SCIENTIFIC DISCUSSION

Name of the veterinary medicinal product:	PIRSUE 5 mg/ml intramammary solution
Active substance / International non-proprietary name INN:	Pirlimycin
Target species	Cattle
Withdrawal period:	Meat and offal: 23 days Milk: 5 days
Therapeutic indication(s):	For the treatment of subclinical mastitis in lactating cows due to Gram-positive cocci susceptible to pirlimycin including staphylococcal organisms such as *Staphylococcus aureus*, both penicillinase-positive and penicillinase-negative, and coagulase-negative staphylococci; streptococcal organisms including *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*.
ATCvet code	QJ51FF90
Pharmacotherapeutic group:	Antibacterial for intramammary use
Marketing Authorisation Holder:	Pfizer Limited Ramsgate Road Sandwich Kent CT13 9NJ United Kingdom

I OVERVIEW OF SCIENTIFIC DATA

Part I - Summary of the Dossier

PIRSUE is a sterile aqueous solution formulation for intramammary use and was first authorised in USA in a slightly different formulation in November 1993. The active ingredient is pirlimycin hydrochloride, a lincosaminide antibiotic active against Gram-positive bacteria. Pirlimycin hydrochloride has been evaluated in the course of the MRL procedure according to Council Regulation (EEC) No. 2377/90 and the Committee for Veterinary Medicinal Products has recommended the inclusion of pirlimycin in Annex I of Council Regulation (EEC) No 2377/90.
PIRSUE is intended for the treatment of subclinical mastitis in lactating cows due to Gram-positive cocci susceptible to pirlimycin including staphylococcal organisms such as *Staphylococcus aureus*, both penicillinase-positive and penicillinase-negative, and coagulase-negative staphylococci; streptococcal organisms including *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*.
Treatment consists of eight infusions every 24 hours of 50 mg pirlimycin per 10 ml sterile aqueous solution and contained in a syringe.

For the treatment of subclinical mastitis by 8 infusions at 24 hour intervals, a withdrawal period of 23 days for edible tissues and 5 days for milk was accepted. The withdrawal periods were estimated according to the Notes for Guidance EMEA/CVMP/036/95-Final and EMEA/CVMP/473/98-Final.

II. CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL DOCUMENTATION

II A - Composition

II A 1. Composition of the medicinal product

Each ml of intramammary solution contains:

Ingredient	Quality	Quantity [mg/ml]	Function
Pirlimycin (as hydrochloride)	Applicant's specification	5.00	Active ingredient
Sterile Aqueous Solution		q.s. ad 1 ml	Vehicle

II A 2. Container

The product is packed in a 10 ml mastitis syringe (max. volume: 14 ml) composed of:
- polyethylene barrel with a 4 mm LDPE cannula
- polyethylene tip cap
- polyethylene plunger rod with rubber stopper

II A 3. Clinical trial formulations

Two batches used in clinical studies are identical to the proposed formulation. Two further early dose-titration studies were conducted using a non-sterile PIRSUE aqueous gel formulation. Subsequent efficacy and milk/liver residue studies were conducted using a formulation marketed in the US up to 2000.

II A 4. Development pharmaceutics

The proposed formulation is based on a non-sterile aqueous gel previously marketed in the USA since 1993 with modifications introduced mainly due to the sterile single dose preparation.

No critical steps in the manufacturing process have been identified, as the raw materials are readily soluble in water. Data are provided to show that both sterilisation by autoclaving and by gamma-irradiation cause degradation of pirlimycin hydrochloride. Therefore, the product is manufactured by double sterile filtration and aseptic filling, using a restricted access barrier system (RABS), specifically designed for sterile filling applications.

Compatibility of the packaging materials with the drug product has been established, taking into account that additives in the plastic could cause oxidation of pirlimycin.

II B - Method of preparation

II B 1. Manufacturing formula

The manufacturing formula is given for a batch size of 1460 litres.

II B 2. Manufacturing process and in process controls

The buffer is dissolved in water for injection, pirlimycin hydrochloride is added and the solution brought to volume. After sterile filtration into a sterile holding tank, the solution is brought to a restricted access barrier system (RABS), where filling and sealing operations take place. The design of this RABS excludes operator intervention, except through the use of half-suit and glove ports, thus providing an increased level of sterility assurance for the finished product. The solution is filtered a second time, filled immediately in pre-sterilised syringes and stoppered. Outside the restricted access barrier, plunger rods are inserted into the stoppers, barrels are labelled and packaging is completed.
Fill volume has been adjusted to fulfil both the requirements on extractable volume and on dose uniformity.

Pre-assembled syringes are sterilised by gamma-irradiation; stoppers are washed, siliconised and steam sterilised.

During the manufacturing process, controls on dissolution of ingredients, mixing time, pH, specific gravity and fill volume are performed; bioburden of the bulk solution is determined prior to filtration, filter integrity is tested.

II B 3. Validation of the manufacturing process

Pilot-scale batches of 160 litres have each been produced at Pharmacia, Puurs, Belgium. These batches were representative of commercial batches, as they were manufactured using the final formula, the final manufacturing process and similar, if not identical, equipment. The only differences are batch size and the use of a RABS unit at Kalamazoo.

As post-authorisation commitment, final process validation has been done at the intended production site in Kalamazoo, Michigan, USA on three full scale production batches. Extensive information on the manufacturing procedures related to sterility assurance (sterile filtration, sterilisation of equipment, environmental controls, etc.) is provided.

II C - Control of starting materials

II C 1. Active ingredient not described in a Pharmacopoeia

II C 1.1 Specifications and routine tests

For the active ingredient, pirlimycin hydrochloride, physical characteristics, tests for identity, impurities, residual solvents and potency are described and specification limits were provided.
The routine tests and specification limits defined in the Applicant's testing instructions show conformity to pharmacopoeial standards. Specifications for individual residual solvents are in agreement with the draft VICH guideline "Impurities: Residual solvents". The tests and specifications provided are considered sufficient to assure constant quality of the drug substance.

II C 1.2 Scientific data

II C 1.2.1 Nomenclature and description

International non-proprietary name:	Pirlimycin
United States Adopted Name:	Pirlimycin hydrochloride
Chemical name:	L-*threo*-α-D-*galacto*-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[(4-ethyl-2-piperidinyl)-carbonyl]amino]-1-thio-, monohydrochlorid, monohydrate, (2*S*-*cis*)-
Laboratory code:	PNU-57930E
Proprietary name:	PIRSUE is Pharmacia's proprietary name for products containing this compound
Physical form:	White to off-white powder
Molecular formula:	$C_{17}H_{31}ClN_2O_5S \cdot HCl \cdot H_2O$
Relative molecular mass:	447.42 (anhydrous)
Chirality:	9 asymmetric carbons

II C 1.2.2 Manufacturer; in process controls

Pirlimycin hydrochloride is manufactured by Pharmacia, 7171 Portage Road, Kalamazoo, Michigan 49001, USA, in 4 steps. For all raw materials, reagents and solvents used during synthesis, routine tests and specifications are provided. In addition, the synthesis of the key starting materials is described. The specifications given for these starting materials relate to assay, residue on ignition and loss on drying.

II C 1.2.3 Development Chemistry

Evidence of chemical structure is supported by ^{1}H-NMR, IR and mass spectra as well as elemental analysis and the route of synthesis. Pirlimycin hydrochloride has 9 asymmetric centres. However, in the manufacturing process a single stereo isomer of pirlimycin (2S-cis) is synthesised and used as active drug substance. No stereo isomer formation is expected to occur in either solid-state bulk drug or in formulated drug product. Real time and accelerated stability data (60 months at room temperature; 12 months at 40 °C) for bulk drug show no change in content of process related impurities.

At 25 °C pirlimycin hydrochloride is soluble in propylenglycol and methanol, and slightly soluble in water. The melting range is 210.5 - 212.5 °C with decomposition; the pKa value is 8.4 and the partition coefficient (n-octanol/pH 7 buffer at 37 °C) is 0.6. In humid atmosphere (31 - 75 % RH) a monohydrate is formed.

The same impurities present in the Reference Standard are also observed in bulk drug batches. Pirlimycin Chromatographic Resolution Material is a bulk drug sample, which contains each of the HPLC impurities for which a structure has been identified or which is being reported by relative retention time (RRT).

Analytical validation data have been provided for assay, impurities, residual solvents, water and bacterial endotoxins. Details of the reference standards are given.

II C 1.2.4 Impurities

Nine potential by-products originating from the route of synthesis have been identified. Batch analysis data from 17 batches show that the identified impurities in pirlimycin hydrochloride range from "none detected" to 0.5 % for single impurities and from 0.6 - 2.1 % for the total amount. With these data the limits set in the specification were considered as justified.

Furthermore, in order to minimise the formation of in-process impurities and to improve process quality, the Marketing Authorisation Holder submitted in November 2001 a variation changing some of the sequences of adding the materials in the end phase of the synthesis process.

II C 1.2.5 Batch analysis

Certificates are provided for 17 batches of pirlimycin hydrochloride, ranging from 38 to 86 kg. All testing results are in conformity to the specifications provided.

II C 1.2.6 Potential risk of Bovine Spongiform Encephalopathy (BSE)

A declaration was provided, confirming that pirlimycin hydrochloride does not contain, and is not derived from specified risk material as defined in the Commission Decision 97/534/EC (adopted 30 July 1997).

II C 2. Excipients

For all other ingredients, starting materials defined by the relevant monographs of the European pharmacopoeia are used. In addition, these raw materials must meet internal standards for microbial content. Certificates of analysis are provided.

II C 3. Packaging material

The product is packaged in a disposable mastitis syringe consisting of a barrel with a 4 mm LDPE-cannula and a LDPE tip cap. The syringe is closed with a siliconised rubber stopper and a HDPE plunger that screws into the stopper. For all components testing specifications and batch analysis data are provided. HDPE, LDPE and the rubber closures comply with the relevant Ph. Eur. monographs.

II D - Control tests on intermediate products

Not applicable

II E - Control tests on the finished product

II E 1. Specifications and routine tests for release and end of shelf-life

The finished product specifications and routine tests comprise appearance, clarity/opalescence, degree of coloration, identification, assay, degradation impurities, unspecified degradation products, pH, extractable volume, sterility and bacterial endotoxins. The tests and specifications provided are regarded as sufficient to assure adequate and constant quality of the finished product.

II E 2. Validation of analytical methods

Assay and degradation products are determined by a gradient reversed-phase liquid chromatography method nearly identical to the method used for control of the starting material. This method is validated with respect to specificity, linearity and precision for pirlimycin hydrochloride and its degradation products.

The membrane filtration method is used to determine sterility of the finished product. Testing is validated according to pharmacopoeial requirements.

II E 3. Batch analysis

Certificates of analysis are provided for 5 batches. All results are in accordance with the specification and show uniformity from batch to batch. Two of the bulk solutions were intentionally produced with pH values at the upper and lower limits of the specified range.

II F - Stability

II F 1. Stability tests on active ingredient(s)

Solid pirlimycin hydrochloride proved to be stable under normal storage conditions, at elevated temperature and humidity and under fluorescent light. Exposure to UV light for several weeks caused some degradation.

In aqueous solution the predominant pathway of pirlimycin hydrochloride degradation is hydrolysis. Degradation rate is increased with increasing pH and also occurs via oxidation.

Long term stability studies on 11 batches were done at room temperature and ambient relative humidity in (double) polyethylene bags inside a fibrepack over periods of up to 60 months. No significant changes in content of pirlimycin hydrochloride, degradation products or other parameters were observed. With the data provided, the proposed 24 month retest period and 60 month expiry date when stored at ambient room temperature seem justified.

II F 2. Stability tests on the finished product

Stability studies have been projected with 3 batches of bulk solution for up to 5 years. Two different types of rubber stoppers are used, resulting in a total of 5 batches of PIRSUE intramammary solution.
Testing results are available for 36 months at 25 °C/60 % RH and 18 months at 30 °C/60 % RH. Studies at 40 °C/ambient humidity for 12 months are finished.

At 25 °C/60 % RH PIRSUE intramammary solution is stable and specifications are fulfilled for all parameters tested over a period of 36 months.

A shelf life of 36 months is supportable when stored at or below 25 °C in the carton.

III. SAFETY AND RESIDUE DOCUMENTATION

III.A Safety

III.A.2 Pharmacological studies

III.A.2.1 Pharmacodynamics

Pirlimycin is a lincosaminide antibiotic, which acts by inhibition of RNA dependent protein synthesis at the ribosomes of sensitive bacteria. Several reports on the *in vitro* susceptibility testing of various bacteria against pirlimycin between 1981 and 1997 are presented. Experiments were carried out in the USA, New Zealand and several European counties. Bacteria examined were mainly derived from bovine udders including those bacteria for which the product is intended for both, penicillinase-positive and penicillinase-negative *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*. Moreover, activity was examined against methicillin-resistant staphylococci and *Mycoplasma* from humans and against Gram-negative pathogens.

Studies on human polymorphonuclear leukocytes indicated that pirlimycin at low levels (1/3 MIC) can produce changes in bacteria by increasing their susceptibility to host defences.

The antibiotic activity of pirlimycin was compared to that of other lincosaminide antibiotics and other classes of relevant antibiotic compounds. MIC values were determined by agar dilution method or microbroth dilution method. An inhibition zone diameter of = 12 mm was defined as being resistant, and the MIC breakpoint was determined as 2 µg/ml. No bactericidal data were provided.

For *in vitro* susceptibility data, only bacteria isolated from European countries have been taken into account.

Overall data indicated that most Gram positive bacteria were susceptible to pirlimycin whereas Gram negative were not. Activity of pirlimycin was principally high against *Streptococcus agalactiae* and *Streptococcus dysgalaciae* with MIC_{50} and MIC_{90} values below 1.0 µg/ml. Activity against *Streptococcus uberis* showed greater variance (MICs from < 0.06 to 4 or > 32 µg/ml). The majority of *Staphylococcus aureus* isolates from veterinary origin were susceptible against pirlimycin with MICs = 1.0 µg/ml. However, there were also some non-sensitive strains among this species.

Some ß-lactamase producing *Staphylococcus aureus* strains were found to be both resistant to pirlimycin and erythromycin, i.e. to lincosaminides and to macrolides due to the similar mechanism of action of both classes of compounds, i.e. inhibition of RNA dependent protein synthesis at the ribosomes and the potential of cross-resistance. Nevertheless, ß-lactamase production by itself did not affect susceptibility of staphylococci towards pirlimycin. Pirlimycin was effective against (coagulase-negative) *Staphylococcus epidermidis* with MIC_{90} values of 0.5 µg/ml.

It is known from clinical experience that many antibiotics are not very active against bovine *Staphylococcus aureus* mastitis for several reasons. Several studies have shown that pirlymicin as many other antibiotics, does not kill intracellular *Staphylococcus aureus* at 100 x MIC, revealing the limited capability of pirlimycin to reach intracellular staphylococci. Such activity against intracellular staphylococci was only been exerted by naphtalenic ansamycin antibiotics (rifampicin, streptovaricins) and quinolones (ciprofloxacin).

In vivo efficacy of pirlimycin was tested using a *Staphylococcus aureus* in a mastitis model. Infected mice developed mastitis and the doses of pirlimycin needed to protect 50 % of the animals from clinical symptoms *(protective dose, PD_{50})* and to remove *staphylococci* from the mammary glands in 50 % of the animals were determined. Given intramammarily 0.2 to 0.6 mg/kg were sufficient to protect 50 % of the mice from clinical signs of mastitis. Higher doses were, however, needed for bacteriological cure in 50 % of the animals (1.3 to 2.7 mg/kg). An intramammary dose of 1 - 0.8 mg/kg pirlimycin *(protective dose, PD_{50}*) represented the median protective dose against clinical mastitis signs by 6 days post-infection. The median protective dose against *Staphylococcus aureus* infection in the gland (PD_{50s}) was 4 fold the PD_{50m}, 5.8 - 10 mg/kg after intramammary administration.

III.A.2.2 Pharmacokinetics

GLP-compliant pharmacokinetic studies with pirlimycin were carried out in cows via intramammary infusion (50 to 200 mg per quarter) or intravenous injection (800 mg per animal). The amount of microbiologically active residue was determined via bioassay. Parent compound was identified with HPLC methods.

After two intramammary infusions of 50 mg pirlimycin at an interval of 24 hours milk levels of the parent compound were high at the first post-treatment milkings, but fell below 1 µg/ml in most cows at the second post-treatment milkings. Milk levels of pirlimycin were slightly higher and longer maintained after 8 infusions.

Approximately one half of radioactive labelled pirlimycin entered systemic circulation 6 to 12 hours after infusion of the therapeutic dose and reached plasma C_{max} values of 0.012 to 0.025 ppm. Compared to intravenous injection, the bioavailability of parent pirlimycin accounted for approximately 40 %.

Around 50 % of the dose was excreted via the milk, mainly during the first post-treatment milking. Absorbed pirlimycin was primarily excreted via the faeces and to a lesser extent via the urine both after intravenous and after intramammary administration. The drug was cleared from plasma with mean terminal elimination half-lives of 10 to 12 hours for the parent compound and of approximately 60 hours for the total residue. Residue studies have identified sulfoxide pirlimycin as the major metabolite and the prolonged half-life of the total residue was attributed to the elimination of the metabolite.

III.A.3 Toxicological studies

Pirlimycin hydrochloride has been evaluated in the course of the MRL procedure according to Council Regulation (EEC) No. 2377/90. A summary report has been prepared on the basis of the dossier presented by Pharmacia. This was adopted by the CVMP on 7 July 1998 (EMEA/CVMP/365/98). It was agreed that the MRL dossier should be used for the toxicity section of the PIRSUE application. The Applicant provided a supportive expert report on the toxicity of pirlimycin hydrochloride in January 1999.

III.A.3.2 Single dose toxicity

An oral LD_{50} of 2524 mg/kg pirlimycin hydrochloride was established in rats. Congestion of the glandular stomach and the kidney medulla was found at necropsy in non-surviving animals.

III.A.3.3 Repeat dose toxicity

Subchronic toxicity studies were performed in rats and dogs with daily doses up to 500 mg/kg in rats and up to 300 mg/kg in dogs.

In rats, sGOT and sGPT plasma levels were slightly elevated at all doses (50 to 500 mg/kg) in the 30 day study. Stomach lesions were found at 160 and 500 mg/kg (no examination at lower doses) and some morphological changes of hepatocytes were seen at 500 mg/kg. From the 13 week study in rats, a NOEL of 10 mg/kg was established. At higher doses (30 to 300 mg/kg), MCHC was increased and total protein, globulin, albumin and creatinine were reduced. Relative liver weights were lower in males at 30 mg/kg and above.

In dogs, doses of 100 and 300 mg/kg caused increased GOT and GPT plasma levels and morphological changes of hepatocytes in the course of the 30 day study. In the 13 weeks study, salivation and vomiting occurred in dogs at 40 and 160 mg/kg and in females gastric irritation accompanied by lymphoid hyperplasia was seen additionally. A NOEL of 16 mg/kg was determined in this experiment.

III.A.3.4 Target species tolerance

Several studies were performed on the tolerance of Pirlimycin in dairy cows treated by intramammary infusion of an aqueous gel formulation.

In experimental, GLP-compliant trials on cows with no signs of clinical mastitis, equivocal signs of udder irritation were observed at the dose of 200 mg/quarter, including an increase of slightly abnormal milk at strip cup examination and a sporadic increase of elevated somatic cell count.

Target animal tolerance studies conforming to guidelines were conducted with the 2 X treatment regimen. In one study, treatment with the original aqueous solution was accompanied by dramatic increases of somatic milk cell counts in several cows. In some of these animals, basic cell counts had not been regained at the end of the observation period. Other effects were a slight and transient fall in milk production and in milk fat. Coliform mastitis had developed in one cow from another tolerance study and was also reported from a few cows under treatment in the field. Marked elevations of somatic cell counts (SCCs) in individual cows observed in one study can be attributed to opportunistic infections with environmental pathogens. Since these effects were found to be almost negligible in a more recently conducted study that followed a standard operation procedure for proper udder infusion technique, introduction of environmental pathogens into the udders by improper teat handling is assumed to be the cause for the unfavourable effects referred to above. Pirlimycin was shown to have little if any activity against coliforms including *E. coli* and *Klebsiella* spp. and this may lead to the progression of more severe mastitis forms requiring other therapy. *E. coli* infections in cows with subclinical mastitis are often secondary and can be avoided by proper infusion technique. Detailed advice for teat cleansing and proper infusion of the product in order to avoid infections with environmental bacteria such as *E. coli* is therefore given in the SPC.

For the 8 X treatment, no specific tolerance study was submitted, but two clinical efficacy studies employing this treatment regimen provide supportive data. Although a new tolerance study should have been provided, the well conducted clinical efficacy studies have been analysed for udder tolerance of 8 daily infusions of the product. This approach is not in line with the EU Guideline on the local tolerance of intramammary preparations since cows with mastitis and initial cell counts of at least 300,000 per ml were included in these studies and milk samples were not collected during the treatment period. Nevertheless, this study supports the conclusion that pirlimycin administered once daily for 8 times is well tolerated. In these studies, a marked decline of somatic cell counts from initial high numbers to levels at or below 100,000 per ml at the 12th or 13th post treatment milking, i.e. 6 or 7 days after the last treatment was observed in cows which had been successfully treated for subclinical mastitis with 8 infusions of the product. This was measured 1 to 2 days after the recommended 5 day milk withdrawal period. Along with the tolerance studies described above, these results support the hypothesis that cell count rises seen in some animals under treatment with pirlimycin are related to opportunistic udder infections rather than to an irritating potential of the product itself.

Pirlimycin in an aqueous gel formulation has been marketed in the USA since November 1993. During that time, more than 8 million tubes have been sold. The Applicant provided 4 adverse reaction reports on a total of 11 cows covering the period 1994 - 1997. The incidence of adverse reaction is thus low. Effects reported were related to allergic reactions in 3 to 4 cows and to coliform mastitis, which developed under treatment in 10 cows. One cow with a suspected allergic reaction and 4 cows with coliform mastitis died.

III.A.3.5 Effects on reproduction

In the course of the MRL procedure on pirlimycin hydrochloride, Pharmacia presented a 2-generation study in rats with oral doses of 100 to 400 mg/kg. A NOEL of 100 mg/kg was determined. At higher doses, salivation and reduced body weight gains were found in adults.

III.A.3.6 Embryotoxicity / foetotoxicity including teratogenicity

Embryotoxicity / foetotoxicity and teratogenicity studies were performed in rats and mice with oral doses of 200 to 800 and 100 to 1600 mg/kg, respectively.

In rats, soft stools, salivation and reduced body weight gains occurred in dams at 400 and 800 mg/kg. No NOEL for embryotoxicity / foetotoxicity could be derived, since a significant dose-related increase of minor skeletal anomalies of the sternebrae of foetuses was seen at all doses.

In mice, a NOEL of 400 mg/kg was retained for both maternotoxic and foetotoxic effects. The highest dose (1600 mg/kg) produced severe diarrhoea and some fatalities in dams and reduced foetal weight in the offspring.

It was concluded that pirlimycin hydrochloride is devoid of any teratogenic potential.

III.A.3.7 Mutagenicity

Gene mutation assays in prokaryontes (Ames-test) and eukaryontes (HPRT-test in two cell lines) were presented. Furthermore, micronucleus tests in rats and mice, an unscheduled DNA synthesis assay (UDS) in primary rat hepatocytes and a recessive lethal assay in *Drosophila melanogaster* have been provided. Negative results were obtained in all tests. Pirlimycin hydrochloride was classified as devoid of any mutagenic or genotoxic potential.

III.A.3.8 Carcinogenicity

Pirlimycin is not mutagenic or genotoxic nor is it related to any known carcinogen. Carcinogenicity studies have therefore not been performed.

III.A.4 Studies on other effects

III.A.4.1 Local irritation studies

Pirlimycin hydrochloride bulk powder was found to seriously irritate rabbit eyes at a single dose of 100 mg causing severe conjunctivitis and corneal opacity. Corneal opacity persisted up to the end of the 21 day observation period. Repeated administration of 20 mg pirlimycin produced moderate conjunctivitis and corneal opacity, which worsened with each subsequent application. Effects persisted up to the end of the 21 day observation period.
Effects were less severe when the eyes were rinsed after application of the powder. Although formulated as a sterile solution, human eye contact should be avoided. In case of accidents, eyes should be rinsed immediately.

Intact skin did not show any irritation after single or repeated treatment. However, pirlimycin was moderately irritating to abraded skin at a single dose of 500 mg or repeated daily doses of 100 mg causing moderate erythema, slight oedema and exfoliation along the scratch lines. Although the appearance of the skin had normalised on day 4, rabbits exhibited severe diarrhoea and died later. This was attributed to absorption of the drug from the application site and subsequent disturbance of the gastrointestinal flora.
Humans should avoid contact to the bulk drug powder in case of wounds or abraded skin, although the effects are not believed to be as serious as in rabbits, which are highly sensitive towards a change of their intestinal flora.

The effects of pirlimycin relative to eye and skin irritation are addressed in the SPC and the package insert:
"Avoid contact with the solution. Wash hands and any exposed skin with soap and water and remove contaminated clothing immediately after use. Flush eyes with water for 15 minutes immediately after exposure. Hold eyelids open to ensure complete contact with water."

III.A.4.2 Immunotoxicity

No specific studies on the potential immunotoxicity of pirlimycin hydrochloride were provided with the MRL dossier. In the MRL summary report it is pointed to the stomach irritation accompanied by lymphoid hyperplasia, which was observed in the course of the 13 weeks toxicity study in female dogs treated with 40 mg/kg and higher. The NOEL for this effect was 16 mg/kg.

III.A.4.3 Observation in humans

In the MRL summary report on pirlimycin hydrochloride, reference is made to an experiment in healthy male human volunteers who received oral doses of 0.83 to 8.3 mg/kg pirlimycin hydrochloride (5 subjects per dose). *Clostridium difficile* was found in stools from 2 to 5 volunteers at each treatment dose and in only one of the control subjects. In treated volunteers, eosinophils, inorganic phosphorus and specific gravity of urine were significantly elevated.

Headache was observed in 10 human volunteers after oral administration of a single dose of 1.8 mg/kg.

III.A.5 Ecotoxicity

The phase I assessment results were as follows: The different Predicted Environmental Concentrations for the compartment soil (PEC_{soil}) calculated for the various exposure scenarios were below the trigger value of Phase I, which is 10 µg/kg. Using the TGD model the $PEC_{ground\ water}$ is calculated to be 2.8 µg/l. This value exceeds the trigger value of Phase I, which is 0.1 µg/l and indicated a Phase II assessment for the compartment ground water. This assessment started with a refinement of the exposure assessment using the German Pesticide Leaching Model (PELMO). The PELMO simulation did not confirm the potential of pirlimycin to leach into the ground water. Thus the Phase II assessment for the compartment ground water was stopped.

The predicted concentration in fresh dung is 950 µg/kg; this means that the $PEC_{fresh\text{-}dung}$ exceeds the trigger value of Phase I by far, which is 10 µg/kg. Therefore, a Phase II - Tier A assessment focusing on the specific issues relating to the presence of residue in fresh dung was required.

The effect data indicate that the active ingredient pirlimycin hydrochloride is phytotoxic. Although pirlimycin was toxic on algae as well as on terrestrial plants none of the PEC/PNEC ratios exceeded the trigger values for the related compartments. In contrast to that pirlimycin has a low acute toxicity on fish, daphnia and earthworms. The toxicity test with daphnia gives no indication of a specific insecticidal activity for pirlimycin.

The environmental risk assessment for non-target species according to the CVMP Note for guidance - Phase II concludes, that pirlimycin hydrochloride is not expected to negatively impact the environment when used per label instructions. Therefore there is no need for any specific label instruction regarding environmental concerns.

III.B Residues

III.B. 2.1 Pharmacokinetics in the target species

The pharmacokinetic studies were conducted with an aqueous gel formulation rather than the claimed aqueous solution. However, the comparison of two formulations revealed, that there were no substantial effects on milk and plasma kinetics of pirlimycin when the gelling agent was present or absent.

The pharmacokinetic behaviour of pirlimycin in cows after intramammary treatment was investigated in 3 comprehensive radiometric studies.

- In one study 12 healthy lactating cows (Holstein) were infused into each quarter with a 4-fold overdose of 200 mg pirlimycin per quarter (i.e. 10 ml of an aqueous gel formulation of 20 mg/ml ^{14}C-pirlimycin). This was done twice. Milk samples (composite samples) were collected at 12 hour intervals. Analysis of milk, blood and urine samples as well as faecal waste was carried out up to 7 days post treatment or until the animal was slaughtered. Three animals each were sacrificed at day 4, 6, 14 and 28 post treatment. Liver, kidney, muscle, abdominal fat and udder were harvested.

- Additional pharmacokinetic data were obtained in another radiometric study, which was conducted at the recommended dose of 50 mg pirlimycin free base per quarter and per infusion.

- In a third study, pharmacokinetics in 3 healthy cows after intravenous injection of 800 mg ^{14}C-pirlimycin and intramammary administration of 200 mg ^{14}C-pirlimycin free base per quarter were compared. Blood samples were collected at various time points for 7 days post treatment. Both treatments were administered immediately after milking. Milk (at 12 hour intervals), urine and faeces were collected for 7 days following treatment.

In another non-radiometric study 2 groups of randomly assigned lactating cows (10 animals/group) were compared, which had received two formulations of 100 mg pirlimycin per quarter with or without the gelling agent. Milk and plasma samples were obtained for 96 hours post treatment and tested for bioequivalence. Three experimental endpoints were tested: the plasma AUC, the amount of pirlimycin recovered in milk and the time it took to reach a concentration of 0.40 µg/ml in milk.
Furthermore, a study was conducted in 12 rats orally treated with ^{14}C-pirlimycin hydrochloride, in order to compare the metabolite profiles in liver and excreta to the profiles found in the lactating cow. Finally, the microbiological activity of pirlimycin sulfoxide relative to pirlimycin was also investigated.

The results obtained at 4 X dose rate were in good agreement with the results obtained at the recommended dose rate of 50 mg pirlimycin free base per quarter. Following two intramammary administrations (1 X or 4 X the recommended dose rate), a considerable amount of ^{14}C-pirlimycin was absorbed. Approximately one half of the radioactive dose reached systemic circulation. The mean absorption half-life was 2.9 hours. Maximum concentrations in whole blood were obtained 6 - 12 hours after treatment. There was an approximate 1.5 fold increase in the blood concentration levels after the second administration. For example, the mean C_{max} after the first dose was 0.083 µg equivalents per ml and the mean C_{max} after the second dose was 0.131µg equivalents per ml (4x dose rate). The concentrations of total residues found in whole blood were slightly higher than the concentrations measured in plasma.

The comparison of the AUCs for total ^{14}C-residues obtained after intramammary treatment and after intravenous treatment revealed, that about 60 % of the intramammary dose was absorbed and reached systemic circulation. This percentage was in good agreement to previous observations concerning the accountability of total radioactivity.

The absorbed portion of ^{14}C-pirlimycin was distributed to all edible tissues. Highest total residue concentrations were found in liver followed by kidney and mammary gland, and then by fat and muscle. Approximately 92 - 95 % of total radioactivity in milk was identified as parent pirlimycin throughout the sampling periods. The percentage of parent pirlimycin in plasma varied somewhat. Concentrations in the range of 40 - 80 % of total residue were detected in plasma by microbiological analysis against *M. luteus*. The main metabolite in liver and kidney was pirlimycin sulfoxide (having very little microbiological activity), which made up approximately 62 % or more of total residue in liver and 46 % in kidney (across all time points investigated). Pirlimycin itself accounted for approximately 25 % of total residue in liver and 43 % in kidney. Pirlimycin sulfone accounted for 9.8 % and 7.2 % of the total residue in liver and kidney, respectively. The composition of fat and muscle radioactivity was not profiled. The main metabolites (parent pirlimycin and its sulfoxide) found in liver and excreta of the cow were the same as those found in the rat.

Elimination of radioactivity was biphasic from blood as well as from milk. In plasma, the elimination of total residue after single intramammary treatment had an initial depletion half-life of 3.6 - 4.9 hours. This was followed by a terminal elimination half-life of 58 - 69 hours. A terminal elimination half-life of 37.6 hours was also reported. Excretion of absorbed radioactivity was mainly via faeces. Faeces excretion in ratio to urine excretion was about 2.5. Approximately 80 % of total residue in urine and 45 % in faeces were identified as parent pirlimycin. Pirlimycin sulfoxide comprised approximately 8 % and 1.5 % of total residue in urine and faeces respectively.

The comparison of two pirlimycin formulations resulted in somewhat equivocal effects. While the plasma AUCs and the residue concentrations determined in milk indicated, that both formulations were bioequivalent, a higher amount of pirlimycin was recovered from the milk of animals dosed with the gelling agent formulation. However, since this finding was attributed to the amount of pirlimycin recovered in the first milking post treatment, both formulations were seen as bioequivalent with respect to total residue depletion from milk.

III.B. 2.2 Depletion of residues

Several residue depletion studies (n = 20 - 57 animals per study) were performed following repeated intramammary treatment of lactating cows at a dose of 50 mg pirlimycin free base per quarter and treatment. In all studies, pirlimycin was applied in the form of hydrochloride (either in the ^{14}C-labelled or in the unlabelled form). In all studies, the cows were milked twice daily. The residue concentrations were determined by LSC or by the Microbiological Cylinder Plate assay or by the HPLC/TSP/MS method (the proposed routine analytical method). In some cases, the assay methods were used in parallel.

Almost all of the residue depletion studies were carried out following two successive infusions into all four quarters. These studies were conducted either with the aqueous gel formulation or with the intended sterile aqueous solution. However, pharmacokinetic data revealed that there was no substantial effect on milk and plasma kinetics of pirlimycin when the gelling agent was present or absent. Only one residue depletion study was provided on the 8 X treatment period. This study was conducted by using the intended sterile aqueous solution.

Edible tissues

Five residue depletion studies for edible tissues following intramammary treatment of dairy cows were provided. In three of these studies, parent pirlimycin was analysed in liver only. In two studies, parent pirlimycin was analysed in all edible tissues. During the course of these studies, it became obvious, that the concentrations of parent pirlimycin in liver increased when samples were incubated at 37° C for 24 hours. This increase was attributed to a conversion of the metabolite pirlimycin sulfoxide back to parent pirlimycin.

Liver was the last tissue to drop below the MRL. Since the bioassay provided substantially lower residue concentrations in liver than the HPLC/TSP/MS method, the determination of the withdrawal period for edible tissues was based on liver concentrations measured by HPLC/TSP/MS. The differences in the assay results were attributed to the more efficient sample extraction in the HPLC methods.

Milk

Four extensive residue depletion studies for milk following intramammary treatment of dairy cows were provided.

The residue depletion data showed, that all three analytical methods used (radioactive assay, microbiological cylinder-plate assay and HPLC/TSP/MS) were appropriate for determination of the marker residue pirlimycin. This was because the total residue determined in milk consisted primarily of parent pirlimycin. Furthermore, the instrumental HPLC/TSP/MS method and the bioassay essentially gave the same results for milk.

Milk was analysed up to the ninth milking after the end of treatment. Cows with one or more udder quarters affected with signs of clinical mastitis revealed a similar depletion profile. For 8 X treatment, the ninth milking was the first time point after end of treatment when the residue concentrations in all milk samples were below the MRL.

Commercially available screening assays were applied to milk samples from both healthy and diseased cows after 2 X treatment. With respect to healthy cows, the *B. stearothermophilus* Disc assay (BSDA) and the Delvotest-P showed some positive results through the sixth and eighth milking, respectively. The Charm II Macrolide assay was positive for seven milkings. In the case of cows with one or two affected udder quarters, some positive results (probably false positive results) were found through eight milkings using the Delvotest-P as well as the BSDA method. The Charm II Macrolide assay was positive for six milkings. Compared to the *M. luteus* cylinder-plate assay, the Delvotest turned positive at a milk pirlimycin concentration of approximately 0.06 µg/ml . The BSDA method turned positive at a milk pirlimycin concentration of approximately 0.1 µg/ml . The limit of detection of the Charm II Macrolide test was reported to be 0.03 - 0.04 µg/ml.

III.B. 2.3 Maximum residue levels

The Committee for Veterinary Medicinal Products recommended on 12 January 2000 the inclusion of pirlimycin in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table.

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Pirlimycin	Pirlimycin	Bovine	100 µg/kg 100 µg/kg 1000 µg/kg 400 µg/kg 100 µg/kg	Muscle Fat Liver Kidney Milk	

III.B. 2.4 Withdrawal period

Twenty healthy lactating cows were infused into each quarter with the recommended dose at 24-hour intervals for eight consecutive days (using the sterile aqueous solution). Milk samples were collected for 8 days post last dose at regular 10 – 14 hour intervals. Groups of 5 cows were slaughtered at 21, 28, 35 and 42 days after the last treatment. Milk samples were analysed by microbiological cylinder-plate assay. Edible tissues were analysed by HPLC/TSP/MS.

Residue concentrations in kidney, muscle, fat and udder were below the reported LOQ of 0.025 µg/g in all samples analysed. Only one cow in the 21-day group had measurable concentrations in fat and udder. All liver samples (incubated at 37°C) had residue concentrations below the MRL of 1 µg/g but above the LOQ of 0.025 µg/g. The ninth milking was the first time point after end of treatment when the residue concentrations in all milk samples were below the MRL of 0.1 µg/g.

For 8 X treatment, a withdrawal period of 23 days for edible tissues and 5 days for milk has been accepted. The withdrawal periods were set according to the Notes for Guidance EMEA/CVMP/036/95-FINAL and EMEA/CVMP/473/98-FINAL.

III.B. 3 Analytical Methods

Tissue and milk samples were analysed by HPLC/TSP/MS (the proposed EU-regulatory methods) and /or by microbiological cylinder-plate assay. The instrumental methods for edible tissues and for milk have already been accepted by the CVMP as routine analytical methods (see Summary Report EMEA/MRL/ 719/99-FINAL).

While the two analytical methods (microbiological cylinder-plate assay and HPLC/TSP/MS) were found to be appropriate to determine the marker residue pirlimycin in milk, the bioassay provided substantially lower residue concentrations in liver than the HPLC/TSP/MS method. Therefore, the assessment of marker residue concentrations in liver was based on data obtained by HPLC/TSP/MS.

III.B.3.1. Analytical method for liver

The determinative and confirmatory analysis of pirlimycin in liver was based on its detection by HPLC Thermospray Mass Spectrometry (HPLC/TSP/MS). The method used a chromatographically resolved stereoisomer of pirlimycin as internal standard. This isomer produced a thermospray mass spectrum identical to that of pirlimycin.

Quantitation was based on m/z 411, the pseudomolecular ion for pirlimycin. The pirlimycin concentration in liver was calculated from the peak area ratio of pirlimycin to isopirlimycin compared to standard solutions, which were prepared in injection diluent. The identification of pirlimycin was verified by the detection of the 4 diagnostic ions for both pirlimycin and isopirlimycin (m/z 411.4, 413.4, 375.4 and 158.2) and by their relative intensity.

Liver was first incubated before the internal standard solution was added. Extraction was performed followed by filtration, and release of pirlimycin from the organic solvent for final HPLC/TSP/MS analysis.

The modified procedure (modified by the incorporation of the incubation step) was tested with 3 sets of incurred liver samples as well as with fortified control samples.

The incurred liver samples were derived from 3 cows slaughtered at 2, 7 and 14 days after end of treatment. At 5 different days, 5 replicates of each sample were analysed per day. The overall precision (CV%) was in the range of 11.7 % - 12.9%. In addition, 4 replicates of control liver samples were fortified at concentrations at 0.54 µg/g (= 1/2 MRL), 1.08 µg/g (= 1 MRL) and 2.16 µg/g (= 2 MRL) and analysed at 5 different days. The overall recovery was in the range of 75% - 80%, precision (CV%) was in the range of 3% - 8.5%.

III.B.3.2 Analytical method for muscle, kidney and fat

The HPLC/TSP/MS method that was developed for liver was applied to kidney, muscle and fat. However, the method did not include the incubation step. The validated limit of quantification, defined as the lowest concentration that could be analysed with acceptable accuracy and precision was determined to be 0.65 µg/g for all three tissues.

III.B.3.3. Analytical methods for milk:

III.B.3.3.1 HPLC method

The determinative and confirmatory analysis of pirlimycin in milk was based on its detection by HPLC Thermospray Mass Spectrometry (HPLC/TSP/MS). As in the case of liver, the pirlimycin concentration in milk was calculated from the peak area ratio of pirlimycin and isopirlimycin compared to standard solutions.

Five sets of fortified control milk samples at three concentrations (0.05, 0.10 and 0.2 µg/ml) were analysed (the internal standard concentration was 0.5 µg/ml). The mean recovery was found to be 85% at 0.05 and 0.10 µg/ml, and 98% at 0.2 µg/ml. The day-to-day precision was 7.4%, 4.2% and 6.5%, respectively. The day-to-day precision achieved for an incurred milk sample at a concentration of about 0.05 µg/ml was 10.4%. With respect to these data, the LOQ of the method, defined as the lowest concentration that may be determined with acceptable accuracy and precision, can be seen as 0.05 µg/ml.

III.B.3.3.2 Microbiological cylinder-plate assay

The fluid part of milk after centrifugation was collected without the top fatty layer, pH adjusted and assayed directly by the cylinder plate method (organism *M. luteus*). Six cylinders were placed on each assay plate. The diameters of the zones of inhibition were measured by an automated zone reader.

The concentration range of the standard samples (prepared in milk) was from 0.020 to 0.320 µg/ml pirlimycin free base. The LOD of the method was defined as the lowest concentration of the standards, being 0.02 µg/ml. Samples showing inhibition zones smaller than the 0.02 µg/ml standard solution were considered to have pirlimycin concentrations undetectable by this method. Assay accuracy (as percent recovery) was 90 - 97 % at the tested concentrations of 0.03, 0.08 and 0.200 µg/ml pirlimycin free base (n=6 per concentration level). The precision of the assay was demonstrated by three replicates of fortified control milk samples at 0.04 and 0.16 µg/ml pirlimycin free base which were assayed in triplicate on three different days. The within-day precision was, respectively, 2.5% and 5.8 %, the between-day precision was, respectively, 7.9 % and 8.0 %.

IV. PRECLINICAL AND CLINICAL DOCUMENTATION

IV.A Pre-Clinical Documentation

IV.A.1 Pharmacodynamics

See Safety file, III A 2.1

IV.A.2 Pharmacokinetics

See Safety file, III A 2.2

IV.A.3 Target species tolerance

See Safety file, III A 3.4

IV.A.4 Resistance

The following median Minimum Inhibitory Concentrations (MIC_{50}) of pirlimycin for microorganisms causing mastitis were:

- *Staphylococcus aureus*: 0.25 – 0.50 µg/ml (96 % to 100 % susceptible)
- *Streptococcus agalactiae*: 0.06 µg/ml (99 % susceptible)
- *Streptococcus dysgalactiae*: 0.06 µg/ml (96 % to 100 % susceptible)
- *Streptococcus uberis*: ≤ 0.06 µg/ml (84 % to 92 % susceptible)

MIC_{90} values were less than 1.0 µg/ml for *Staphylococcus aureus, Streptococcus agalactiae* and *Streptococcus dysgalactiae*. For *Streptococcus uberis*, MIC_{90} values were between 0.25 and 32.0 µg/ml.

- Coagulase-negative Staphylococci (*S. chromogenes, S. epidermidis, S. hominis, S. hyicus, S. simulans, S. warneri, S. xylosus*): 0.13 – 0.50 µg/ml (95 % to 96 % susceptible)

Gram-positive isolates with an MIC > 2 µg/ml are considered resistant.

Some ß-lactamase producing *Staphylococcus aureus* strains were found to be both resistant to pirlimycin and erythromycin, i.e. to lincosaminides and to macrolides due to the similar mechanism of action of both classes of compounds, i.e. inhibition of RNA dependent protein synthesis at the ribosomes and the potential of cross-resistance. An appropriate reference to this is made in the SPC and product information. Nevertheless, ß-lactamase production by itself did not affect susceptibility of staphylococci towards pirlimycin.

Enteric bacteria such as *E. coli* are intrinsically not susceptible to pirlimycin. Therefore, advice and/or warnings have been included in the SPC and product information:
"Treatment of infections due to enteric bacteria such as E. coli." (Contraindication)
"Susceptibility testing should be carried out prior to treatment." (Special precaution(s) for use):
Furthermore, detailed instructions regarding hygienic measures are described under posology.

IV.B Clinical documentation

Pirlimycin was originally proposed by the Applicant to be indicated for the treatment of clinical and subclinical mastitis in lactating cows and a number of studies were submitted. However, based on the studies submitted, the Committee agreed that the efficacy of pirlimycin was provided only for the treatment of subclinical mastitis in lactating cows.

IV.B.1 Dose and duration of treatment

An infection model study including 25 lactating dairy cattle with induced *Staphylococcus aureus* infection was conducted in the USA to evaluate dosages of pirlimycin for the treatment of model induced clinical and subclinical mastitis. A regime of two intramammary infusions of 10 ml formulations of pirlimycin at doses of 25, 50 or 100 mg per quarter was compared to no treatment. Treatment results indicate that pirlimycin at doses of 50 or 100 mg/quarter was significantly better than no treatment or 25 mg pirlimycin.

Although this study is only of limited value, the conduct and design of the study is however acceptable since cows were routinely clinically monitored and milk samples for SCC and bacteriology were collected up to 28 days following the last infusion. In addition, the dose level of 50 mg/infected quarter was confirmed. Cure rates in two clinical dose determination studies following 2 x 100 or 2 x 200 mg pirlimycin were not increased when compared to 2 x 50 mg pirlimycin, although higher pirlimycin concentrations in milk were obtained.

The Applicant provided study results of several researchers who investigated extended duration of therapy with pirlimycin in cows subclinically infected with *Staphylococcus aureus* in the USA (experimental or field conditions). Data show that the extended treatment duration resulted in significantly improved quarter cure rates. Mastitis of chronically infected cows was not improved by extended therapy duration. The Applicant has therefore proposed an extended therapy duration with 8 infusions of pirlimycin at 24 hour intervals.

IV.B.2 European multicentric field study

A first GCP European multicentric field study conducted in a total of 54 herds and 397 cows (376 quarters) was submitted by the Applicant. However, this study did not prove the satisfactorily efficacy of pirlimycin in the treatment of subclinical mastitis.

The rather low percentages of cure rates in this study in treated cows was considered to be related to the high number of cows of the 4th or 5th parity, i.e. old cows known to have more chronic infections. Nevertheless, the SCC decline in bacteriologically cured quarters in treated young cows exceeded that observed in cows of fourth or fifth parity.

A further GCP compliant European multicentric field study was conducted in order to investigate whether an 8-day treatment was associated with higher cure of subclinical mastitis when compared to a 2-day treatment. In addition, an authorised reference product of known therapeutic value containing cefazolin was used for control as required in Council Directive (EEC) 81/852, Part IV, Chapter 2 (Clinical requirements).

The study included 57 dairy herds in 8 European countries (The Netherlands, Sweden, Denmark, Spain, Italy, France, Germany and The United Kingdom) and it was performed according to the EC intramammary guidelines. Cows with clinical mastitis and cows with clinical palpable udder lesions indicative of chronic infections were excluded. The study enrolled a total of 801 quarters from 481 cows with a bacteriologically positive culture result and SCC >300.000 cells/ml on day –8 pre-treatment in at least one quarter.

Prior to treatment milk samples were collected for bacteriology and SCC. Cows with subclinical mastitis were randomly allocated to treatment groups including (1) pirlimycin, 50 mg, 8 daily infusions, (2) pirlimycin, 50 mg, two infusions at 24 h interval, and (3) positive control. Milk samples for bacterial culture and SCC were collected up to 30 days after the day of enrolment. “Subclinical cure” was classified as both bacteriologically and cytologically cured with no clinical signs.

The incidence of clinical mastitis was low with 1.8, 5.2 and 4.9 % for pirlimycin 2 X treatment, pirlimycin 8 X treatment and the control group, respectively. A total of 532 quarters from 364 cows remained for analysis according to the current EC intramammary guidelines. Cytological cure rates of 21, 38.5 and 43.4 % were obtained after pirlimycin 2 X treatment, pirlimycin 8 X treatment and in the control group, respectively. The overall bacteriological cure rates were 37.4, 60.4 and 55.5 % for pirlimycin 2 X treatment, pirlimycin 8 X treatment and the control group, respectively. The subclinical cure rates were 15 % for pirlimycin 2 X treatment compared to 30.2 % and 35.8 % for pirlimycin 8 X treatment and the control group, respectively.
Pirlimycin 8 X treatment had a numerically greater bacteriological cure rate compared to the positive control, but was numerically less for cytological and subclinical cure rates. Both pirlimycin 8 X treatment and the positive control had significantly higher cure rates than pirlimycin 2 X treatment. Post-treatment SCC was significantly lower for pirlimycin 8 X treatment and the positive control than pirlimycin 2 X treatment. The post-treatment SCC did not differ significantly when considering the 14 - 21 days post treatment.

As a result, the study confirms that the administration of eight daily infusions of pirlimycin is more efficacious in the treatment of subclinical mastitis caused by major pathogens than two infusions at 24 hour interval and is as efficacious as the administration of a registered intramammary product used for control. The Applicant’s proposal of extended therapy regime in the treatment of subclinical mastitis caused by major pathogens is therefore accepted.

The results of the European study on subclinical mastitis show that the bacteriological cure rate for coagulase-negative streptococci (CNS)-induced subclinical mastitis was not higher with lengthening of treatment duration. However, the decrease in milk somatic cell count associated with bacteriological cure following 8-day treatment exceeded by far that following a 2-day treatment with pirlimycin. Therefore, the Committee decided in favour of an 8-day rather than a 2-day for the treatment of subclinical mastitis caused by coagulase-negative staphylococci as well.

V. RISK – BENEFIT ASSESSMENT AND CONCLUSIONS

Pirlimycin is intended for the treatment of subclinical mastitis in lactating dairy cows. 50 mg pirlimycin in 10 ml sterile aqueous vehicle shall be infused into each infected udder quarter. Eight infusions 24 hours apart are intended for the treatment of subclinical mastitis due to *Staphylococcus aureus*, *Streptococcus agalactiae, dysgalactiae* and *uberis*.

The final product contains pirlimycin (as hydrochloride) in a buffered aqueous solution. Stability data for the drug substance cover up to 60 months at room temperature. For the drug product a shelf life of 3 years can be retained when stored at or below 25 °C in the carton. Additional information on packaging materials, validation data for the manufacturing process from the intended production site and stability data of the first 3 production batches were provided by the Marketing Authorisation Holder as post-authorisation commitments.

Pirlimycin was considered to be without effects on reproduction, embryonic and foetal development in rats and mice. It was devoid of mutagenic properties and is not related to a known carcinogen. Pirlimycin was severely irritating when applied into the eyes or onto the abraded skin of albino rabbits. An appropriate user warning has been included in the SPC and package insert.

Pirlimycin hydrochloride has been evaluated in the course of the MRL procedure according to Council Regulation (EEC) No. 2377/90. The determination of the withdrawal period for edible tissues was based on liver concentrations measured by HPLC/TSP/MS. A withdrawal period of 23 days for edible tissues and 5 days for milk has been established.

Although a Phase II - Tier A assessment focusing on the specific issues relating to the presence of residue in fresh dung was required, data indicate pirlimycin hydrochloride is not expected to negatively impact the environment when used per label instructions. Therefore there is no need for a specific label instruction for environmental caution.

Pirlimycin did not elicit signs of udder irritation or clinical adverse effects in cows. Somatic milk cell count rises were seen in some animals under treatment and coliform mastitis was reported in a few cows. However, these effects were found to be almost negligible in studies following standard operating procedures for proper udder infusion technique. Therefore, introduction of environmental pathogens into the udder by improper teat handling is assumed to be the cause for the unfavourable effects referred to above. Detailed advice for teat cleansing and proper infusion of the product in order to avoid infections with environmental bacteria such as *E. coli* is therefore given in the SPC and the product information.

Antimicrobial resistance risk has been assessed according to the recommendations and conclusions of the CVMP's Risk Assessment on Antimicrobial Resistance. It was noted that efficacy of pirlimycin against *Streptococcus uberis* is variable. However, the Committee agreed that this would be taken into account by an adequate recommendation, that "susceptibility testing should be carried out prior to treatment" in the SPC and product literature.

The Committee acknowledged the diagnosis "Subclinical mastitis" based on bacteriological identification would be achieved in normal veterinary practise, since it is common practice in dairy production to perform somatic cell counts on a routine basis.

A European multicentric field study was conducted to investigate whether an eight-day treatment was associated with higher cure of subclinical mastitis when compared to a 2-day treatment. In addition, an authorised reference product of known therapeutic value was used for control as required in Council Directive (EEC) 81/852, Part IV, Chapter 2 (Clinical requirements). The study confirmed that the administration of eight daily infusions of pirlimycin is more efficacious in the treatment of subclinical mastitis caused by major pathogens than two infusions at a 24 hour interval and is as efficacious as the administration of a registered intramammary product used for control. The Committee therefore agreed to an extended duration of treatment of eight days.

Based on the original and complementary data presented the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC.