrations far exceeding the estimated PEC_{soil}. The RQ values for both earthworms and plants were less than 1 indicating an acceptable level of risk. For groundwater the metamodel prediction indicated that the $PEC_{groundwater}$ would be less than 0.01 µg/l and no further risk characterisation was required. For surface water a PEC value was calculated using the approach recommended by CVMP. Risk characterisation indicated that aquatic organisms would not be at risk. The CVMP concluded that the use of the product would not pose a risk for the environment when used as recommended. No risk mitigation measures were considered necessary.

In 2009, the marketing authorisation was extended to add a **42.5 mg/g oral powder for pigs.** The indications and dosing regimen for the oral powder are identical to those of the authorised Aivlosin 42.5 mg/g Premix for Medicated Feeding Stuff for Pigs. As a consequence, the risk for the environment will be no greater than that posed by the use of the premix product. The oral powder does not pose an unacceptable risk for the environment when used as recommended

In 2009, the marketing authorisation was also extended to add a new target species **(pheasants)** for the **granules for use in drinking water**. Information provided by the applicant demonstrated that pheasants are raised under similar conditions to chickens and that the PEC_{soil} values for pheasants will not exceed those calculated for broilers. Therefore, the exposure of the environment from use of the product in pheasants will not be greater than the exposure from its use in chickens.

B. RESIDUE ASSESSMENT

Depletion of residues

A number of residue depletion studies were provided which have been assessed previously for the MRL applications.

Pigs

Premix and Oral Powder:
The pivotal residue depletion study was conducted in pigs fed twice daily, for 10 days, with feed medicated to provide a dose of 5 mg tylvalosin/kg bodyweight/day. The pigs were slaughtered at 2, 12, 24, 72 or 120 hours after withdrawal of the medicated feed. Samples of liver, kidney, muscle and skin + fat were analysed for residues of tylvalosin and the main metabolite 3-O-acetyltylosin (3-AT). The limit of quantification was 25 µg/kg for each analyte. Quantifiable residues were found only in

some samples of liver and kidney taken at the first two time points, i.e. 2 hours and 12 hours after withdrawal of medicated feed.

Another study on which the MRL was based was conducted in piglets given the recommended oral dose of 2.5 mg ^{14}C-tylvalosin tartrate/kg bodyweight/day on 7 consecutive days in gelatin capsules. The pigs were slaughtered at 12 hours, 1, 3 and 5 days after the last dose. Total residues in tissues were determined by combustion and liquid scintillation counting. Total residues were highest in liver and depleted from mean values of 483 µg equivalents/kg at 12 hours to 234 and 90 µg equivalents/kg at 1 and 3 days. Mean total residues in kidney depleted from 308 µg equivalents/kg at 12 hours to 202 and 107 µg equivalents/kg at 1 and 3 days respectively. Mean total residues in muscle depleted from 29 µg equivalents/kg at 12 hours to 11 µg equivalents/kg at 1 day. Mean total residues in composite fat samples were 187 µg equivalents/kg at 12 hours, 64 µg equivalents/kg at 1 day and 43 µg equivalents/kg at 3 days. Mean total residues in samples of skin with fat were 110 µg equivalents/kg at 12 hours, 120 µg equivalents/kg at 1 day and 47 µg equivalents/kg at 3 days.

All samples of fat, kidney, liver, muscle and skin with fat were analysed for residues of tylvalosin and 3-AT using the proposed routine analytical method based on HPLC with mass spectrometric detection. In all samples, concentrations of each analyte were below the limit of quantification (25 µg/kg).

The total residue in all tissues was rapidly depleted so that at 12 hours, 24 and 72 hours after treatment the amount of residues likely to be ingested by consumers represents 134, 66 and 30% of the ADI respectively.

Granules for use in drinking water
A new residue depletion study was submitted in accordance with current requirements. The pigs were housed individually and received a higher dose of 7.5 mg tylvalosin/kg BW/day (range: 5.3 to 14.2 mg tylvalosin/kg bw) than the recommended dose of 5 mg tylvalosin/kg bw/day over 5 days in medicated water via a nipple-drinker with an overhead reservoir. Non medicated water was available *ad libitum* during acclimatisation and after the end of the test period to determine the withdrawal period. The animals were slaughtered at various time points after administration and tissue samples collected (0, 12, 24, 48 and 72 hours). Results showed that residues in all tissues were below their respective MRLs after 12 hours.

Poultry
The pivotal residue depletion study was conducted in chickens in accordance with current requirements. Time points that extended beyond what was necessary were selected. Residues of both marker residues (tylvalosin and 3-AT) were measured in the target tissues (liver and skin + fat). The animals were dosed with higher than the recommended dose (mean dose of 32.8 mg tylvalosin/kg/day instead of 25 mg tylvalosin/kg/day) and for longer periods of time (5 days instead of 3 days). The results indicate that residues in liver were below the MRL after 12 hours and in skin and fat after 24 hours. One animal had residues of tylvalosin in skin and fat which were close to the MRL after 12 hours and residues of 3-AT which were below the LOQ for 3-AT. The combined residues could exceed the MRL after 12 hours. The first time point where residues in all animals fell below their respective MRLs was, therefore, 24 hours.

MRLs

Tylvalosin is included in Annex I of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal Species	MRLs	Target tissues	Other provisions
Tylvalosin	Sum of tylvalosin and 3-O-acetyltylosin	Porcine	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Skin and fat Liver Kidney	
Tylvalosin	Sum of tylvalosin and 3-O-acetyltylosin	Poultry	50 µg/kg 50 µg/kg	Skin and fat Liver	Not for use in animals from which eggs are produced for human consumption

Withdrawal periods

Pig
In the pivotal residue depletion study in pigs, one liver sample contained 37.1 µg/kg tylvalosin and less than 25 µg/kg 3-AT at 12 hours. This could add up to a concentration at 12 hours of marker residue of more than the MRL of 50 µg/kg. Thus, the first time point when residues in all tissues were known to fall below the MRLs is 24 hours. Although it is noted that a higher dose was used in the pivotal residues depletion study, it is nevertheless considered that a safety span is required. Since it is not practicable to set a meat withdrawal period in terms other than whole days, this safety span would have to be 100%, giving a withdrawal period of 2 days.

In 2006, the Marketing Authorisation Holder extended the indications for two further claims (Porcine Proliferative Enteropathy (ileitis) and Swine Dysentery). The new indications use **twice the dose** rate used for the initial indication over 10 days (i.e. 3 days longer than the initial indication). In the original pivotal residues depletion study, pigs received 5 mg tylvalosin/kg bodyweight/day (in the feed) for 10 days, therefore no new data were submitted. The CVMP concluded that the currently approved withdrawal period for pig meat and offal of 2 days to be adequate for the new indication and dosing regime. The CVMP took into account the fact that the dose used in the study was higher than the highest proposed for the new indications and by 24 hours, all edible tissues contained concentrations of the marker residues (tylvalosin and 3-AT) below the MRLs. The withdrawal periods thus have an 'uncertainty factor' of 100%.

In 2009, the marketing authorisation was extended to a new pharmaceutical form (**granules for use in drinking water).** In support of the extension, the marketing authorisation holder provided a new residue depletion study. Results showed that residues in all tissues were below their respective MRLs after 12 hours. Therefore, a withdrawal period of 1 day for meat and offal was considered acceptable.

In 2009, the marketing authorisation was also extended to a new pharmaceutical form **(oral powder)**. As the proposed product formulation and dosage regime (route/dose/frequency/duration) for this extension are identical to those already authorised, the proposed withdrawal period of 2 days for meat and offal (as already approved for the premix) was also considered acceptable.

Chicken
In chickens, the first time point where residues in all animals fell below their respective MRLs was 24 hours. Addition of any 'uncertainty factor' would increase the meat withdrawal period to 2 days, as meat withdrawal periods should be expressed in full days. Therefore, a 2-day chicken meat withdrawal period was considered to be appropriate for this presentation. The product is not authorised for use in birds laying eggs for human consumption. The time period during which treatment is not allowed before onset of egg laying for human consumption is 14 days.

Pheasant
In 2009, the marketing authorisation was extended to a new target species (pheasants). The same meat withdrawal period, as already approved for chickens (2 days), was accepted because the same product is used for the new, minor species. The product is not authorised for use in birds laying eggs for human consumption. The time period during which treatment is not allowed before onset of egg laying for human consumption is 14 days. Also, an additional warning was added to the SPC and product literature that animals should not be released until 2 days after treatment.

Analytical Methods

The Applicant provided a description of the routine analytical methods, which were approved by the CVMP during its consideration of the respective pig and poultry MRL application.

Overall Conclusion on Safety and Residues

After oral administration to pigs and chickens, tylvalosin is rapidly absorbed, metabolised and distributed into tissues. The main metabolite 3-O-acetyltylosin (3-AT) shows similar antimicrobial activity to the active substance. Excretion is rapid with the major route of elimination via the faeces.

Tylvalosin is of low to moderate oral toxicity. Studies in laboratory animals have not produced any evidence of teratogenicity and maternal toxicity has only been observed in rodents at high doses. However, since no reproductive safety studies have been performed in pigs, an appropriate warning has been included in the SPC to use the product in pregnant animals only in accordance with a risk/benefit assessment by the responsible veterinarian.

People may be exposed to tylvalosin via inhalation, by accidental ingestion or by skin/eye contact. In animal tests, tylvalosin was slightly irritating to eyes but the extent of this irritation was insufficient to warrant classification as an irritant. It is, however, capable of causing skin sensitisation and relevant warnings regarding user safety with respect to accidental ingestion, skin sensitisation and protective clothing have been included in the product literature. The impact of the use of Aivlosin in relation to development of resistance in human medicine was considered low.

With respect to environmental safety, the Committee concluded that tylvalosin was shown to be of low toxicity in earthworms, terrestrial plants and water fleas and unlikely to move to groundwater, and it agreed that Aivlosin, if used according to the recommended posology for pigs, chickens and pheasants, is expected to be of low risk to the environment.

MRLs for tylvalosin of 50 µg/kg for porcine muscle, skin and fat, liver and kidney have been included in Annex I of Council Regulation (EEC) No. 2377/90. MRLs for poultry were established of 50 µg/kg for skin, fat and liver. No MRLs were established for eggs and as a result tylvalosin is not authorised for use in birds from which eggs are produced for human consumption. The Applicant provided approved analytical methods.

Based on the data provided and taking into account a sufficient safety span, a withdrawal period of 2 days for meat and offal was considered acceptable in pigs (premix and oral powder), chickens and pheasants (granules for use in drinking water). The marketing authorisation holder provided a new residue depletion study for the granules for use in drinking water for pigs. Results showed that residues in all tissues were below their respective MRLs after 12 hours. Therefore, a withdrawal period of 1 day for meat and offal was considered acceptable for this presentation.

4. EFFICACY ASSESSMENT

Pharmacodynamics
Tylvalosin is a macrolide, which is mainly active against Gram-positive bacteria and mycoplasma. No other major pharmacological effects are known. The mode of action is to inhibit protein synthesis by reversibly binding to the 50S ribosome subunit.

Tylvalosin showed activity towards various gram-positive strains (e.g. *Staphylococcus*, *Micrococcus*, *Microbacterium*, *Bacillus*, *Corynebacterium*, *Aerococcus*, *Arthrobacter and Streptococcus*, *Campylobacter*, *Enterococcus and Clostridia*). The substance was not active against most of the gram-negative strains (including *Escherichia coli*, *Serratia*, *Klebsiella*, *Proteus*, *Salmonella*, *Shigella* and *Pseudomonas*). The main metabolite, 3-O-acetyltylosin (3-AT), showed similar antimicrobial activity.

A study investigating the prevalence of *Enterococcus* in faeces of pigs treated with the therapeutic dose of 50 mg tylvalosin tartrate/kg feed over 7 days showed an increase of tylvalosin resistance amongst intestinal *Enterococcus* populations of the medicated animals, but this increase in resistance may be transient.

Mycoplasma
Tylvalosin is primarily mycoplasmastatic. Killing rates *in vitro* have been shown to be concentration dependent and time dependent. Mycoplasmas are slow growing organisms, so at least 24-48 hours of exposure to tylvalosin are required for an effect to be observed.

No breakpoint is set for tylvalosin. Tylvalosin is more potent than tylosin and thus the breakpoint for resistance is likely to be lower. The clinical cut-off value (i.e. the highest MIC for a strain still treatable using the recommended dose regime for tylvalosin) is not known. Pivotal for the dose determination are, therefore, the clinical studies.

Mycoplasma hyopneumoniae (Swine Enzootic Pneumonia)
The Minimum Inhibitory Concentration (MIC) of tylvalosin against *M. hyopneumoniae*, was determined in field isolates from lungs of pigs with porcine respiratory disease. Isolates were collected from Germany, the Netherlands and the United Kingdom between 1997 and 2003 and the MIC_{90} of 0.06 µg/ml was determined for tylvalosin. Due to the additional antimicrobial activity of the active metabolite(s), the MIC against *M. hyopneumoniae* is likely to be higher than that of the parent molecule alone.

Mycoplasma gallisepticum
All *M. gallisepticum* stock isolates tested can be considered to be susceptible to tylvalosin. The MIC-values are generally low. However, the distribution is bimodal with a genetically modified subpopulation with higher MIC-values MICs were also determined for recent isolates from the clinical studies conducted in the EU. Although MICs for tylvalosin were elevated for the isolates that were resistant to tylosin, the isolates could still be regarded as sensitive to tylvalosin. The distribution profiles suggest that isolates with an MIC>0.06 µg/ml are outside the wild type distribution and that they are likely to carry a resistance mechanism, yet tylvalosin maintains activity against these isolates.

Brachyspira hyodysenteriae (Swine Dysentery)
Tylvalosin is bacteriostatic against *B. hyodysenteriae* in a concentration dependent manner, but due to the slow growth of the organism, it is also time dependant. A post antibiotic effect is not considered to be a significant factor. *Brachyspira* is difficult to culture and there is only limited information available on the antimicrobial effects of tylvalosin towards *B. hyodysenteriae*. MIC values range from 0.25 to 128 µg/ml with a proposed MIC_{50} of 2 µg/ml. No breakpoint has been established concerning the susceptibility of *B. hyodysenteriae* to tylvalosin.

Lawsonia intracellularis (Porcine Proliferative Enteropathy)

Two studies were provided to determine the antimicrobial activity of tylvalosin against *L. intracellularis*. The CVMP acknowledged that there are limited data available on *L. intracellularis* as the organism can only be propagated in enteric cell lines and few laboratory isolates exist. Data regarding the *in vitro* sensitivity for *L. intracellularis* are difficult to obtain as this organism is intracellular. However, intracellular MIC-values of approximately 30 µg/ml have been reported.

Impact of resistance development onto efficacy

***Mycoplasma (M. hyopneumoniae* ; *M. gallisepticum*)**

Although resistance by *M. hyopneumoniae* to tylvalosin can be induced *in vitro* after several passages at increasingly high antibiotic concentrations, there have been no *in vivo* reports of resistance of *Mycoplasma* derived from either pigs or chickens to tylvalosin.

All *M. gallisepticum* stock isolates tested were considered to be susceptible to tylvalosin. MICs were also determined for isolates from the clinical studies conducted in the EU. MICs for recent isolates against tylvalosin are shown in Fig 1.Although MICs for tylvalosin were elevated for the isolates that were resistant to tylosin, the isolates could still be regarded as sensitive to tylvalosin. The distribution profiles suggest that isolates with an MIC>0.06 µg/ml are outside the wild type distribution and that they are likely to carry a resistance mechanism, yet tylvalosin maintains activity against these isolates.

MIC distributions for tylvalosin (acetylisovaleryltylosin) and tylosin against *M. gallisepticum isolates.*

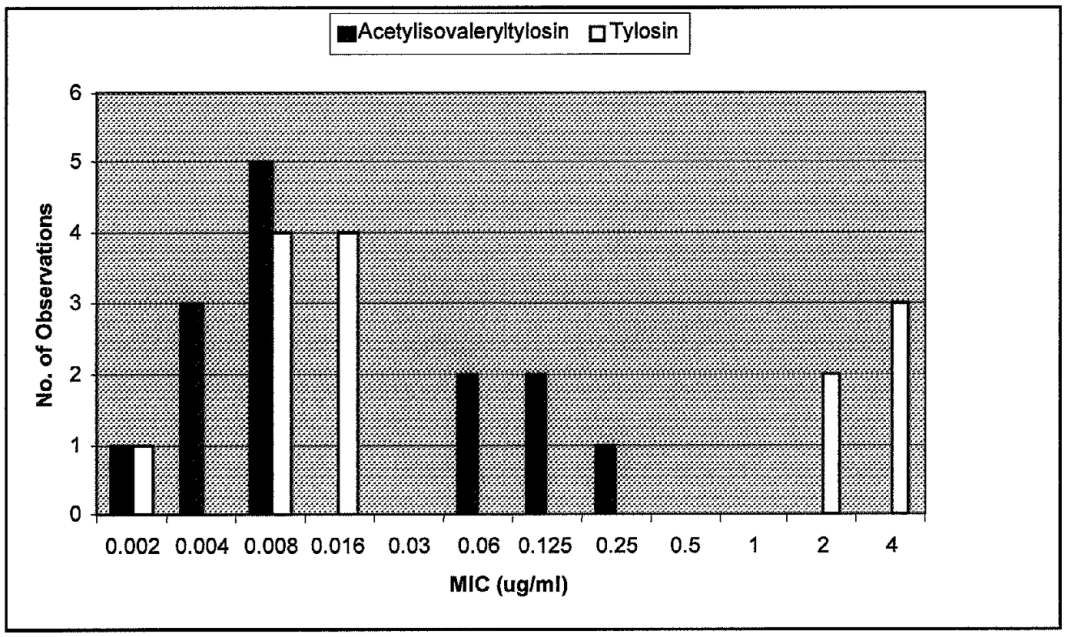

Fig. 1. Distribution of MIC-values for tylvalosin and *Mycoplasma gallisepticum* (Hungary and Slovakia, 2005-2006). The MIC-values are generally low, however the distribution is bimodal with a genetically modified subpopulation with higher MIC-values.

Resistance development in *Mycoplasma* seems to be slow and associated with the very slow growth rate of *Mycoplasma* (3 - 7 days *in vitro*), and consequently a slow appearance of mutations in the field in both pigs and man.

The Committee concluded that tylvalosin would not carry a greater risk of development of resistance than other macrolides authorised for veterinary use, especially for the treatment of *Mycoplasma*.

Lawsonia intracellularis

To date resistance of *L. intracellularis* to tylvalosin has not been reported or found in the field since its introduction to the market in Japan in 1989. No readily available method exists for performing susceptibility tests for this organism.

Brachyspira hyodysenteriae
The CVMP discussed a possible link of resistance developing to tylvalosin in tylosin-resistant *B. hyodysenteriae*. The Committee concluded that there might be a risk of cross-resistance with other macrolides and agreed to include an appropriate warning in the SPC. Also, a statement was introduced into the product literature regarding good management and hygiene practices designed to reduce the risk of infection, which will also aid in the management of potential build up of resistance.

In connection with the extension for use of granules in drinking water for pigs, the Committee included further warnings to the SPC and product literature in relation to the potential of resistance development: "Use of the product deviating from the instructions given in the SPC may increase the risk of development and selection of resistant bacteria and decrease the effectiveness of treatment with other macrolides due to the potential for cross-resistance."

Target animal safety

Pigs
Two tolerance studies were presented in pigs receiving up to 250 mg tylvalosin/kg feed (inclusion rate) over 10 days and in pigs receiving 500 mg tylvalosin/kg feed (inclusion rate) for 14 consecutive days (i.e. five-times the recommended dose for an extended treatment duration). Tylvalosin had no negative effect on the health of the pigs. There was no variation in food consumption between groups and no differences in body weight. No intolerance to the diet was observed. No necropsy findings could be attributable to tylvalosin administration.
It was concluded that tylvalosin administration via feed to growing pigs, was safe at up to five times the proposed dose.

In support of the extension application for granules for use in drinking water, the marketing authorisation holder provided in 2009 a new GLP-compliant target animal safety study in 6-week old pigs. The study was conducted in USA in 2006. As concentrations over 200 ppm cause palatability issues, most pigs were medicated by gavage. No adverse effects were observed and it was concluded that Aivlosin Granules for use in drinking water are safe when administered at up to 10 x the recommended dose level of 5 mg/kg for 3 x the recommended duration of treatment, and at up to 20 x the recommended dose level for the recommended duration of treatment.

Chickens
Groups of chickens were medicated with doses of 30 mg, 90 mg or 150 mg tylvalosin/kg bodyweight for three days, or with 30 mg/kg bodyweight for 15 days, i.e. 5 x the recommended duration. The results demonstrated that the administration of high doses of tylvalosin had no adverse effect on the birds. Similar observations were made in the clinical studies. The CVMP concluded, therefore, that tylvalosin has a wide safety margin and is considered to be safe when administered at the proposed dose regimen in chickens as young as 1 day old.

Pheasants
As the proposed dose rate in pheasants is the same as for chickens, the CVMP agreed that the findings in chickens could be extrapolated to the minor species pheasant and that no extra tolerance data in pheasants were required.

Clinical Documentation

Pigs

Choice of feeding stuffs (pigs)

In the laboratory animal studies tylvalosin was usually given by gavage, but various commercially available pig feeding stuffs were used in the clinical trials. The feed content varied between countries, due to the use of locally available ingredients. In view of the variability of the diets used in the efficacy studies, it was concluded that feed ingredients would not interfere with the efficacy of tylvalosin. The feed inclusion rates in the clinical trials were based on tylvalosin tartrate and were, therefore, slightly higher than the actual content of tylvalosin, e.g. those trials using 50 mg tylvalosin tartrate/kg feed would have provided an inclusion rate of 42.5 mg tylvalosin/kg feed tylvalosin.

Enzootic Pneumonia

Dose determination studies

Two dose determination studies were provided demonstrating the efficacy of 50 mg tylvalosin tartrate/kg feed (i.e. 42.5 mg tylvalosin/kg feed).

In a GLP-compliant dose titration study in **Japan**, pigs receiving 10, 20, 50 or 100 mg tylvalosin tartrate/kg feed (i.e. 0.625, 1.25 , 2.5 or 100 mg tylvalosin tartrate/kg bodyweight) and 2 control groups (unmedicated or tylosin medicated) were experimentally infected with *M. hyopneumoniae* into the nasal cavity at 10 days of age. Medicated feed was given for 7 days, starting 3 days after infection (i.e. the study aimed to determine the appropriate dosage for prevention of clinical disease).
All challenged pigs had lung lesions at necropsy. However, the extent of lung lesions in the groups receiving 20, 50 or 100 mg tylvalosin tartrate/kg feed (i.e. 1.25, 2.5 or 5 mg tylvalosin tartrate/kg bodyweight) or tylosin was significantly lower than in non-medicated pigs.
None of the doses of tylvalosin or tylosin completely prevented establishment of the pathogen. However, 50 mg tylvalosin tartrate/kg feed (i.e. 2.5 mg tylvalosin tartrate/kg bodyweight) gave an 86% reduction of lung lesions when compared with the unmedicated controls, and this was therefore proposed as the dose for prevention of Swine Enzootic Pneumonia.

Another study was conducted in the **UK** in 4-5 weeks old pigs, allocated to 5 treatment groups and one unmedicated control. The pigs were challenged on 2 consecutive days with pneumonic lung material containing *M. hyopneumoniae*. One group was used to investigate the prevention of Swine Enzootic Pneumonia using an inclusion rate of 50 mg tylvalosin tartrate/kg feed (i.e. 2.5 mg tylvalosin tartrate/kg bodyweight) for 7 days, starting 1 day before challenge with *M. hyopneumoniae*. In three other groups treatment of Swine Enzootic Pneumonia was investigated using inclusion rates of 50 or 100 mg tylvalosin tartrate/kg feed (i.e. 2.5 mg and 5 mg tylvalosin tartrate/kg bodyweight, respectively) or a positive control (valnemulin) for 7 days, starting 3 days after challenge with *M. hyopneumoniae.* The remaining group was challenged with *M. hyopneumoniae* but left unmedicated.
No clinical signs were observed in the unchallenged group indicating that infection had not spread between groups. Coughing was greater in the pigs in the infected, unmedicated control group than in any other challenged group.

Prevention of experimentally induced swine enzootic pneumonia
Coughing was observed in both challenged groups (medicated and unmedicated animals) until the end of the study; however, in the 50 mg/kg feed group (i.e. 2.125 mg tylvalosin /kg bodyweight) it was first observed 7 days after challenge while in the infected, non-medicated group it was seen 4 days after challenge. The mean lung lesion score in tylvalosin medicated pigs was significantly reduced (-45%) in comparison with the infected, non-medicated controls.

Treatment of experimentally induced swine enzootic pneumonia

Coughing was observed in all groups until the end of the study and at necropsy the majority of pigs in the medicated groups showed lesions typical of enzootic pneumonia. However, the inclusion rate of 50 mg tylvalosin tartrate/kg feed (i.e. 2.125 mg tylvalosin/kg bodyweight) showed a significantly better reduction of lung lesions when compared with the non-medicated control group (-51%), and numerically better than the positive control group. The Feed Conversion Ratios (FCR) and average daily live weight gains were not significantly different, but the small number of pigs and the short time period influenced this result.

As there was very little difference between the medicated groups, and the results for tylvalosin were comparable to those for valnemulin (authorised for the treatment and prevention of enzootic pneumonia), it was concluded that a dose of 50 mg tylvalosin tartrate/kg feed (equivalent to the proposed dose of 2.125 mg tylvalosin/kg bodyweight) was effective in treating experimentally induced enzootic pneumonia.

Field trials

Four GCP compliant field trials were carried out in Europe to confirm the efficacy of tylvalosin for the treatment and prevention of Swine Enzootic Pneumonia, compared with positive controls (two with tylosin, as the phosphate salt, and two with valnemulin, as HCl salt). Negative control groups were not used at the request of the trial farmers and the investigators on the grounds of animal welfare and the possible induction of long-term production depression.

Comparison of the efficacy of tylvalosin with tylosin (I)

Pigs (25 - 60 kg bodyweight) from a number of suppliers were used in a multi-source fattening unit with the disease well established on the farm. Pre-trial checks at the slaughterhouse had shown that 79% of the pigs had lesions of enzootic pneumonia and 87% were seropositive for *M. hyopneumoniae*. The pigs were placed in two groups; one group was medicated with 50 mg tylvalosin tartrate/kg feed (i.e. 2.125 mg tylvalosin/kg bodyweight) for 7 days and the other group with 100 mg tylosin/kg feed for 21 days. The identity of the treatment groups was blinded throughout the trial.

Eighty five percent of pigs were seropositive to *M. hyopneumoniae* at the end of the trial confirming the extensive presence of the infection. The necropsy results showed an 8.4% reduction in lung lesions in the tylvalosin group but 56% of lungs showed signs of lesions (compared with 51% in the tylosin medicated group). There was a reduction in lung lesion score and incidence in comparison with the pre-trial findings but no statistically significant differences between the two treatment groups. There were no significant differences between the two treatment groups with respect to weight gain and feed conversion efficiency.

It was concluded that both treatment groups showed the same efficacy in treatment of enzootic pneumonia and the prevention of further clinical cases.

Comparison of the efficacy of tylvalosin with tylosin (II)

Pigs from a farm with previous history of enzootic pneumonia and weighing on average 40 kg, were used in this trial: 50 % of fattening pigs from this farm previously examined for enzootic pneumonia lesions at the abattoir had lesions with an average lung lesion score of 10%. Pleurisy and lung abscesses were noted in about 15% of lungs. The pigs were allocated to two groups; one group was medicated with 50 mg tylvalosin tartrate/kg feed for 7 days and the other group with 100 mg tylosin/kg feed for 21 days. The pigs were clinically scored during the trial but there were no severe disease problems.

At slaughter, lungs were examined and scored. The lung scores in the tylvalosin medicated group and the tylosin medicated group were not significantly different. The performance data showed no statistical differences between the two treatments concerning weight gain, feed conversion efficiency, and mortality. There was no significant difference between the mean lung scores of tylvalosin and tylosin medicated groups. It was concluded that both treatment groups showed the same efficacy in treatment and prevention of enzootic pneumonia.

The results of the two field trials with tylosin as positive controls were analysed together (statistical meta-analysis). The combined results for performance showed no significant differences in the treatment effects although the group with 50 mg tylvalosin tartrate/kg feed (i.e. 2.125 mg tylvalosin /kg bodyweight did show a numerical improvement over tylosin phosphate at 100 mg/kg feed.

Comparison of the efficacy of tylvalosin with valnemulin (I)

Pigs (44 – 72 kg) were used in a study on a multi-source fattening unit with the disease well established on the farm. Pre-trial checks at the slaughterhouse had shown that 59% of the pigs had lesions of enzootic pneumonia with an average lung lesion score of 5.5%. In addition, presence of *M. hyopneumoniae* was confirmed by PCR and isolation.

The pigs were allocated to two groups; one group was medicated with 50 mg tylvalosin tartrate/kg feed (i.e. 2.125 mg tylvalosin/kg bodyweight) and the other group with 200 mg valnemulin HCl/kg feed (i.e. 10 mg/kg bodyweight). The duration of treatment was 7 days for both groups and animals were observed for a further 14 days. The identity of the treatment groups was blinded throughout the trial. The inclusion rate of both antimicrobials was increased by 50% above the nominal initial inclusion rate to compensate for reduced feed intake; i.e. giving inclusion rates of 64 mg/kg for tylvalosin and 300 mg/kg for valnemulin.

There were no significant differences between the two treatment groups for lung scores or clinical data. There were however significant differences in feed intake during the treatment period and Feed Conversion Efficiency over the period from treatment to slaughter in favour of tylvalosin, probably due to the increase in the inclusion rate for valnemulin (from 200 to 300 mg/kg feed) reducing its palatability.

It was concluded that both treatment groups showed the same efficacy in the treatment and prevention of enzootic pneumonia. In the case of feed conversion from treatment to slaughter and feed intake during treatment, tylvalosin was superior to valnemulin.

Comparison of the efficacy of tylvalosin with valnemulin (II)

In addition to the above study, more recent results from a second trial site using pigs (29 – 75 kg) with the same study profile, were provided, i.e. two groups medicated for 7 days with either 2.125 mg tylvalosin/kg bodyweight/day (with an inclusion rate of 64 mg/kg feed) or 10 mg valnemulin/kg bw/day. The presence of *M. hyopneumoniae* on the farm before the trial started was confirmed by culture and susceptibility to tylvalosin was confirmed. Post-mortem investigations on pigs that died during the study, or were euthanased, indicated that they were infected with *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and *Streptococcus suis* type 3. These bacteria are not susceptible to tylvalosin.

There was a clear reduction in the clinical scores of the tylvalosin group compared with the positive control, which reached statistical significance after 7 days of treatment. However, the statistical meta-analysis and lack of inferiority tests carried out on the results from this trial and the data of the first trial site showed no statistical difference in any of the parameters measured between tylvalosin and valnemulin other than the inter-trial difference.

It was concluded that both treatment groups showed the same efficacy in treatment and prevention of enzootic pneumonia.

Assessment of the field studies

The CVMP considered that although the presence of the disease in the field studies was confirmed by serological and post-mortem findings, the studies included only a few pigs with clear clinical signs of enzootic pneumonia thus making it difficult to demonstrate the efficacy of the "treatment" of the disease. Also, the field trials investigated enzootic pneumonia only in animals from farms with a history of infection with enzootic pneumonia and not from pathogen-free farms (which are not available under field conditions) thus making it difficult to demonstrate the efficacy of the claim "prevention".

However, the Committee acknowledged the difficulties in diagnosing infections with Swine Enzootic Pneumonia under field conditions since clinical signs of this disease are often mild and chronic and infections with secondary bacterial pathogens frequently complicate the clinical picture. Inevitably, some apparently healthy animals will be sub-clinical carriers and some animals are not infected since there is no reliable method of determining the stage of infection and lesion development for an individual (live) pig. In addition, the lack of possible routine analysis of *Mycoplasma hyopneumoniae* in most laboratories was considered. Furthermore, in the most recent study clinical scoring showed a significant difference between tylvalosin and valnemulin treatment.

The Committee, therefore, accepted that in addition to the results obtained from scoring of clinical signs, the efficacy of the treatment could be based on post-mortem examination of lungs, PCR results, culture and positive serology from contemporaneous batches of pigs. Furthermore, the Committee acknowledged that the applicant was able to demonstrate in the challenge studies the efficacy for the prevention of the disease with tylvalosin.

The Committee noted the widespread prevalence of the pathogen in Europe and considered that the clinical trials would probably reflect the current situation in the majority of pig farms in Europe, i.e. a history of infection with enzootic pneumonia.

Also, the Committee considered that neither tylvalosin, nor the positive controls were able to eliminate the infection with *Mycoplasma hyopneumoniae*. This was confirmed in those field studies where serological examinations for *M. hyopneumoniae* before and after treatment showed no change in the number of seropositive pigs. The Committee, therefore, concluded that the antimicrobial therapy for enzootic pneumonia would neither eliminate nor totally prevent infection and subsequent challenge. An appropriate statement was, therefore, included in the SPC and product literature.

In 2006, the Marketing Authorisation Holder extended the indications for two further claims (Porcine Proliferative Enteropathy (ileitis) and Swine Dysentery).

Swine dysentery

Dose determination

The applicant submitted two dose determination studies performed in the UK with pigs challenged with *Brachyspira hyodysenteriae* by oesophageal tube on 2 consecutive days demonstrating the efficacy of an inclusion rate of 75 ppm tylvalosin tartrate (the product prevented the appearance of clinical signs and clinical disease did not recur when medication was withdrawn). However, the bacterial strain used in these studies was not representative of those found under field conditions and the studies were therefore only considered supportive.

Duration of treatment

The applicant proposed a treatment duration of up to 21 days to cover the period of risk. However, the CVMP considered that this was not acceptable since clinical data were only provided supporting the efficacy of the use of tylvalosin of up to 10 days. In addition, the maximum duration of dosing in the residue depletion studies and in the environmental risk assessment was 10 days.
The CVMP concluded therefore that the duration of treatment should be limited to 10 days.

Field studies

The pivotal field studies were conducted in Ireland in 2004 and 2005.

A double blinded GCP-compliant controlled clinical trial investigating the efficacy of Aivlosin in the **treatment** of naturally occurring swine dysentery was conducted in Ireland in 2005. The trial was conducted on a commercial pig farm with pigs from various different sources. Presence of disease was confirmed by positive *B. hyodysenteriae* isolations and clinical signs of disease in the herd. Animals were medicated with either 4.25 mg tylvalosin /kg bw/day for 10 days or valnemulin at 3.75 mg/kg bw/day for 7 days. The trial also included an unmedicated negative control group.

Pigs medicated with tylvalosin showed slightly improved growth rates and weight gains though no statistically significant differences were shown between the treatment groups with respect to pig weight, daily live weight gain, feed consumed or feed conversion ratio. However, no firm conclusions on efficacy could be drawn, as the unmedicated control animals remained healthy throughout the study period. The study was initiated when most animals were healthy and the Committee concluded that the study demonstrated the efficacy of the prevention of swine dysentery rather than a treatment effect.

Results of another GCP-compliant trial investigating the efficacy of tylvalosin in the **prevention** of naturally occurring swine dysentery were submitted**.** The study was conducted on a finishing unit in Ireland in 2004 from two swine dysentery positive breeding herds. Clinical swine dysentery was present in one of the donor herds when the pigs were transferred to the trial site. The presence of a high level of *Brachyspira hyodysenteriae* in finishing pigs on one of the farms of origin was confirmed by a combination of clinical history, post mortem examinations, PCR analyses and culture for *Brachyspira hyodysenteriae*.

Animals were medicated with either 2.125 mg tylvalosin/kg bw/day for 10 days, a positive control (valnemulin at 1.34 mg/kg bw/day for 7 days) or remained unmedicated (negative control). The study results demonstrated that the productivity of the medicated pigs was better than unmedicated control pigs. The level of infection of *Brachyspira hyodysenteriae* was low and although faecal consistency improved in the medicated groups, it was not possible to relate this to prevention of swine dysentery. By days 14 and 21, cases of swine dysentery were identified in all groups.

Based on the data submitted, the CVMP concluded that the only recommended dose would be 4.25 mg tylvalosin/kg bodyweight for the treatment of clinical outbreaks of swine dysentery in herds where the disease had been diagnosed and for the prevention of further clinical cases. Severely affected pigs showing inappetence should be treated with an injectable product.

Porcine Proliferative Enteropathy (PPE)

Dose determination Studies

The Applicant submitted two dose determination studies, both performed in the USA with pigs orally challenged on 2 consecutive days with intestinal mucosal homogenate containing high infective doses of *Lawsonia intracellularis*.

The pivotal GCPv study was conducted in 2000 in pigs receiving either 2.125 mg or 4.25 mg tylvalosin/kg bodyweight/day for 10 days. This study was well performed and the infection well established. The data demonstrated that tylvalosin in feed significantly reduced the severity of clinical signs and the gross and microscopic lesions of PPE, especially when included at a level of 4.25 mg/kg bodyweight/day for 10 days. No mortality occurred in the 4.25 mg/kg bodyweight/day group, the lowest incidence and severity of ileal/jejunal lesions was seen in this group, average daily weight gain was not reduced in this group, diarrhoea scores and general demeanour were best in this group and there was a lower percentage of tissue immunohistochemistry (IHC) - positive intestines in this group. Statistical analysis demonstrated superiority of the 4.25 mg/kg bodyweight/day treatment over the proposed 2.125 mg/kg bodyweight treatment.

Since the highest dose (4.25 mg/kg bodyweight/day) performed significantly better than the proposed therapeutic dose of 2.125 mg/kg, the Committee concluded that a **dose of 4.25 mg/kg** is more likely to ensure the effective treatment of PPE.

In support of the extension application for granules for use in drinking water, a number of new dose determination / confirmation studies were provided, all conducted in 2005 and 2006 in the USA/Canada. In the dose determination studies, pigs received doses of 0, 2.7 – 2.88 mg/kg BW, 4.2 mg/kg BW, 5.2 – 5.95 mg/kg BW and 11.17 mg/kg BW in the drinking water daily for 5 consecutive days. It was demonstrated that:

- Compared to a negative control group, tylvalosin at an inclusion rate of approximately 4 mg/kg BW), was effective in the treatment of PPE, in terms of reducing clinical signs (demeanour, faecal score, abdominal score), reducing the severity of PPE lesions in the intestine and improving performance (average daily gain (ADG), feed efficiency).
- Treatment with tylvalosin at approximately 2.8 mg/kg BW resulted in a numerical improvement in mortality compared to an untreated control group.
- There was no evidence for a statistically or clinically significant difference in the response between inclusion rates of 37.5 ppm vs. 50 ppm, or 50 ppm vs. 100 ppm.
- All inclusion rates led to a decrease in shedding of *L. intracellularis* as determined by PCR. There was also an association between the dose rate and reduction in identification of *L. intracellularis* in the ileum as determined by immunohistochemistry.

Dose confirmation studies evaluated inclusion rates of 50 ppm (equivalent to approximately 5 mg/kg BW with a range of 4.3 to 6.7 mg/kg BW) and 75 ppm (equivalent to approximately 7.5 mg/kg BW with a range of 5.9 to 9.1 mg/kg BW) as compared to a negative control group.
The studies were conducted to a common protocol and it was intended that the results would be pooled for statistical analysis. When these 3 studies were analysed separately, the 50 ppm group did not meet the clinical efficacy criteria in one of the dose confirmation studies and the performance criteria in another study. The 75 ppm rate met both sets of efficacy criteria in all 3 studies. However, the 50 ppm rate met the efficacy criteria for both clinical and performance variables when the pooled results from all 3 studies were analysed, and there was no statistically significant difference between the two inclusion rates.

Based on these studies, the CVMP concluded that a dose of approximately 5 mg/kg bodyweight demonstrated efficacy for the control of porcine proliferative enteropathy (PPE, ileitis) in pigs experimentally infected with *Lawsonia intracellularis.*

Field studies

Premix / oral powder
The applicant submitted two field studies from Ireland and Mexico on farms with known history of PPE to demonstrate the efficacy of 2.125 mg tylvalosin/kg bodyweight in-feed against Porcine Proliferative Enteritis (PPE). However, in view of the unsatisfactory results from these field studies and the clear results from the dose determination studies (where a higher dose of 4.25 mg/kg bodyweight demonstrated significant improvements), the CVMP concluded that the proposed dosage regime of 2.125 mg tylvalosin/kg bodyweight for the treatment of PPE was not sufficiently supported by the studies and asked for further data.

In the responses to questions, the Applicant proposed two different dose rates for the treatment of PPE, both given for 10 days; a low dose rate of 2.125 mg tylvalosin/kg bodyweight/day for the treatment of mild to moderate herd infections (chronic) PPE and a higher dose of 4.25 mg tylvalosin/kg bodyweight/day for the treatment of acute or more severe cases of PPE.

In support of the proposed dosing scheme, the results of two further field trials were provided, one from Ireland and another one from Denmark.

A masked controlled GCP compliant clinical trial was conducted in 2005 on a commercial pig farm in **Ireland.** The presence of disease in the herd was confirmed by positive *L. intracellularis* PCR results prior to the trial starting and a clinical history of endemic herd infection . The trial was initiated when 23.8% of the pigs were exhibiting clinical signs of PPE. Animals were medicated with either tylvalosin at 48 mg/kg feed (2.09 mg tylvalosin/kg bodyweight) or 83 mg/kg feed (4.25 mg tylvalosin/kg bodyweight) for 10 days, 93.75 mg/kg feed valnemulin (3.75 mg tylvalosin/kg bodyweight/day) for 7 days (positive control) or remained unmedicated (negative control).

The trial demonstrated that the tylvalosin medicated groups performed better than the unmedicated group for several of the parameters measured. However, the positive control group showed signs of an adverse reaction to valnemulin, resulting in the removal of the group from the trial. Therefore, no comparison to a positive control could be made.

The Committee noted that there was no significant difference between the groups medicated with different dose rates of tylvalosin and noted several deficiencies in the studies performed. The CVMP, therefore, considered that this study did not sufficiently demonstrate the efficacy of Aivlosin in the treatment of PPE.

A further clinical study, a masked blinded, controlled trial in accordance with GCP, was conducted in 2005 on a commercial pig herd in Denmark. The herd was endemically infected with *Lawsonia intracellularis* with pigs showing signs of disease at 7 – 12 weeks of age. The herd diagnosis was based on previously recorded clinical signs in the herd and earlier laboratory examinations. Pigs received feed *ad libitum*, either unmedicated (negative control) or feed medicated either with Aivlosin for 10 days or tylosin (positive control) for 21 days. The recovery rate for tylvalosin was low; therefore, the calculated daily intake was 1.43 mg/kg bodyweight rather than the intended 2.125 mg tylvalosin/kg bodyweight.

As the actual dose rate of Aivlosin was lower than that proposed one, the Committee concluded that it was not possible to draw definite conclusions from this study.

The CVMP agreed that based on the results from the clinical studies, it was not possible to conclude on the efficacy of Aivlosin at the initially proposed dose rate of 2.125 mg/kg. A dose of 4.25 mg tylvalosin/kg bodyweight appeared to be more efficacious than 2.125 mg/kg. Taking into account the clear results from the pivotal dose determination study where the higher dose of 4.25 mg tylvalosin/kg bodyweight performed significantly better than the dose of 2.125 mg/kg, the Committee concluded that the dose of 4.25 mg tylvalosin/kg bodyweight should be used for the treatment of PPE.

In 2006 and 2009, the CVMP approved extension applications for a new pharmaceutical form, 8.5 mg/g (and later 42.5 mg/g) oral powder for pigs, to allow the treatment of individual pigs on farms where only a small number of pigs are to receive the medicine. Larger groups of animals should be treated with medicated feeding stuff containing the premix. The product is to be thoroughly mixed into the daily feed ration for each individual pig. The CVMP noted that 8.5 mg/g and 42.5 mg/g oral powder are identical to the respective premix and the pharmacokinetic profile of tylvalosin would be expected to be similar for both.

Granules for use in drinking water for pigs

The efficacy of the 5 mg/kg BW dose rate was investigated in a GCP-compliant field trial on a grower unit in Denmark in 2007 where there was a natural outbreak of PPE in a herd of 8-week old, pigs. The trial was designed as a non-inferiority study with tylosin as the comparator. Pigs were treated for 5 consecutive days with medicated drinking water containing either tylvalosin (approximately 5 mg/kg BW) or tylosin (at approximately 10 mg/kg BW).

Of the pigs enrolled, 27% with faecal consistency scores of at least 2 (i.e. “loose”) were assigned to the treatment group for analysis and about 50 % with faeces scores of 0 (i.e. “normal”) were assigned to the prevention claim. Approximately 22% of pigs with faeces scores of 1 (i.e. “soft and unformed”) were treated but not included in the analysis. Pigs were observed individually and each pen contained pigs from both treatment and prevention analysis groups so that pigs included for the prevention claim were exposed to infection. For the treatment claim, the primary efficacy parameter was “*faecal consistency score on Day 5”* and for the prevention claim it was *“percentage of pigs developing clinical signs of PPE on any observation day after start of treatment”*. The primary treatment variables were compared between the tylvalosin and tylosin groups for non-inferiority.

For the treatment claim, non-inferiority was demonstrated for the primary parameter, “*faecal consistency score on Day 5”*, and at Days 14 and 21, with a 97.5% confidence interval.

In the prevention group, *“abnormal condition”* and *“abdominal appearance”* scores on Day 1 suggested that pigs had sub-clinical disease. Both treatments were very effective in preventing the development of abnormal faeces over the 21-day observation period, with only one animal in each group developing a faecal score of at least 1. Non-inferiority was demonstrated between tylvalosin and tylosin for this primary parameter (with a 97.5% confidence interval for the difference between groups). .

Based on the results from these studies, the CVMP concluded that tylvalosin (5 mg/kg bw) was as effective as an approved product containing tylosin in the treatment and prevention of porcine proliferative enteropathy (PPE) in pigs caused by *Lawsonia intracellularis,* when given via the drinking water over 5 consecutive days.

Chickens

Dose determination / confirmation

For the **treatment** claim, groups of chickens experimentally infected with *M.gallisepticum* were medicated for 3 days with one of four different proposed dose rates (10, 20, 30 or 40 mg/kg) and compared to groups either medicated with tylosin or left unmedicated. Tylvalosin at an analytical dose of approximately 35 mg/kg gave in the majority of parameters such as bodyweight, clinical scores, necropsy lesion scores, histological lesion scores and isolation rates of *M. gallisepticum* better results compared to tylosin and tylvalosin at any lower doses. Although dose rates over 19 mg/kg showed improvement of clinical signs, the optimum dose rate in this study was shown to be an analytical dose rate of 35 mg/kg administered for 3 days. The applicant considered that the correct dose rate for use in the clinical trials was 25 mg/mg – the dose rate that improved the same proportion of clinical signs in the infected birds as the positive control using tylosin.

For the **prevention** claim, four different doses were administered to chickens for a duration of 4 days, starting one day before experimental challenge with *M. gallisepticum*. The tylvalosin groups were compared with groups either medicated with tylosin or left unmedicated. The actual dosage drunk was considerably lower than the predicted proposed dose. The results of this study demonstrated that tylvalosin showed a dose-dependent improvement in clinical and pathological parameters and, at an analytical dose of approximately 23 mg tylvalosin/kg bodyweight gave, for the majority of parameters such as bodyweight, clinical scores, necropsy lesion scores, histological lesion scores and isolation rates better results than lower concentrations of tylvalosin, and slightly better results than those obtained with tylosin. The results for the group medicated with approximately 11 mg tylvalosin per kg showed similar results to tylosin. A dose rate of approximately 23 mg tylvalosin per kg (nominal dose of 25 mg/kg) for 4 days was shown to be the ‘optimum’ dose to prevent infection.

To confirm the result of the dose determination prevention studies, a dose confirmation study was provided to evaluate tylvalosin's effectiveness at preventing experimental mycoplasmosis in chickens at a dose of 15 mg tylvalosin per kg for 4 days, starting one day before challenge and compared with a challenged, unmedicated group. Although this dose showed some positive effect, the results from the previous dose determination study at the higher dose rate (25 mg/kg) demonstrated better results. The CVMP, therefore, considered a higher dose (of 25 mg) as the effective dose for both claims, prevention and treatment.

Field trials

For the **treatment** claim, the applicant submitted the results of two controlled studies conducted on farms in Hungary and Slovakia, in flocks where mycoplasmosis was present prior to medication. Chickens were either medicated with 25 mg tylvalosin per kg bodyweight administered in the drinking water for 3 days, although the actual dosage taken in by the birds was only either 80.3 or 83% of the proposed dose. The results in the tylvalosin group were compared with the results from chickens medicated with tylosin (positive control) or chickens without medication (negative control).
The results showed similar or better results in the tylvalosin group compared with those medicated with tylosin. Treatment did not completely eliminate all pathogens in the respiratory system and diseased birds were present in all groups, but at lower numbers in both, the tylvalosin and tylosin medicated groups.

For the **prevention** claim, the applicant initially suggested a different dosing scheme and conducted two studies in Hungary and Slovakia with this dosage; i.e. a dose of 25 mg tylvalosin per kg bodyweight administered in the drinking water for the first 3 days of life followed by 15 mg tylvalosin per kg for 4 days at 16 - 19 days of age. The time of the second medication was chosen as a typical time of "management stress" in a flock, e.g. at the time of routine vaccination. Infection was likely to be present in the flocks as both the parent stock and a sample of one day old chicks were sero-positive. Results indicated that for several parameters, tylvalosin was similar or better than tylosin and superior to no medication. Tylvalosin had a positive impact on the respiratory lesions.

The CVMP agreed that a dose of 25 mg tylvalosin per kg bodyweight administered in the drinking water for 3 days was efficacious in the treatment of infected chickens as well as in the prevention of an outbreak in newly hatched chickens. However, the Committee disagreed with a lower dose for chickens medicated for a second time after about 14 days. The Committee considered that the significantly better results of the higher dose in the dose determination studies, would not support the proposed lower dose and concluded that the same dose of 25 mg should also be used when treating a second time.

It was noted that the actual intakes of product were, in the majority of studies, considerably less than the proposed dose rates. The applicant explained that samples of medicated water were taken prior to the product being fully dissolved in the drinking water. Appropriate mixing instructions have, therefore, been included in the product literature.

Pheasants

Dose determination / confirmation

Groups of pheasants experimentally infected with *M.gallisepticum* were medicated for 3 days with one of four different proposed dose rates (10, 20, 30 or 40 mg/kg) and compared to groups either medicated with tylosin or left unmedicated. Bodyweights were measured at day -1, on each of the medication days and at the end of the study. Efficacy parameters were clinical scores, weight gain, feed conversion efficiency, gross pathology scores and isolation/detection of *M.gallisepticum*.
Although all dose rates over 20 mg/kg showed improvement of clinical signs, at 20 mg/kg there was some evidence of reinfection/recrudescence of disease toward the end of the study period, and a dose rate of 25 mg/kg administered for 3 days was considered to be the optimum dose. This dose was shown to be efficacious in a dose-confirmation study.

Field Studies

No field studies were presented. However, the applicant had previously received scientific advice from the CVMP regarding this limited market application stating that either a dose confirmation study or a field trial should be conducted to support this application. The applicant followed this advice and chose to conduct a dose confirmation study, as indicated in the previous paragraph. Therefore, no clinical field trials were submitted which is acceptable. The applicant described the epidemiology and treatment of mycoplasmosis in pheasants and included some additional statements in the SPC regarding practical and effective treatment of a flock of pheasants.

OVERALL CONCLUSION ON EFFICACY

Tylvalosin is a macrolide, which is mainly active against Gram-positive bacteria and mycoplasma. It is bacteriostatic but can be bactericidal with concentration dependant killing rates. European isolates of *M. hyopneumoniae* showed an MIC_{90} of 0.06 µg/ml for tylvalosin. The risk of resistance development by *M. hyopneumoniae* against tylvalosin is not different to that of other authorised macrolides.

Only limited microbiological data on *Lawsonia intracellularis* are available, as the organism can only be propagated in enteric cell lines and few laboratory isolates exist. However, MIC-values of approximately 30 µg/ml have been reported. Resistance of *L. intracellularis* to tylvalosin has not been reported or found in the field since its introduction on the market in Japan in 1989.

Since *Brachyspira hyodysenteriae* is difficult to culture, only limited information is available on the antimicrobial effects of tylvalosin against *B. hyodysenteriae* and no breakpoint has been established. However, in view of reported cross-resistance in macrolides and known resistance of other macrolides (tylosin) against *B. hyodysenteriae*, the Committee concluded that the SPC should include a relevant warning concerning possible cross resistance.

Target animal tolerance was demonstrated in two studies in pigs receiving tylvalosin in feed at dose rates up to five times the highest recommended dosage for an extended treatment duration.

Enzootic pneumonia in pigs

Two experimental challenge studies and four European field trials supported the clinical evaluation.

The dose titration studies using inclusion rates of 50 and 100 mg tylvalosin tartrate/kg feed (i.e. doses of 2.125 and 4.25 tylvalosin mg/kg bodyweight) showed significant reductions in lung lesions with the higher inclusion rate of 100 mg/kg feed not showing superior results. In addition, 50 mg/kg feed showed similar results to an authorised positive control (used in the studies) and other authorised products (literature comparison). The inclusion rate of 50 mg tylvalosin tartrate/kg feed resulted in concentrations of 0.06 µg/g in lung, i.e. in the range of the MIC_{90}. The Committee, therefore, concluded that the proposed dose of 50 mg tylvalosin tartrate/kg feed (i.e. 42.5 mg tylvalosin/kg feed, equivalent to 2.125 mg tylvalosin/kg bodyweight) has been sufficiently justified for treatment and prevention of Swine Enzootic Pneumonia.

The Committee noted that there are difficulties in diagnosing enzootic pneumonia under field conditions. However, the use of tylvalosin for treatment and prevention of Swine Enzootic Pneumonia showed significant improvements in clinical signs and / or post-mortem findings compared to unmedicated controls in the laboratory studies and at least the same improvement in clinical signs and / or post-mortem findings as positive controls in both field and laboratory trials. The CVMP, therefore, concluded that a dose of 50 mg tylvalosin tartrate/kg feed is as effective in the treatment and prevention of enzootic pneumonia as two other veterinary medicinal products authorised for this indication.

Taking into account that complete cure of diseased animals has not been adequately demonstrated, as *Mycoplasma hyopneumoniae* was not eliminated, the following sentence was added to the SPC (indications): “At the recommended dose, lung lesions and weight loss are reduced but infection with *Mycoplasma hyopneumoniae* is not eliminated.”

Swine Dysentery
The applicant proposed two different dosages; a low dosage of 2.125 mg tylvalosin/kg bodyweight/day for the prevention of swine dysentery and a higher dose of 4.25 mg tylvalosin/kg bodyweight/day for the treatment of swine dysentery. To support the proposed dosage, the results of two dose determination studies and six clinical field trials were submitted. Due to inconclusive results, the Committee considered the dose determination studies and some of the clinical studies only supportive for the claim.

However, based on the results from clinical studies, the CVMP concluded that the only recommended dose is 4.25 mg tylvalosin/kg bodyweight for the treatment of clinical outbreaks of swine dysentery in herds where the disease has been diagnosed and for the prevention of further clinical cases. Severely affected pigs showing inappetence should be treated with an injectable product.

Porcine Proliferative Enteropathy (PPE)
The applicant initially proposed a dose of 2.125 mg tylvalosin/kg bodyweight/day for 10 days for the treatment of Porcine Proliferative Enteritis (PPE). To support the dosage scheme, the results of two dose determination studies and two clinical field trials were submitted. Further to the initial assessment of this application by the CVMP, the results of two further field trials were submitted and the dosing scheme was amended; i.e. proposing two different dosages for the treatment of PPE both given for 10 days; a low dosage of 2.125 mg tylvalosin/kg bodyweight/day for the treatment of chronic PPE and a higher dose of 4.25 mg tylvalosin/kg bodyweight/day for the treatment of acute or more severe cases of PPE.
However, based on the results from the clinical studies, the CVMP concluded that it was not possible to conclude whether tylvalosin is efficacious at the proposed dose rate of 2.125 mg/kg. Taking into account the clear results from the pivotal dose determination study where the higher dose of 4.25 mg tylvalosin/kg bodyweight performed significantly better than the dose of 2.125 mg/kg bodyweight, the Committee concluded that the dose of 4.25 mg/kg bodyweight for 10 days should be used for the treatment of acute and chronic PPE.
Since pigs with reduced feed intake will not always consume a sufficient amount of active substance, additional recommendations were included in the SPC and product literature to increase inclusion rates, or to treat severe cases parenterally.

In connection with the extension for **granules for use in drinking water**, the applicant submitted a number of new dose determination and dose confirmation studies, as well as a new field study. Based on the results from these studies, the CVMP concluded that tylvalosin at a dose of 5 mg/kg bw was as effective as an approved product containing tylosin in the treatment and prevention of porcine proliferative enteropathy (PPE) in pigs, when given via the drinking water over 5 consecutive days.

Mycoplasma gallisepticum in chicken
The applicant initially proposed different dosage schemes; i.e. a higher dose of 25 mg/kg bodyweight over 3 days for an initial administration; and for prevention, a lower dose of 15 mg/kg over 4 days for a second medication after 14 days. To support the proposed dosage schemes, the results of two dose determination studies and two clinical field trials were submitted.

Tylvalosin was shown to be effective against *Mycoplasma gallisepticum*, both in challenge studies and under field conditions in comparison with placebo and a positive control (tylosin) at the dose rate of 25 mg/kg bodyweight administered over 3 days. At this dosage, tylvalosin was either non-inferior or superior to tylosin. However, results with the lower dose of 15 mg/kg bodyweight were reduced relative to the higher dose and the Committee considered that only the higher dose of 25 mg/kg bodyweight could be supported.

No data were provided on the performance of tylvalosin against infections with reduced susceptibility to tylosin; therefore, the effectiveness of tylvalosin against such mycoplasma strains could not be concluded and an appropriate warning was included in the product literature.

The preventive effect of tylvalosin in newly hatched chicks is limited, as a proportion of the samples taken were still positive for *Mycoplasma* after treatment. The Committee considered that other measures than medication alone are needed to control the disease. Preventive treatment was therefore restricted to flocks where infection *in ovum* with *Mycoplasma gallisepticum* is likely because the disease is known to exist in the parent generation. The prevention strategy should also include efforts to eliminate the infection from the parent generation and appropriate information was added to the SPC and product literature.

Mycoplasma gallisepticum in pheasants
In experimentally infected pheasants, treatment with Aivlosin at a dose rate of 25 mg/kg over 3 days was shown to be efficacious in the treatment of *M. gallisepticum*.

5. BENEFIT RISK ASSESSMENT

Tylvalosin is a macrolide antibiotic with *in vitro* activity against Gram-positive bacteria, mycoplasma and some Gram-negative bacteria, including *Lawsonia intracellularis.*

BENEFIT ASSESSMENT

Direct benefits

Aivlosin in different formulations (premix for medicated feeding stuff, oral powder, granules for use in drinking water) has been shown to be efficacious in the treatment and / or prevention of a number of indications in pigs, i.e. enzootic pneumonia, porcine proliferative enteropathy and swine dysentery; and in respiratory disease associated with *Mycoplasma gallisepticum* in chickens and pheasants.

Efficacy of the proposed dose and duration in the treatment of the respective diseases has been demonstrated in a number of pre-clinical and clinical studies.

Indirect benefits

The Committee noted that the inclusion rate of active substance in the 42.5 mg/g premix in feed would be 0.1%. Since inclusion rates of less than 0.5% are not permitted in some EU Member States, the lower strength premix (8.5 mg/g premix for medicated feeding stuff), introduced as an extension in 2005 achieved higher inclusion rates in feed, which are in accordance with recommendations in the European Pharmacopoeia and national legislation in the EU concerning the medication of feed.

Granules for use in drinking water have the advantage that even animals with reduced appetite due to illness might still drink and receive medication via drinking water. Also, the inclusion rate can be adjusted daily according to water intake to achieve the correct dose. The oral powders permit treatment of individual animals where necessary.

RISK ASSESSMENT

Tylvalosin has a wide therapeutic margin in target animal safety studies. This is supported by the clinical studies (no serious adverse reactions). The risk of adverse effects is low and no special warnings are required in the SPC.

Safety of the product has not been established in pregnant or lactating pigs; however, these animals are not at high risk of developing the disease and are unlikely to be treated with the product. An appropriate warning in section 4.7 of the SPC addresses any potential risk.

Tylvalosin is of low toxicity and poses low risk to users of the product. However, people may be exposed to tylvalosin via inhalation, by accidental ingestion or by skin contact and hypersensitivity reactions are a possible effect of contact with the product. However, satisfactory user warnings are included in the SPC and product literature explaining how to avoid such contact.

The environmental risk assessment has demonstrated that the risk for the soil and aquatic environments is acceptable and it is concluded that the product will not pose a risk for the environment when used according to the recommended posology for pigs, chickens and pheasants.

MRLs for tylvalosin for pigs and poultry have been included in Annex I of Council Regulation (EEC) No. 2377/90. Based on the data provided and taking into account a sufficient safety span, a withdrawal period of 2 days was considered acceptable for chickens and pheasants, as well as for the premix and oral powder formulations for pigs, and 1 day for the granules for use in drinking water formulation for pigs.

Consumer safety is assured by a withdrawal period for meat and offal of 1 to 2 days for chickens, pheasants and pigs (depending on the different presentations).
Resistance may be a hazard. Tylvalosin has no activity against *Salmonella* spp. and variable activity against *Campylobacter* spp., and concentrations reached in the ileum and colon of pigs may be sufficient to lead to selection for resistant strains, which could be transferred to humans. An appropriate warning has been added to the SPC and product literature. The indication for the product advises that the presence of disease should be established in a herd before preventive use.

EVALUATION OF THE BENEFIT - RISK BALANCE

The product is considered to be appropriately formulated. It is manufactured and controlled in accordance with relevant EU and VICH quality guidelines and current scientific knowledge.

The indications for Aivlosin represent serious diseases in terms of the effect they have on pig, chicken and pheasant welfare and production losses (mortalities and reduced feed efficiency).

Dose determination and confirmation studies demonstrated the efficacy of Aivlosin in the treatment of clinical signs, improving performance and reducing mortality. The product has been shown to be efficacious for a number of indications.

The therapeutic margin for the product is very good, with no adverse reactions at up to 5 x of the recommended daily dose. Residues also deplete quickly, leading to practicable withdrawal periods. Sufficient warnings have been included in the SPC and product literature in relation to mitigation against the risk of resistance development and risk for the user.

The overall benefit-risk evaluation is deemed positive with a sufficiently clear and complete SPC and product literature.

OVERALL CONCLUSIONS

Based on the original and subsequent data presented, the Committee concluded that the quality, safety and efficacy of Aivlosin were considered to be in accordance with the requirements of Council Directive 2001/82/EC, as amended.

This module was last updated in November 2009