SCIENTIFIC DISCUSSION

Invented name:	Dicural	
International Non-proprietary Name:	Difloxacin hydrochloride	
Target species (with associated Pharmaceutical form):	Chickens (broilers and future breeders):	Oral solution
	Turkeys (young, up to 2kg bodyweight):	Oral solution
	Cattle (calves and young cattle):	Solution for injection
	Dogs:	Solution for injection; Coated tablets

Therapeutic indications:

Chickens and Turkeys:

- for treatment of chronic respiratory infections caused by sensitive strains of *Escherichia coli* and *Mycoplasma gallisepticum*.

Turkeys:

- for the treatment of infections caused by *Pasteurella multocida*.

Cattle:

- for the treatment of bovine respiratory disease (shipping fever, calf pneumonia) caused by single or mixed infections with *Pasteurella haemolytica*, *Pasteurella multocida*, and/or *Mycoplasma spp*.

Dogs:

- for the treatment of acute uncomplicated urinary tract infections caused by *Escherichia coli* or *Staphylococcus spp.*
- for the treatment of superficial pyoderma caused by *Staphylococcus intermedius.*

Withdrawal periods:	Chicken and turkeys:	Meat: 24 hours
	Cattle:	Meat and offal 46 days

ATCvet code and Pharmacotherapeutic Group:	QJ01MA94 General anti-infectives for systemic use; antibacterials for systemic use; quinolone antibacterials.
Marketing Authorisation Holder:	Fort Dodge Animal Health Holland

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I SUMMARY OF THE DOSSIER

Dicural was eligible for the granting of a Community marketing authorisation via the centralised procedure as it is a product intended for food-producing animals and its active ingredient, difloxacin, had not been authorised for use in food-producing animals on the date of entry into force of Council Regulation (EEC) No 2309/93 (i.e. on January 1, 1995), as provided for under the last indent of Part B of the Annex to that Regulation.

On 16 January 1998 the European Commission issued the initial marketing authorisation, valid throughout the European Union, for the veterinary medicinal product Dicural 100 mg/ml Oral Solution for Chickens and Turkeys. This decision was based on the favourable opinion and the assessment report adopted by the Committee for Veterinary Medicinal Products (CVMP) on 11 June 1997. The Marketing Authorisation Holder was Fort Dodge Animal Health Holland.

The Marketing Authorisation was subsequently extended to both dogs and cattle, with different pharmaceutical forms. For dogs: Dicural 15 mg, 50 mg, 100 mg and 150 mg coated tablets; and, Dicural 50 mg/ml solution for injection. For cattle: Dicural 50 mg/ml solution for injection.

The marketing authorisation was renewed on 16 January 2003 and on 16 January 2008.

Dicural contains difloxacin (as the hydrochloride), which is an antibiotic of the fluoroquinolone group. Fluoroquinolones exert their antibacterial effect against both replicating and dormant micro-organisms. Difloxacin hydrochloride can be bactericidal in activity and acts primarily through inhibition of bacterial DNA gyrase.

No raw materials of bovine, ovine or caprine origin are used in the production of Dicural Oral Solution and Dicural Solution for Injection; in Coated Tablets lactose is used. The lactose is derived from milk sourced from healthy animals in the same conditions as milk collected for human consumption and no other ruminant materials are used during its manufacturing. Dicural products do not represent a risk for human or animal health regarding a possible transmission of TSE. The TSE status is unchanged since original authorisation.

Based on the original and complementary data presented the Committee for Veterinary Medicinal Products has concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Directive 2001/82/EEC of the European Parliament and of the Council.

II DICURAL 100 MG/ML ORAL SOLUTION FOR CHICKENS AND TURKEYS

QUALITY ASSESSMENT

Dicural oral solution is a clear yellowish solution containing difloxacin hydrochloride (100 mg difloxacin base per ml) (10%) as the active ingredient for administration via the drinking water.

The product contains per ml:

Active Ingredient:		Quality standard
Difloxacin hydrochloride, corresponding to Difloxacin	100.0 mg	monograph

Excipients:

Propylene glycol, benzyl alcohol, potassium hydroxide, edetic acid, purified water.

Container:

The oral solution is presented in 250 and 1000 ml plastic (HDPE) white bottles with tamper evident polypropylene screw caps. The bottles are packed in individual cartons, as well as in 6-bottle pack for the 1000 ml bottles.

Product Development Studies:

This product is a high pH product, which can be diluted at all concentrations with water of different temperature, hardness and pH without precipitation.

METHOD OF PREPARATION

All raw materials are weighed after control and release of the starting materials. They are transferred to a tank for mixing. The mixture is cooled to room temperature, pH adjusted, and made up to volume prior to filter and filling in the final containers.

The actual batch size will be multiples of 100 litres. The batch size will be maximally 5000 litres.

As the product is a true solution, the critical step of total dissolution of all ingredients is checked during in-process control.

CONTROL OF STARTING MATERIALS

The active ingredient difloxacin hydrochloride is not described in a pharmacopoeia but a monograph has been supplied.

A description of the assay method for difloxacin-HCl and related substances was provided. The method is validated for precision (RSD repeatability = 0.7%, RSD reproducibility = 0.7%), accuracy (99.2%), linearity and selectivity. The limit of detection for difloxacin is 32.2 ng/ml and for the related substances 0.04% relative to the nominal difloxacin concentration. The limit of quantitation is for difloxacin 53.6 ng/ml and for the related substances 0.07% relative to the nominal difloxacin concentration.

A number of impurities are tested and in particular the six by-products of the synthetic route by HPLC.

The supplier of the raw material difloxacin-HCl, determined the amount of total impurities in different lots. The total amount varied from 0.4 - 0.8%, with a mean of 0.6% and a standard deviation of 0.165%. According to these results the applicant proposes a limit equivalent to 1.1% (being the mean of 0.6% with 3 times the standard deviation of 0.165%).

CONTROL AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

The weighing of bottles, the total dissolution, the temperature and the pH are controlled.

CONTROL OF THE FINISHED PRODUCT

The finished product is checked for:

- characteristics: clear yellowish solution, free from visible suspended particles;
- identity of difloxacin-hydrochloride: by HPLC and TLC as a second independent identification method;
- assay of difloxacin: 95-105 mg/ml (95-105%), by HPLC.

However, as the finished product specifications were extended during the assessment process by a specification for the related substances (below 0.5% for every individual impurity and below 1.1% for the sum of total impurities), the results of three batch analyses submitted initially were no longer relevant and the Committee requested that the results of a minimum of five production batches analysed for these parameters be submitted when available.

STABILITY

Finished Product

Stability studies were performed with three batches kept for 18 months at 25°C and at 60% relative humidity and for 12 months at 40°C and 75% relative humidity. The appearance, identity and assay of the active ingredient, pH and relative density of the product were determined at different control points (initial and after 1, 2, 3 6, 9, 12 and 18 months).

As a consequence of the change of the analytical procedure during the assessment process, a new stability study was started with three pilot batches. The protocol of the study was set up for real time (25°C and 60% relative humidity) and accelerated time (40°C and 75% relative humidity). The interim-results submitted at week 26 indicate that the product is stable under both conditions. Based on this study and on the 18 months results of the earlier study provided in the original dossier, a shelf-life of 24 months for this product, if stored below 25°C, was accepted.

Further stability data from these three pilot production batches, stored in both 250ml & 1000ml HDPE bottles at 25°C/60%RH for 36 months and at 40°C/75%RH for 6 months, were provided in support of a Type I variation (approved November 1999). The test methods and specification limits accepted during the registration procedure were applied. No changes were observed in the parameters tested and all results were within the agreed specifications. The proposed increase in shelf life to 36 months was therefore justified. The recommended storage conditions remain unchanged.

As no data on the light stability of the product were provided, the Committee agreed that the product should be protected from light and that a statement to this effect should appear on the labelling.

In addition, the applicant was requested, and agreed to submit results of the on-going real time stability study. These data have since been provided (as part of the supporting data for the variation application to increase the shelf life to 36 months).

In-use shelf-life

As no data had been originally provided to justify the proposed in-use shelf-life of one year after first opening of containers, the applicant submitted preservative efficacy data at the request of the Committee, and revised its original claim to 3 months.

However, as the microbial quality of the product had not been tested during this in-use stability study, the Committee could not confirm that the microbial quality of the product was in compliance with the Ph. Eur. requirements. Therefore the Committee agreed that the in-use shelf-life should be limited to one month.

The applicant was invited to submit additional data on the microbial quality of the product after first opening of the container with a view to extend the in-use stability beyond 1 month.

Medicated drinking water

As Dicural Oral solution is intended for administration via drinking water, data demonstrating stability of the product in drinking water was requested by the Committee.

The applicant performed such a study focusing in particular on the possible effects of contact between the medicated drinking water and different materials used in water supply systems of commercial poultry units.

Based on the results of this study, a stability of 24 hours for the medicated drinking water was agreed to. However, the Committee required that the SPC include the following statement: *medicated water should be prepared daily*.

SAFETY ASSESSMENT AND RESIDUES

Consumer safety

Acute oral toxicity comprised decreased activity, ataxia, squinting, dyspnoea, tremors and a decreased body weight. The oral LD_{50}s (males-females) for mice and rats were 1.38-1.60 and 5.51-6.27 g/kg bw, respectively, so difloxacin was considered slightly to moderately toxic.

Several oral repeated dose toxicity studies were performed with rats and dogs. Decreased food consumption and body weight, convulsions, dehydration and effects on haematology and clinical chemistry were noted in both species.

An overall NOEL of 1 mg/kg bw/day has been established, based on effects on articular cartilage in immature dogs, which was observed in a 3-month repeated dose study and was the most sensitive parameter.

Reproductive toxicity of difloxacin was studied in rats after oral administration. In a fertility and general reproductive performance study and a peri- and postnatal study no significant effects were found on reproductive performance, on physical development, reflex responses and behaviour of pups. Effects were seen on maternal body weight, food and water consumption and foetal and pup body weight at 45 and/or 150 mg/kg. In a 3-generation reproduction study, a number of effects on parents and pups were seen and fertility was strongly affected at 100 mg/kg (the highest dose). The NOEL for reproductive and neonatal toxicity in this study was 50 mg/kg bw/day and for parental toxicity 25 mg/kg bw/day. Embryo- and foetotoxicity, including teratogenicity was studied in the rat and the rabbit. In the rat, litter resorption occurred and foetal survival was decreased at the highest dose (275 mg/kg). An ossification delay, apparent from unossified and unfused sternebrae, was noted at 65 and 275 mg/kg, but this was most probably due to inhibition of pup growth. In the rabbit study, no teratogenic effects were observed at 15 and 35 mg/kg. As a result of increased embryotoxicity at the highest dose level (75 mg/kg), the number of foetuses was too low for a teratogenic examination.

Genotoxicity of difloxacin was only tested *in vitro*. Tests in bacterial systems were not conducted in view of the mechanism of action of the compound. Difloxacin was negative in three of the mutagenicity tests. Only in some indicator-tests (UDS, SCE, DNA-repair, alkaline elution) did difloxacin give positive results. However, due to the limited solubility of difloxacin, precipitates were formed at relatively low doses, which in UDS and DNA tests may have interfered with tritiated thymidine as a result of intracellular trapping of thymidine by microaggregates of the drug. Remarkably, when the precipitate was removed, the UDS test was negative. Therefore, these results might have been false positives. Furthermore, an indirect effect on DNA replication is not uncommon for fluoroquinolones, since they act as a gyrase-inhibitor. Sarafloxacin, a structural analogue (N-desmethyl-difloxacin), showed also some positive results in the *in vitro* UDS tests. However, an *in vivo* UDS test with sarafloxacin gave negative results, and sarafloxacin was considered not to be a genotoxic agent.

In view of the explanations above, and the fact that difloxacin gave negative results in the mutagenicity tests and does not contain a structural alert, difloxacin is considered a non-genotoxic compound.

Carcinogenicity studies with difloxacin were carried out in the rat and in the mouse. The tumour incidences in the rat and in the female mouse were not affected. In the male CD-1 mouse a shift from alveolar/bronchiolar adenomas to carcinomas was observed which was only statistically significant in the highest dose group (150 mg/kg bw/day). At lower doses also a slight increase in the incidence of carcinomas was found, but this effect was not considered toxicologically significant since the mice strain used in this study is known for relative high incidences of spontaneous lung tumours. Given the results of the rat and the mouse carcinogenicity studies, in spite of the low survival in male rats, difloxacin is considered not carcinogenic.

The toxicological Acceptable Daily Intake (ADI) for difloxacin was calculated to be 10 µg/kg bw, based on the overall No Observed Effect Level of 1 mg/kg bw/day, derived from the 3-month oral toxicity study in dogs, and a safety factor of 100.

The microbiological ADI is higher than the toxicological ADI as it was calculated to be 40.6 µg/kg.

On the basis of this toxicological ADI, MRLs have already been established by the Community for difloxacin which has been included into Annex I of Council Regulation (EEC) No 2377/90 as indicated with the following table:

Pharmacologically active substance(s)	**Marker residue**	**Animal species**	**MRLs**	**Target Tissues**	**Other provisions**
Difloxacin	Difloxacin	Chicken, turkey	300 µg/kg 400 µg/kg 1900 µg/kg 600 µg/kg	Muscle Skin/fat Liver Kidney	

These MRLs will lead to a maximum daily intake of 399 µg/person (66% of the toxicological ADI of 600 µg/person), and provide a safety margin for other uses.

Operator safety

The active substance, difloxacin, is non-corrosive and non-irritant in ocular and dermal irritation studies in rabbits, and its oral acute toxicity is low. However due to the high pH (11-12) of the finished product, Dicural should be considered to be a dermal and ocular irritant. Therefore the following special precautions have to be taken by persons administering this product to animals:

- *Persons with known hypersensitivity to quinolones should avoid any contact with the product.*
- *In order to avoid irritation of skin and/or eyes, use gloves and a face-protecting device, when handling this product.*

Environmental safety

Data were provided to address predicted concentrations in the environment, fate in the terrestrial environment, effects in the terrestrial environment, and effects in the aquatic environment. The predicted concentrations in the environment are summarised below:

Species	Environmental compartment	Assumptions	Predicted Environmental Concentration
Broiler	soil	Ploughed to 25cm, nitrogen applied at 170 kg/ha, 65% of finishing weight	126 µg/kg
Broiler	water		0.6 µg/l
Turkey	soil	Ploughed to 25cm, nitrogen applied at 170 kg/ha, 65% of finishing weight	31 - 77 µg/kg

The predicted environmental concentration of difloxacin in soil was determined to be higher than 0.01 mg/kg and therefore a Phase II assessment of difloxacin hydrochloride was requested. For the Phase II assessment a comprehensive set of studies of good quality were submitted, generated according to recognised guidelines and Good Laboratory Practice.

Toxicity tests with 6 plant species revealed a lowest NOEC of 18 mg difloxacin hydrochloride per kg soil. Difloxacin hydrochloride was non-toxic to earthworms because a nominal concentration of 1100 mg/kg revealed no effect on the earthworm *Lumbricus terrestris* during a 28 days test period. Tests with 3 fungal species showed no effect on these organisms at a concentration of 1000 mg difloxacin hydrochloride per litre agar.

Aquatic toxicity tests with difloxacin hydrochloride and bluegill sunfish (*Lepomis macrochirus*), water fleas (*Daphnia magna*) and algae (*Selenastrum capricomutum*) revealed No Observed Effect Concentrations of 146, 29.8 and 1.0 mg/l respectively. These concentrations were much higher than the predicted environmental concentration (worst case) of 0.6 µg/l and therefore no effects of difloxacin hydrochloride on aquatic organisms were predicted if and when manure might enter into the aquatic compartment.

The results of 3 different bacterial growth inhibition tests revealed a lowest NOEC for *Bacillus megaterium*. An estimate of the NOEC of difloxacin hydrochloride for this species was 0.026 mg difloxacin hydrochloride per litre agar.

Difloxacin was found to be persistent in soil and MIC data using various media indicated a risk to soil micro-organisms as the PEC/MIC for bacteria was superior to 0.1. However, when soil was used as a medium the MICs for bacteria were > 1000 mg/kg given a PEC/MIC of 0.0002. In view of this, the Committee considered the risk to micro-organisms to be acceptable.

The phytotoxicity data together with the estimated PECs indicate that it is likely that effects on following crops may occur during seedling growth. Data on this is to be provided at renewal of the marketing authorisation.

The further phytotoxicity data requested were provided at time of the Renewal and demonstrated that no significant effects on seedling growth for eight out of nine of the tested plant species are expected at 510 µg.kgsoil^{-1}, equivalent to 4xPEC after one year of application. No effect on Glycine max is expected at 250 µg.kgsoil-1, equivalent to 2xPEC after one year of application. It was noted that in 64 days no appreciable degradation of difloxacin occurred under experimental conditions and therefore there might be a concern about persistence of difloxacin residues in soil.

However, the data provided represented field conditions and therefore the twofold margin of safety between the experiment and the maximum PEC is sufficient.

RESIDUES

Pharmacokinetic studies

In a first pharmacokinetic study, 40 broiler chickens (Cornish Rock, 10/sex/group, 35 days of age, body weight 1.14-1.51 kg for females and 1.26-1.668 kg for males) and 40 growing turkeys (Nicolas Broad Breasted White, 10/sex/group, 57 days of age, body weight 2.82-3.78 kg for females and 3.4-4.33 kg for males) received either a single i.v. dose of Dicural Injection at 5 mg difloxacin/kg bw into the right wing vein or a single oral (gavage) dose of Difloxacin Hydrochloride Liquid Concentrate at 5 mg difloxacin/kg bw.

Blood samples were collected at several time points up to 24 hours post-dosing. Difloxacin was determined in the plasma samples by HPLC fluorescence detection (LOQ 0.010 mg/ml). Pharmacokinetic parameters were determined for both routes.

In a second pharmacokinetic study, broiler chickens (Cobb500, n=28, 31 days of age, body weight 580-900 g) and turkeys (Hybrid, n=28, 80 days of age, body weight 3450-4940 g) received water medication with Dicural Oral solution at a dose of 0 (n=4/sex), 10 (n=6/sex) or 20 (n=4/sex) mg difloxacin/kg bw/day for 5 consecutive days. These animals were also part of a residue study (see Residue depletion studies).

Blood samples were collected at 0, 24, 48 and 120 hours after the start of medication and at slaughter at 0.5/1 and 24 hours after cessation of medication. Difloxacin was quantified in plasma by means of HPLC with fluorescence detection (LOQ 4.5 ng/ml for broiler plasma and 5.04 ng/ml for turkey plasma).

From these studies, it appeared that maximum plasma levels of difloxacin are reached within 1-2 hours after oral administration. Elimination half-lives were approximately 7 hours in these species. At the same dose level, difloxacin plasma levels in turkey are about 3-4 times lower than those in broilers. This might be explained by the 2-fold larger distribution volume and the lower bioavailability in turkeys (57.4%) compared with broilers (96.4%).

Pharmacokinetics in target species do not differ substantially from pharmacokinetics in laboratory animals, in which is was also shown that difloxacin is subjected to enterohepatic cycling.

Residue depletion studies

In a first residue depletion study, 500 Cobb broilers received water medication with Dicural Oral solution at a dose of 0, 10 or 20 mg difloxacin/kg bw/day for 5 consecutive days. Another unmedicated group of 10 animals/sex served as replacement and for QC samples and calibrators. The medicated drinking water was prepared once daily depending on the water consumption of the previous day. Based on this water consumption the calculated average daily intake was 9.95 ± 0.57 mg difloxacin/kg bw/day.

At day 0, 1, 3, 5 and 7 the animals were slaughtered and samples of blood, kidney, liver, abdominal fat, breast muscle, skin and adhering fat, and in some cases lung, were collected and analysed for their difloxacin content by means of HPLC with fluorescence detection. In liver and kidney also the difloxacin and sarafloxacin concentrations without and with a deconjugation step were determined. No tissue samples were collected from the high dose animals.

In a second residue depletion study, turkeys received water medication with Dicural Oral solution at a dose of 0, 10 or 20 mg difloxacin/kg bw/day for 5 consecutive days. Another unmedicated group of 10 animals/sex served as replacement and for QC samples and calibrators. The medicated drinking water was prepared twice daily depending on the water consumption of the previous day.

Based on this water consumption the calculated average daily intake was 10.15 ± 0.15 and 20.50 ± 2.92 mg difloxacin/kg bw/day.

At day 0, 1, 3, 5, 7 and 10 the animals were slaughtered and samples of blood, kidney, liver, abdominal fat, breast muscle, skin and adhering fat, and in some cases lung, were collected and analysed for their difloxacin content by means of HPLC with fluorescence detection. In liver and kidney also the difloxacin and sarafloxacin concentrations with and without a deconjugation step were determined. No tissue samples were collected from the high dose animals.

These studies showed that highest residues in broilers and turkeys were found in liver and kidney, but that residues in skin were most persistent. Residues in skin were still above 10 µg/kg at 7 and 10 days after treatment at the recommended dose for chickens and turkeys, respectively. Only in the first few days after treatment small amounts of sarafloxacin (broiler/turkey), N-oxide difloxacin (turkey) and conjugates (broiler/turkey) could be detected in the tissues (mainly liver).

Radio-labelled studies were conducted with 7 groups of 3 male and 3 female broiler chickens (35 days of age, body weight approximately 1.5 kg) received Dicural Oral solution fortified with ^{14}C-difloxacin by gavage at a dose rate of 10 mg difloxacin/kg bw/day for 5 consecutive days. Two additional groups, consisting of 2 males and 2 females, received gavage doses with the same test substance for 5 consecutive days at a dose equivalent with 17 ppm difloxacin in the drinking water (on body weight basis the dose was equal to approximately 3 mg/kg bw). One extra group (3M/3F) served as control. The daily dose was administered as 4 equal partial doses at 7 hour intervals. The 17 ppm groups were slaughtered at 6 and 18 hours after the last administration, and the 10 mg/kg bw groups were slaughtered at 6, 12, 24, 48, 72 and 96 hours after the last administration.

Radio-labelled studies were also conducted with 6 groups of 3 male and 3 female Nicholas turkeys, one group of 2 male and 3 female, two groups of 2 male and 4 female and one group of 2 male and 6 female turkeys received Dicural Oral solution fortified with ^{14}C-difloxacin by gavage at a dose rate of 10 mg difloxacin/kg bw/day for 5 consecutive days. Two additional groups, consisting of 2 males and 2 females, received gavage doses with the same test substance for 5 consecutive days at a dose equivalent to 28 ppm difloxacin in the drinking water (on body weight basis the dose was equal to approximately 2.3-2.5 mg/kg bw). One extra group (3M/3F) served as control. The daily dose of difloxacin was administered as 4 equal partial doses at 7 hour intervals. The 28 ppm groups were slaughtered at 6 and 24 hours after the last administration, and the 10 mg/kg bw groups were slaughtered at 6, 12, 24, 48, 72, 96, 120, 144 and 168 hours after the last administration.

These radio-labelled studies revealed that extractable residues were the predominant fraction of the total radioactive residues in tissues (>90%) and excreta (>99%), and difloxacin was the predominant radioactive compound. In tissues the major residue was difloxacin or its conjugates (60-90%) with a smaller portion of its demethylated form, sarafloxacin (5-15%). In excreta difloxacin was demethylated to sarafloxacin or N-oxidised to N-oxide-difloxacin, all of which were conjugated to base and/or acid-labile conjugates.

Routine analytical method for the detection of residues

For the determination of difloxacin in tissues of chicken and turkey a HPLC/fluorescence method was developed. This method is validated, suitable for residue surveillance, and is described in the ISO 78/2 format. The limit of quantification (LOQ) is 10 µg/kg in all tissues, and the limits of detection were all well below 2.3 µg/kg, even after deglucuronidation. The method also provides in measuring glucuronides in liver samples, and has the capability of measuring sarafloxacin.

Withdrawal period

Taking into account the toxicological ADI of 10 µg/kg bw (=600 µg for a 60 kg person), the tissue distribution of difloxacin and the concentration of residues at 1-6 hours and 24 hours, the following MRLs for chicken and turkey tissues were established for the marker difloxacin: liver 1900 µg/kg, muscle 300 µg/kg, kidney 600 µg/kg, and skin + adhering fat 400 µg/kg.

Taking into account that residue depletion between 0 and 24 hours is very rapid and that residues in all samples are sufficiently below the MRLs at day 1, the Committee concluded that a withdrawal period of 24 hours for both species can be regarded as safe for Dicural oral solution being administered via drinking water to broilers and turkeys at the recommended dosage of 10 mg difloxacin/kg bw/day for 5 consecutive days.

EFFICACY ASSESSMENT

Pharmacodynamics

The mechanism of action for difloxacin is similar to that of the other fluoroquinolones i.e. it binds to the A subunit of bacterial DNA gyrase.

A summary of the most relevant MIC data is given below:

Species	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)
Campylobacter	0.06	0.12	0.02-0.5
E. coli **(broiler)**	≤0.06 0.06	0.12 1.65	≤0.06-0.12 0.03->8
E. coli **(turkey)**	≤0.06 1.65	0.12 2	≤0.06-0.12 0.03-2
M. gallisepticum	0.25	0.5	0.12-0.5
P. multocida	≤0.06 0.01	≤0.06 0.01	≤0.06 ≤0.01-0.03
Salmonella	0.25	0.25	0.12-4

Many organisms were received from referral centres and include organisms recently isolated in Europe and the USA.

Pharmacokinetics

Lung and plasma tissue ratios of difloxacin in broilers and poults immediately after cessation of medication with difloxacin at 10mg/kg for 5 days are shown in the table below.

Tissue	Broilers		Poults	
	tissue conc*	tissue/plasma	tissue conc*	tissue/plasma
Lung	302±1022.4		148±1383.7	
Plasma	125±39	1	31±11	1.0

*ng/ml or g

Distribution in the body is good in both species with adequate tissue levels being maintained.

Tolerance in the target species

In the original application there were two target animal safety studies. The first was a broiler study involving a total of 700, twenty one day old broilers. Birds were dosed at 0, 30, 90, 150 and 300 ppm difloxacin in the drinking water for 15 days, i.e. approximately x0.7, x1.5, x3 and x6 the recommended dose for three times the recommended treatment duration. The only abnormality was a decrease in feed and water intake in the highest dose level attributed to poor palatability. Otherwise the product was well tolerated.

The second study involved 350 8 week old turkeys. Dicural was administered in the drinking water at 30, 90, 150 and 300 ppm for 15 days, i.e. approximately x0.4, x1.2, x2 and x4 the recommended dose for three times the treatment duration. No adverse clinical signs were noted or gross effects found on post-mortem.

The Committee asked the applicant to justify the safety of proposed treatment in turkeys in view of histopathology effects being recorded in the liver. The applicant has answered these points satisfactorily by having slides reanalysed independently and concluding that there was no treatment effect on the livers.

The Committee asked the applicant to provide more evidence that Dicural would not cause joint lesions in birds. The applicant responded to this by submitting a new safety study involving a total of 1000 1 day old broilers. One group remained as controls and the other received treatment at 2x recommended dose for 10 days i.e. 2x recommended duration. No lesions were observed macroscopically or microscopically using the stifle joint for pathological examination.

The Committee were still concerned that the answer was insufficient in relation to turkeys since the applicant had only argued that it was reasonable to extrapolate from broilers. In an oral presentation the applicant mentioned that the growth rate of chickens and turkeys was very similar until 4-6 weeks and that the lack of adverse effects in field studies involving 23,000 turkeys indicated the product to be safe. However, the applicant proposed a restriction for use in turkeys only up to 2 kg body weight. The Committee were satisfied with the response.

Resistance

A report on resistance monitoring to Dicural of 971 bacterial isolates from diseased poultry in the Netherlands from 1993-1996 showed low levels (5-6%) of resistance for *E. coli*, *Salmonella spp*. and *Pasteurella multocida* isolates. The animal species of origin were approximately 75% chickens and 25% turkeys. No significant change in annual resistance levels for difloxacin could be seen during the years studied, despite the introduction and widespread use of quinolones in Dutch veterinary practice since the mid-eighties.

The Committee asked the applicant to comment further on the data concerning resistance and to propose a possible resistance monitoring programme. In their oral presentation the applicant indicated the level of resistance for difloxacin to be 5-6% of isolates as compared to 1-5% for enrofloxacin, 70% for tetracyclines and 40% for trimethoprim/sulphadiazine. The applicant proposed to continue the resistance monitoring programme in the Netherlands and to extend the programme to further European countries.

The Committee recognised that there was published literature showing a high degree of resistance to fluoroquinolones for *E. coli* and the possibility of cross-resistance had to be taken account of.

However, for the time being, the Committee was satisfied with the response of the applicant in view of the whole issue of antimicrobial resistance being reviewed by its ad hoc group and pending the provision of a risk assessment and of recommendations for management of resistance development.

Several members were not in agreement, expressing concerns that the current indication might encourage widespread use of the product which in turn might lead to increased resistance. Their proposal to restrict the use of the product beyond that which now appears on the SPC was not supported by the Committee. The following statement is included in the SPC under 5.2: 'Dicural oral solution should only be used based on susceptibility testing'.

During the first five years post-authorisation a number of EU susceptibility monitoring reports were submitted and considered by the CVMP. During the Renewal assessment the CVMP requested one final report.

The susceptibility data provided post-authorisation demonstrated that the use of quinolones in general in poultry is clearly reflected in significant proportions of resistant *E. coli* strains. In the three countries involved 17 to 43% of the strains were microbiologically resistant to quinolones. Because in the study design an effort was made that the *E. coli* population examined was a random sample of all *E. coli's* from broilers, and each broiler has app.10^6 cfu/gr *E. coli* in its faeces, the data indicate that in each broiler of broiler flock in the three EU member states concerned, in each gram of faeces at least more than 10^5 cfu (=10% of 10^6) quinolone resistant *E. coli* strains are present. This could be considered a very significant effect of quinolone usage.

Global data from monitoring programs (not in this report) demonstrate the effect of the use of fluoroquinolones on resistance development in *Campylobacter* spp. and to a lesser extent in *Salmonella* spp. Examples that cause concern are *S. Paratyphi B var. Java* in broilers in the Netherlands and Germany which is very rapidly becoming resistant to quinolones and DT104 in the UK and many other countries.

It is obvious that certain serotypes or bacteria species become more rapidly resistant than others. For these bacteria one common factor is of importance, which is selection pressure by usage of quinolones.

Although the susceptibility monitoring report contains good quality data, these data cannot in any way be related to the specific use of difloxacin in poultry and therefore they cannot be used by any risk manager to base any action on regarding the use of a certain fluoroquinolone containing product. Specifically oral mass medication with quinolones will have a major contribution to the selection pressure. Both the data on *E. coli* from this report and data from literature on *Campylobacter* and certain *Salmonella* sero- or phage types are enough reason to continue the discussion on prudent use of fluoroquinolones. Such discussions take place on both national and EU-levels. The CVMP also concluded that the data available would need to be considered in the wider context of use of fluoroquinolones in veterinary medicinal products and undertook to do so.

Clinical studies

In both broilers and turkeys the enteric claim was dropped by the applicant, because in the Committee's opinion it had not been supported by sufficient data.

Broiler chickens

Experimental studies involving *Escherichia coli* infection were conducted in the US and a European country and these supported the 10mg/kg dose rate. Similar studies were conducted for *Mycoplasma gallisepticum* infection as well as for a single mixed infection by *E. coli/M. gallisepticum*. Although the data on *Mycoplasma* and mixed infections were less substantive, they were considered adequate to support the recommended dose of 10mg/kg.

The field trials supporting the treatment in broilers in the initial application were not considered adequate, although they provided many indications that the product was effective in terms of reduced mortality, improved clinical signs and pathology and in some cases a reduction in the number of pathogens isolated. The applicant was asked by the Committee to conduct further, better designed field trials.

Seven additional field trials involving *E. coli* and/or *M. gallisepticum* were conducted in Europe. These included (a) one trial in which efficacy of difloxacin treatment was studied versus non-treatment for the control of post vaccinal *E. coli* respiratory disease; (b) one trial comparing the efficacy of difloxacin in drinking water to a reference product (enrofloxacin) to control respiratory disease caused by *E. coli* and/or *M. gallisepticum;(c)* five other trials on the efficacy of difloxacin for the treatment of *E. coli* and/or *M. gallisepticum* infections.

The lesion scores that were present in 20 randomly sacrificed birds before and 5 days after therapy were evaluated in 3 studies. The mean lesion scores for heart and liver were 0.13 and 0.06 respectively before treatment and zero for both tissues following treatment. Airsac and trachea mean lesion scores were both reduced post treatment compared to pre-treatment.

For the same 3 studies the total number of birds was 37,502. As a whole 24 out of 60 randomly selected birds had lesions infected with *E. coli* before treatment, while after treatment only 2 out of 56 birds had lesions infected with *E. coli*. This reduction was highly significant (Chi^2 $p<0.001$).

With regard to mortality the applicant reported determination of the efficacy of Dicural oral solution was difficult because in most of the trials, the total mortality was due to a mixture of causes such as ascites, emaciation due to malabsorption (poor growers) and cardiac arrest.

In four of the studies the growth of the birds was well maintained despite the presence of disease.

The Committee agreed that these data supported the efficacy of the product for *E. coli* respiratory infections but asked for assurances with regard to the *Mycoplasma gallisepticum* data and invited the applicant to comment on the minimal clinical trial data provided in support of the claim for respiratory infections caused by *M. gallisepticum.*

The applicant at an oral presentation stressed the difficulty of isolating *Mycoplasma* organisms, the fact that serological diagnosis of *M. gallisepticum* infection was performed by ELISA techniques and made the point that *M. gallisepticum* may have been present in some of the other studies. The Committee were satisfied with these explanations and agreed that the efficacy of the product for the treatment of *E. coli*/*M. gallisepticum* respiratory infections in broiler chickens had been demonstrated.

The Committee discussed at length whether or not the indications should be for a claim for 'metaphylaxis' in chickens and turkeys as proposed earlier in the evaluation phase. However, having taken account of the fact that the product is to be used in flocks in which signs of the disease have just become apparent, with a view to curing the minority of already clinically affected birds and preventing the spread of infection to healthy or subclinical birds, the majority of members agreed that a claim for 'treatment' would be more appropriate. It was generally agreed that 'treatment' would be a clearer and more accurate reflection of product characteristics since the term 'metaphylaxis' is rarely or ever used in many EU countries, and that the matter would be addressed satisfactorily by the application of Good Veterinary Practice.

Turkeys

Experimental studies were provided only for *Pasteurella multocida* infections (fowl cholera) with justification being provided for extrapolation of data from broilers for *E. coli* and *M. gallisepticum* on the basis of the turkey being a minor poultry species and the provision of pharmacokinetic/MIC data for turkeys. There were 3 experimental infection studies presented which demonstrated efficacy against *P. multocida* at dose levels corresponding to, and below the recommended dose of 10mg/kg. In its response, the applicant replied that it would be almost impossible to conduct field trials to investigate efficacy against fowl cholera in Europe since it is a reportable disease. In addition, the applicant defended the model study confirming that it demonstrates satisfactory efficacy and that European *P. multocida* isolates had shown good sensitivity to difloxacin.

Two trials were carried out in an EU member state involving naturally occurring respiratory diseases to demonstrate efficacy against *M. gallisepticum* and *E. coli.* The first trial was unsuccessful for a variety of reasons including late treatment and a mixed viral infection being diagnosed in the flock. The Committee could not draw any conclusion from this trial. The second field trial, which involved 3000 17 day old turkeys with respiratory disease treated at 10mg/kg for 5 days, demonstrated efficacy against mixed *E. coli*/*M. gallisepticum* infections. *E. coli* was isolated from the affected birds and *Mycoplasma* infection was diagnosed serologically.

The applicant was advised by the Committee that on the basis of these data, only a claim for *Pasteurella* infections appeared supportable but that further data would be required to justify the *E. coli* and *Mycoplasma* claim. Two further trials were then conducted in 16 day old poults. The first trial involved 3000 poults suffering a severe respiratory infection caused by *E. coli*. The second trial involved 4000 poults suffering a moderate to severe respiratory infection caused by *E. coli*.

In the first trial therapy was initiated too late but, in the other, the treatment reduced clinical signs and mortality. The Committee therefore considered that these results were not entirely satisfactory and that further reassurances would be required with regard to the turkey claims. The applicant was consequently invited to provide oral explanations on the clinical data supporting the claim of treatment of respiratory infections caused by *M. gallisepticum* and *E. coli* in turkeys. The Committee also invited the applicant to discuss the fact that the fowl cholera claim was not supported by European data and to further justify the recommended dose.

At the oral presentation the applicant provided a justification for the extrapolation of *E. coli* and *Mycoplasma* data in broilers to turkeys since the respiratory symptoms are comparable in chicks and turkeys, the growth rate and physiology of young turkeys are comparable to chicks, and MIC data are comparable. The applicant indicated that the poor results in some of the field trials could be explained by initiating treatment too late or because birds had other disease problems such as haemorrhagic enteritis or herpes virus infections.

The Committee accepted the explanation of the applicant and considered that in view of the two successful trials involving a total of 7000 birds, the claim for *E. coli/M. gallisepticum* respiratory infections could be granted. A minority of members considered these data to be inconclusive, in that they did not appear to support the claims against *M. gallisepticum* and *E. coli*, and furthermore they could not accept the extrapolation of data from broilers to turkeys particularly as the bioavailability of difloxacin in turkeys is just over half that in chickens.

In relation to fowl cholera, the applicant agreed that 5mg/kg was efficacious but maintained that as the initial symptoms would resemble *E. coli/M. gallisepticum* infections it would be necessary to begin therapy at the highest dose of 10mg/kg. One dose on the label would avoid confusion at user level. The *Pasteurella* claim was defended by the experimental studies and also in relation to MIC data from both US and EU. The Committee considered that in view of these arguments a dose of 10 mg/kg was appropriate in the treatment of both fowl cholera and *E. coli/M. gallisepticum* infections.

RISK-BENEFIT ASSESSMENT AND CONCLUSION

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

Consequently, the Committee decided on June 11, 1997 that the product could be recommended for the granting of a Community marketing authorisation.

III DICURAL 15 MG, 50 MG, 100 MG AND 150 MG COATED TABLETS FOR DOGS QUALITY ASSESSMENT

Dicural Coated Tablets are available in four strengths and contain difloxacin hydrochloride. (The tablet strengths are expressed in terms of the difloxacin content.) The tablets are round, biconvex coated tablets with no break-line. The difloxacin-containing tablet cores are coated (by compression) to protect the active ingredient from light. The coating mixture includes both brewer's yeast and an aromatic liver flavour to improve the palatability to the target species. The tablets are presented in PVC/aluminium heat sealed blister packs which are packed in outer cartons.

Composition of the veterinary medicinal product

All the four strengths of these tablets are manufactured from common blends of both the core powder blend and also the coating powder blend. Their compositions are detailed below:

Active Substance:	**Grade**	**mg/tab**	**mg/tab**	**mg/tab**	**mg/tab**
Difloxacin (as difloxacin hydrochloride)	MS	15	50	100	150
Other Substances:					
Tablet core					
Palatable coating					
Total tablet weight:		180 mg	600 mg	1200 mg	1800 mg
Tablet diameter:		7.5 mm	12.6 mm	16 mm	18 mm

MS = Manufacturer's specification.

No overages were included.

Container

The tablets are packed in polyvinylchloride blister packs (ten tablets per blister strip), sealed with thermo-adhesive lacquered aluminium foil, which are then packaged into a cardboard box. The outer boxes hold ten, twenty or one hundred tablets (as one, two or ten blister strips).

Clinical trial formulations

During the development of the product, different formulations were used in the clinical trials, such as capsules, non-coated tablets and coated tablets. The formulations of these are given.

Product Development Studies

The pre-formulation studies demonstrate that difloxacin hydrochloride is stable under all conditions except light and oxidising agents such as hydrogen peroxide. To protect difloxacin against light a coated tablet was, therefore, developed. Data were submitted to show that the coating did not influence the bioavailability nor the absorption of difloxacin. Because of possible changes on crystal modification of difloxacin hydrochloride due to the influence of moisture and heat, a dry granulation process was developed.

The quantities of the excipients used in Dicural coated tablets were chosen to optimise flowability with binding capacity.

DESCRIPTION OF METHOD OF PREPARATION

The manufacturing formulae for batch sizes of 100 kg core material and 200 kg coating material were presented.

The coated tablets are produced by a compression coating process. Initially, the tablet core excipients are dry mixed to produce the tablet core blend. The palatable coating powder blend is also produced by a simple dry mixing process. The coated tablets are then compressed using a two-stage tablet compression process. A flow diagram and description of the manufacturing process was presented.

Appropriate in-process controls are conducted on both the powder blend for the tablet cores, and also the tablet cores. Maximum holding times of 2 weeks are applied to both the core blend and coating mixtures. The packaging processes and controls were also described. A maximum holding time between manufacture of the tablets and their blister packaging, of 2 weeks, is specified.

Validation of the process

All manufacturing steps in the production of Dicural are standard steps. Data were submitted which indicate that the mixing process is validated satisfactorily.

CONTROL OF STARTING MATERIALS

Active substance

The active ingredient is not described in a pharmacopoeia so a monograph was developed. An Applicant's part of the European Drug Master File (EDMF) was included in the dossier.

Details of the identity, manufacturing site, synthesis and control of the active ingredient were provided. A flow chart, details of the batch size and full details of the specifications and control methods for the starting materials, reagents, catalysts and solvents were provided, with certificates of analysis. Specifications and control methods were also provided for the intermediates.

Evidence of structure data were provided from a variety of techniques, and the physico-chemical properties of difloxacin hydrochloride were described.

Impurities and residual solvents were described, and limits applied in the specification were based on batch analyses data.

The specification agreed for the active ingredient was considered satisfactory and included the following criteria: description, appearance of solution, identification, water content, heavy metals, residue on ignition, impurities and assay.

The difloxacin hydrochloride purity was determined by HPLC and a description of the method was given. This was validated for linearity, precision, specificity and the stability indicating nature of the assay.

Appropriate validation data were provided for all the other assay methods used in the active ingredient specification, and details of the reference standards were provided.

The results of four batch analyses were given. The batches complied with the specifications.

Excipients

All the pharmacopoeial excipients were purchased to the appropriate specification of the current Ph.Eur., and Certificates of Analysis were provided which demonstrated compliance with these specifications.

The first of the two flavours (yeast) complied with a specification developed by the dosage form manufacturer (which includes the Ph.Eur. limits for microbial contamination) which was adequate to control the quality of this excipient. A certificate of analysis was provided which demonstrated conformity with the specification.

The second flavour is an aromatic liver flavour. This flavour constitutes a significant proportion of each tablet (up to 250 mg per tablet), is purchased from a specified manufacturer, and complies with a specification which was developed by the dosage form manufacturer. The pig livers used in the manufacture of this flavour were stated to originate only from Italy, and a Sanitary certificate was provided to certify that the pig livers originate from disease-free herds. Details of the method of production of the pig liver powder were provided, and this powder is now sterilised by gamma-irradiation. A minimum dose of 2.5 MRad was used to provide an adequate level of lethality for all viruses and micro-organisms, however the initial development (laboratory and pilot scale) batches of these products were manufactured using pig liver powder which had not been gamma-irradiated. Details concerning the gamma-irradiation were provided, in accordance with the EU Guideline "The use of ionizing radiation in the manufacture of medicinal products". The specification for this excipient included satisfactory limits on microbial and viral contamination, and a certificate of analysis was provided which demonstrated conformity with the specification.

Packaging material

Specifications were included for the packaging materials, and certificates of analysis were provided. Evidence was provided that the PVC complied with the Ph.Eur. monograph.

CONTROL TESTS CARRIED OUT AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

Not applicable.

CONTROL TESTS OF THE FINISHED PRODUCT

Specification and routine testing

The Finished Product Specifications (FPSs) agreed included Release and Check (shelf-life) limits, which were considered sufficient to assure consistent quality of the finished product. Appearance, identity tests (2) and assay criteria were included.

The use of a slightly wider lower limit in the Check FPS for the active ingredient was justified by the stability data provided.

Limits for impurities/degradation products were included in the Check specification, and the limits proposed were justified by the stability data provided.

Physical properties of the tablets were controlled by the inclusion of tests and limits for uniformity of weight, thickness, diameter, tablet hardness and disintegration. The limits for moisture content in the Release specification were also justified.

Dissolution testing was performed according to the method of the BP. As standard dissolution media resulted in incomplete recovery of difloxacin from the media, an acetate buffer was used, and this was justified.

Details of the methods used were provided and appropriate validation data were supplied.

Scientific data

Some batch analyses data provided referred to product manufactured in 1994, but these were of little value as, firstly, the specification associated with the product at that time was limited, and secondly, the pig liver powder flavour was not gamma-irradiated at that time. Further batch analyses data were provided from recently manufactured batches of three strengths of the product which were manufactured using gamma-irradiated pig liver powder. These complied with the finished product specifications.

STABILITY

Stability studies on active substance

Degradation studies demonstrated difloxacin hydrochloride to be stable in aqueous solution, and weak aqueous acid and base solutions, both at room temperature and after refluxing for 3 hours. Stability was also demonstrated in the dry state at 110°C. However, rapid surface degradation was seen if difloxacin was exposed to high intensity UV light, both in the dry state and in aqueous solution. Acidic solutions were also unstable in intense UV light.

Samples of batches of the drug substance were stored in packs which mimic the commercial packaging at: ambient temperature and humidity for up to 5 years; 30°C for up to 24 months; 40°C/ambient RH for up to 12 months; and, 40°C/75 %RH for up to 12 months. The results showed no decrease in potency or increase in degradation products under any of these conditions. All results remained within specification, including those for moisture content. The proposed retest period of five years appeared justified.

Stability tests on the finished product

No stability data were provided for the 100 mg strength. The Applicant relied on extrapolation from the other tablet strengths. (All four strengths of tablets are manufactured from a common blend.) This was considered acceptable.

The main shortcoming of the long term (up to 36 months) stability studies reported was that the pig liver powder is now gamma-irradiated; whereas the batches of tablets in the initial stability studies included non-irradiated pig liver powder. Gamma-irradiation is known to induce instability by the generation of free radicals, and the aromatic liver flavour (containing the pig liver powder) constitutes a significant proportion of each tablet; up to 250mg per tablet. Stability data were provided from several batches of the tablets incorporating non-gamma irradiated pig liver powder; however, further data were also provided from two batches of the product which did incorporate gamma-irradiated pig liver powder. Data from a third batch was to be supplied when available. Only limited data (up to 13 weeks storage) were available from these batches incorporating irradiated material, from storage at temperatures up to 40°C/75 %RH. Although these data demonstrated that the initial stability of tablets containing gamma-irradiated pig liver powder was not significantly different from batches using non-irradiated flavouring, further data were required when these became available. At the request of the CVMP the Applicant provided a letter of undertaking (dated 18 June 1999) that these data would be provided when available. A shelf-life of only 18 months was, therefore, supported.

The interim 24-month results of this stability study were subsequently submitted and a shelf life extension requested; from 18 months to 24 months.

Two full-scale production batches of each strength of tablets were used in the study, which was on-going for 3 years. The tablets were stored at 25°C/60%RH. The following parameters were tested: appearance, content, purity, disintegration time, dissolution, hardness and uniformity of weight. The test methods were the same as those used for the control of the finished product. These test methods had been described in the original dossier and validated.

The results and conclusions were as follows:
- the appearance and size remained unchanged
- the active substance content remained within the acceptance limits and showed no significant decline
- the amount of degradation products has been shown to be dependent on the batches of difloxacin hydrochloride used for the preparation of the different batches. At the initial time point, about 0.3% of 7-ethylenediaminodifloxacin and about 0.4% difloxacin N-oxide were found in two batches, while no degradation products could be detected in a different two batches. All batches showed only a slight increase in individual impurities; on average: 7-ethylenediaminodifloxacin 0.02%, sarafloxacin 0.05% and difloxacin N-oxide 0.03%. Individual and total amount of impurities stayed within limits
- the disintegration and the dissolution rate remained well within stability specifications. The results indicate faster disintegration on storage
- apart from a minimum individual value of 18.6 N at the 6-months time point (batch 83007), the hardness of the individual tablets and the mean hardness of the tablets remained within the acceptance limits at all further test points. The hardness of the tablets showed no tendency to decrease on storage
- the uniformity of weight remained within stability specifications.

It was agreed that the extension of the shelf life, from 18 months to 24 months, had been satisfactorily explained.
Although the data provided demonstrated the chemical stability of the tablets, physical stability of the products was less comprehensively addressed. However, data were provided which demonstrated that the dissolution rate was not affected after storage of the tablets for 3 years at 25°C/60 %RH in the blister packs. There was some softening of the tablets on storage, particularly at higher temperatures, but the data demonstrated that the tablets remained within specification for hardness, disintegration and dissolution. It was also noted that storage at higher temperatures and humidities resulted in a darkening in tablet colour.

The agreed storage precautions are “Store below 25°C” and “Store in a dry place”.

SAFETY ASSESSMENT

Pharmacodynamics

The active ingredient, difloxacin hydrochloride, is a synthetic fluoroquinolone, which acts by inhibition of bacterial DNA-gyrase (topoisomerase II). Fluoroquinolones cause significantly less inhibition of the corresponding mammalian enzyme.

The secondary pharmacological effects of the active ingredient, difloxacin hydrochloride, on motor activity, cardiovascular system and blood coagulation were sufficiently investigated in various species (rodents, dogs, monkeys) and various dosing regimes (oral, intravenous, intraduodenal).

Mild effects, such as a small decrease in mean arterial blood pressure and reflex tachycardia, were observed in dogs following an intravenous infusion of 6.25 mg/kg bw for 90 minutes; this dose produced plasma concentrations in the anticipated clinical therapeutic range. No effects on blood pressure or heart rate were produced in monkeys given an intraduodenal dose of 20 mg/kg bw. Oral doses of 100 mg/kg bw and above did not affect blood pressure but increased the heart rate in rats; the lower dose of 30 mg/kg bw had no effect on heart rate.

Pharmacokinetics

The bioavailability of a single dose of difloxacin (5 mg/kg) in 3 different formulations was compared in 12 Beagle dogs using a cross-over design study. The following results were obtained:

		AUC_{48} (ng/ml/h)	C_{max} (ng/ml)	T_{max} (h)
Palatable tablet	female	13354	1265	4.8
	male	10323	1095	3.0
Capsule	female	12856	1271	3.4
	male	12621	1235	3.3
Tablet	female	12096	1095	3.7
	male	10011	1041	2.8

Single dose toxicity

The toxicological studies with difloxacin hydrochloride were referenced in the original application for Dicural Oral Solution. The main findings are summarised below.

The acute oral LD_{50} of difloxacin hydrochloride prepared as a suspension in 0.2 % hydroxypropylmethylcellulose was 1380 mg/kg bw for male and 1600 mg/kg bw for female ICR Mice. In Sprague-Dawley rats the acute oral LD_{50} was 5510 mg/kg bw (males) and 6270 mg/kg bw (females).

Signs of toxicity were reduced activity, ataxia, squinting, dyspnoea, tremors and decreased body weight.

Solid difloxacin hydrochloride was tested in New Zealand White rabbits for acute percutaneous toxicity, primary skin irritation and ocular irritation. The acute dermal LD_{50} was greater than 2000 mg/kg bw, the maximum dose applied. The primary irritation score was zero after 500 g was applied to the skin for a 4-hour exposure period. After instillation of 100 mg into the conjunctival sac, transient mild conjunctivitis was observed at 24 hours and had completely resolved by 48 hours.

Repeated dose toxicity

Repeated-dose oral toxicity studies were carried out in rats and dogs. A toxicologically derived ADI of 10 µg/kg bw per day was calculated by applying a safety factor of 100 to the NOEL of 1 mg/kg bw per day, which was established based on effects on the articular cartilage in a 3-month repeated-dose study in immature dogs.

Reproductive toxicity, including teratogenicity

Reproductive toxicity studies were carried out in rats using oral administration. In a fertility and general reproductive performance study and a peri/post-natal study, no significant effects were found on reproductive performance, physical development, reflex responses or behaviour of the pups. There were adverse effects on maternal body weight gain and food consumption and foetal weight and pup body weight gain at 45 and/or 150 mg/kg bw. In a 3-generation study of reproductive performance, fertility was reduced at 100 mg/kg bw but not at 50 mg/kg bw.

Difloxacin was not teratogenic in rats or rabbits. In rats, administration of oral doses of 65 mg/kg bw to the dams caused reduced foetal weight and foetal delayed ossification. Oral administration of 75 mg/kg bw per day to pregnant rabbits caused severe maternal toxicity and consequent foetotoxicity.

Mutagenicity

Difloxacin was considered to be a non-genotoxic substance.

Carcinogenicity

Difloxacin was considered to be not carcinogenic.

Special studies

No specific data concerning the immunotoxicity of difloxacin were provided. The results of the repeated-dose studies in dogs and rodents revealed no haematological or histopathological changes indicative of an immunotoxic effect.

Difloxacin hydrochloride was not tested for skin sensitisation potential. However, negative results were obtained in studies with other fluoroquinolones.

Observations in humans

Difloxacin is not authorised for use in humans. Oral administration of difloxacin to male human volunteers at (single) doses ranging from 100 - 600 mg resulted in a low incidence of adverse reactions including headache, dizziness, disturbed sleep, nausea, vomiting and upset stomach. Some volunteers reported visual disturbances.

Microbiological studies

In vitro MIC data were provided for a range of micro-organisms which were representative of those found in the human gut. Based on these data, a microbiological ADI of 40.6 µg/kg bw per day was established.

Studies on metabolites, impurities, other substances and formulation

All of the excipients are well known substances.

User Safety

The active ingredient, difloxacin hydrochloride, was of slight to moderate acute oral toxicity. It was of low toxicity via the dermal route. It was not a skin or eye irritant. Potential inhalation toxicity was not investigated but user exposure *via* this route was negligible for the tablet formulations.

Difloxacin hydrochloride was not teratogenic. It was considered to be a non-genotoxic substance and was not carcinogenic.

Trials in adult human volunteers indicated that the adverse effects after ingestion of therapeutic doses of difloxacin were mild. A number of fluoroquinolones (but not difloxacin) have been authorised for human use. These are contraindicated for use in children due to the risk of arthropathy and in patients who have shown hypersensitivity to the quinolones.

Exposure to the user and other humans

The difloxacin hydrochloride is contained within the tablet core. As the tablets do not have a break-line and the use of half tablets is not indicated, user exposure to the active substance will be negligible.

Risk management proposals

The main route of user exposure will be dermal; from handling and administering the tablet. However, the nature of the product (the tablets are coated and the use of half tablets is not indicated) means that user contamination will be minimal. Consequently, no special precautions are needed for persons administering the product to animals.

The following phrase is included under Section 5.12 of the Summary of Product Characteristics: *Persons with known hypersensitivity to quinolones should avoid any contact with the product*. It was considered that no other precautions were warranted.

Ecotoxicity

Dicural Coated Tablets are used for individual animal medication. As the products are indicated for dogs and mass treatment is not to be expected, it was concluded that they did not form a potential risk to the environment. Consequently, according to the Phase I decision tree of the Note for Guidance of Environmental Risk Assessment for veterinary medicinal products (EMEA/CVMP/055/96) further assessment of the ecotoxicity was considered unnecessary.

EFFICACY ASSESSMENT

Pharmacodynamics

Antimicrobial activity

The MIC range and the MIC_{90} values for the bacteria, which have been adequately demonstrated as having susceptibility or intermediate susceptibility to difloxacin, are in the following table:

Isolate	**No.**	**Range (µg/ml)**	**MIC_{90} (µg/ml)**
Susceptible in vitro:			
Escherichia coli	35 19	0.031-8.0 0.031-8.0	0.125 0.125
Klebsiella spp.	10 23	0.031-8.0* 0.031-0.25	8.0* 0.125
Pasteurella spp.	20	0.008-0.125	0.031
Pseudomonas spp.	20	0.5-2.0	0.5
Staphylococcus intermedius	114 20	0.12-2.0 0.25-0.5	0.5 0.25
Intermediate susceptibility:			
Proteus spp.	16 20	>8.0 0.125-8.0	2.0 8.0**
Staphylococcus spp.	9 20	0.125-2.0 0.25-0.5	2.0 0.25
Streptococcus canis	17	0.25-4.0	2.0
Streptococcus spp.	20	1.0-2.0	2.0

* Just one of the 10 isolates had a MIC of 8 µg/ml; the MICs of the remaining 9 isolates were < 1 µg/ml (= susceptible)

** The MIC of 3 out of 20 isolates was equal to 8 µg/ml. The remaining 85 % of the isolates had a MIC ≤ 0.5 µg/ml.

With the exception of the MIC values for *E. coli* and *Staph. intermedius* (0.125 µg/ml and 0.5 µg/ml, respectively), values for other bacteria only suggest moderate susceptibility. The indications for skin infections (good susceptibility of *Staph. intermedius*) and urinary tract infections (good susceptibility of *E. coli* and intermediate susceptibility to *Staphylococcus spp.*) were therefore supported by these data.

Secondary pharmacological effects:
Studies investigating the effects of difloxacin on the cardiopulmonary system and on blood coagulation were performed, as effects on these systems have been reported in man, mice and rats.

An intravenous dose of 6.25 mg/kg in anaesthetised dogs induced a minor, compensated vasodilatation whereas a dose of 31.25 mg/kg induced an uncompensated vasodilatation. It was concluded that the recommended oral dose of 5 mg/kg was unlikely to induce any significant cardiopulmonary effects.

Studies conducted with doses higher than the recommended dose, on both normal and Factor VII deficient dogs, revealed no adverse effects.

Secondary pharmacological effects were, therefore, considered to be unlikely.

Pharmacokinetics

Difloxacin was rapidly absorbed and had a high bioavailability after oral administration of the recommended 5 mg/kg dose.

Plasma protein binding is low.

Difloxacin is well distributed into various tissues at 2 hours and there were high concentrations in the target tissues (skin and urinary tract) which remained above the MIC's of most clinically relevant pathogens for a large part of the day. High concentrations were found in the liver and urinary tract.

The maximum concentrations in plasma were 0.59 μg/ml at 0.5 hours, and 0.19 μg/ml at 12 hours. The concentrations in urine were very high up to 24 hours (13.85 μg/ml at 24 hours). The maximum concentrations in skin were 1.78 μg/ml at 3 hours, 0.32 μg/ml at 12 hours and 0.42 μg/ml at 24 hours.

The concentration of difloxacin in plasma and skin was compared with the MIC_{90} values for *E. coli* (0.125 μg/ml) and *Staph. intermedius* (0.5 μg/ml). At 12 hours the concentration in skin had fallen below the MIC_{90} for *Staph. intermedius* and had not risen above it by 24 hours. This was of concern as dosing is once daily, but was consistent with other findings.

Excretion was mainly via the bile into the faeces with some excretion via the urine.

Difloxacin was administered orally to dogs daily for one month in one study. As well as the recommended dose of 5 mg/kg, doses of 25 and 125 mg/kg were also administered, which represent X 5 and X 25 the recommended dose. Blood samples were taken at 1, 3, 6 and 12 hours after the treatment, on Days 1, 15 and 29. Levels of difloxacin in plasma were proportional to the dose given.

Long term studies demonstrated that there was no drug accumulation. The approximate steady state concentration occurred at 3 hours post administration. The estimated terminal phase half lives were independent of dose size and treatment duration.

Whilst the studies give a good indication of the pharmacokinetic properties and parameters, it was considered unfortunate that there was not a pharmacokinetic study with more frequent sampling times up to 24 hours, from which the pharmacokinetic parameters could be more accurately and confidently determined.

<u>Bioequivalence</u>

Bioequivalence studies were conducted according to current Guidelines with plain tablets, capsules and coated tablets. All 3 presentations of difloxacin appeared to be bioequivalent.

An *in vitro* dissolution test also showed bioequivalence between 3 plain tablet strengths and the 4 coated tablets.

It could, therefore, be concluded that studies conducted using the earlier formulations, gelatine capsules and plain tablets, were valid to support the final presentation of coated tablets.

Tolerance in the target species

The proposed dose for difloxacin in dogs was 5.0 mg/kg bodyweight daily for at least 5 days (up to a maximum of 21 days for superficial pyoderma).

A. Studies in young adult dogs

30 day tolerance study with difloxacin hydrochloride tablets in dogs:

Tablets containing difloxacin (as the hydrochloride) were administered for 30 consecutive days to three groups of 8 Beagles (9.5 - 11.5 months old) at dosages of 5 mg/kg, 15 mg/ kg and 25 mg/kg bodyweight. The test product was not the formulation to be marketed. A fourth group of similar animals received a placebo. It was unclear whether the dose was administered with food.

On two of the treatment days, a small number of animals in the difloxacin HCL treatment groups did not receive the full dose of tablets (three animals on day 27 and two animals on day 30).

The incidence of vomiting and diarrhoea (liquid and/or mucoid faeces) was slightly higher in the difloxacin treated groups than in the control animals. Vomit containing dose material was observed occasionally in all dose groups. One animal in the 25 mg/kg dose group developed a caecal intussusception during the study.

Lameness was noted in four animals during the study (one from the 15 mg/kg group and 3 from the 25 mg/kg group). The animal from the 15 mg/kg group was diagnosed as having soft tissue trauma from an unrelated incident. For the remaining three animals the lameness was not confirmed following examination by the veterinary surgeon.

There were no test-substance related effects on bodyweight. The significantly lower mean bodyweight seen for the 25 mg/kg group was attributed to the animal that developed the intussusception. Food consumption was reduced on some days for the 25 mg/kg and 15 mg/kg groups and on only one day for the 5 mg/kg group. The test substance did not appear to cause any consistent electrocardiographic or ocular abnormalities. No apparent effects were seen on the clinical pathology results.

Some differences in organ weights were seen but no differences were seen in these organs on macroscopic or microscopic examination and the differences in weights were considered to be incidental.

Some lesions were found in the tibial tarsal joints of some of the animals in the 5 mg/kg and 15 mg/kg treatment groups. However, lesions were also found in the female control group. No macroscopic joint lesions were found in the 25 mg/kg group. Following microscopic examination of these lesions it was concluded that these findings were not related to the administration of the test substance.

Ten day tolerance study with difloxacin hydrochloride tablets in young adult dogs:

Difloxacin hydrochloride tablets were administered orally to four adult Beagles (aged 9-11 months) once daily for 10 days, at a dose of 50 mg/kg bodyweight (10 x overdose). The test product was not the formulation to be marketed. The animals were observed twice daily for mortality and morbidity. Animals were also observed 1 hour post-dose for signs of poor health or abnormal behaviour. At specified time-points throughout the study, bodyweights, feed consumption, clinical pathology results (haematology, clinical chemistry, faecal analysis and urinalysis) were recorded. At the end of the treatment period the animals were euthanased and a full post-mortem examination was performed, including the examination of humeral heads, femoral heads (three sites) and tibial tarsal bones.

Faecal abnormalities (yellow/orange faeces) and vomiting were recorded. One female vomited nine times during the study. One animal showed excessive salivation immediately following dosing. Individual body weight loss during the dosing period ranged from 2.4 % to 14.0 %. Mean feed consumption showed a large variation between individual animals (three animals showed decreases of 12.5, 21.2 and 66.3 % respectively and one animal showed an increase of 9.2 %). At post-mortem granular-like particles/material was found within the gall bladder of 3 of the animals. It was stated that similar granular material (identified as difloxacin glucuronide) had been observed in previous studies. Gross lesions (thickened mucosa and raised foci) were also identified in the stomachs of two of the animals.

14 day oral toxicity study in young adult dogs:

In this study difloxacin hydrochloride was administered orally in gelatin capsules to groups of two Beagle dogs (11 months old). The dosages administered were 0, 20, 49, 122, 294 and 783 mg/kg bw/day for two weeks. One death occurred during the study (day 13). This animal (from the highest dose group) had had a convulsion on day 12. Convulsions also occurred in three other dogs (one from the 122 mg/kg and two from the 783 mg/kg groups). Spontaneous jerking movements also occurred in one animal in the 783 mg/kg group and ataxia was seen in one female in each of the 294 and 783 mg/kg groups. Shiver-like tremors were also seen in the 20, 294 and 783 mg/kg groups. Vomiting was seen in all difloxacin treatment groups and the three highest dose groups showed intermittent decreased activity, lacrimation, ptosis and salivation at various time points throughout the treatment period. Bodyweight loss and decreased feed intake were seen in the three highest dose groups. Bilirubinuria was observed in the 20, 122 and 294 mg/kg groups and fat droplets were found in the urine of one dog in the highest dose group. Stress induced haematological alterations were seen in one dog in the 783 mg/kg group. Increases in alanine aminotransferase (ALT) were seen in the three highest dose groups but increases in blood urea nitrogen and creatinine were only seen in the two highest dose groups. The highest dose group also showed evidence of liver toxicity: increases in total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), serum cholesterol and albumin. Disturbances in electrolytes were seen in the lowest and the highest dose groups. A granular biliary sediment containing difloxacin glucuronide was found in all difloxacin treatment groups. Pyelonephritis with tubular epithelial cell basophilia and single cell necrosis in the kidney was seen in the two highest dose groups.

One-month oral toxicity study in young adult dogs:

In this study difloxacin hydrochloride was administered orally in gelatin capsules to groups of eight Beagle dogs (9-12 months old). The dosages administered were 0, 5, 25 and 125 mg/kg bw given daily for one month. One dog in the 125 mg/kg group was found dead on day 17. This animal had progressive decreases in bodyweight and was observed to be hypothermic and inactive on the day prior to death. Most of the clinical signs were seen in the 125 mg/kg group. These included emesis, diarrhoea, convulsions and tremors, ataxia, ptosis, decreased activity and salivation. Diarrhoea and emesis also occurred sporadically in the other difloxacin treatment groups. Decreases in bodyweight and feed consumption also occurred in some animals in the highest dose group. Statistically significant electroretinographic changes were also seen in the highest dose group. It was considered that these changes may reflect some changes in the animals' visual ability. Changes were also seen in some animals in the other two dose groups but these were not statistically significant. Signs of renal and hepatic toxicity were also seen in the highest dose group.

Three-month oral toxicity study in young adult dogs:

In this study difloxacin hydrochloride was administered orally in gelatin capsules to groups of eight or fourteen Beagle dogs (9-12 months old). The dosages administered were 0, 5, 20 and 60 mg/kg bw given daily for 91-99 days. For six dogs each from the 0 and 60 mg/kg groups a recovery period of 32-34 days followed the treatment period. Increased incidences of emesis, ptosis, swelling of the ears, salivation and constricted pupils occurred in the highest dose group. No deaths or statistically significant changes in feed consumption or bodyweight occurred in the study.

Electroretinographic changes also occurred in the dogs in the two highest dose groups but no changes were seen following the one month recovery period. Indications of possible hepatotoxicity were seen in the highest dose group but again these changes were not seen following the one month recovery period. A crystalline biliary deposit was also found in the two highest dose groups at post mortem.

B. Studies in immature dogs

14 day oral toxicity study in immature dogs:

In this study difloxacin hydrochloride was administered orally in gelatin capsules to groups of two Beagle dogs (3.5 months old). The dosages administered were 0, 20, 49, 122, 294 and 783 mg/kg bw given daily for 14-15 days. Both dogs from the 294 mg/kg group became moribund and were euthanased during the study (days 8 and 11 respectively) and both dogs from the 783 mg/kg group died following convulsions (days 4 and 5 respectively). Convulsions and ataxia were seen in the two highest dose groups, jerks were seen in the 294 mg/kg group and shiver-like tremors were seen in the 122, 294 and 783 mg/kg dose groups. Vomiting was seen during the first week in all difloxacin treatment groups. Rear leg stiffness, weakness or pain was seen in the 122 and 294 mg/kg groups but flattening of the front feet to radial-carpal joint was seen in the 20, 122 and 294 mg/kg groups. Body weight loss occurred in the three highest dose groups and decrease feed intake occurred in the 122 and 294 mg/kg groups. Increases in ALT and cholesterol were also seen in the 122 mg/kg group. At post mortem bone marrow hypoplasia was seen in the highest dose group and arthropathy was identified in all dose groups except the 20 mg/kg group.

13-week oral toxicity study in immature dogs with a 4-week recovery period:

In this study difloxacin hydrochloride was administered orally in gelatin capsules to groups of twelve or sixteen Beagle dogs (3.5-3.8 months old). The dosages administered were 0, 5, 25, 35, 50 and 125 mg/kg bw given daily for 13 weeks. For four dogs each from the 0 and 125 mg/kg groups a recovery period of 4 weeks followed the treatment period. Test material related observations seen in all difloxacin treatment groups included periorbital swelling, red skin, red hair coat, elevated third eyelid and swelling of the ears, muzzle or conjunctiva. The incidence of vomiting increased at doses over 35 mg/kg and at 125 mg/kg twitching, tremors, convulsions, dehydration, weakness and recumbancy were observed. These observations were not present during recovery (with the exception of red skin in one animal). Decreases in bodyweight occurred for animals in the two highest dose groups and feed consumption was frequently lower in all the difloxacin treatment groups than for the controls. Carpal flattening was seen as a dose related effect in the 25, 35, 50 and 125 mg/kg groups. One animal in the 5 mg/kg group showed subtle carpal flattening. Intermittent lameness was observed in the two highest dose groups. Lower hocks were noted in animals given dosed of 35, 50 and 125 mg/kg. It is stated that there was no correlation between the flattening of the carpus and the microscopic changes recorded at post-mortem. Lower total protein and globulin values were found in all difloxacin treatment groups, except the 5 mg/kg group. The clinical pathology showed signs of hepatotoxicity in the two highest dose groups.

Test substance related effects were seen at post-mortem in the articular cartilage of the femur, proximal tibia, distal radius and carpal region. These changes occurred principally in the two highest dose groups but similar changes did occur in all difloxacin treatment groups. Hepatic biliary hyperplasia was also noted in the 35, 50 and 125 mg/kg groups.

13-week oral toxicity study in immature dogs:

In this study difloxacin hydrochloride was administered orally in gelatin capsules to groups of eight Beagle dogs (3-4 months old). The dosages administered were 0, 0.3, 1.0 and 3.0 mg/kg bw given daily for 13 weeks. Two dogs in the highest dose group showed subtle bilateral carpal flattening during the study, however, at post-mortem no macroscopic or microscopic abnormalities related to the test substance were seen in the joints.

Summary of target species tolerance studies:

None of the products used in any of the target species tolerance studies were the same as the formulation to be marketed. In two studies difloxacin hydrochloride tablets were used which were not the same as the proposed product "Dicural". In the other studies the active ingredient was administered in gelatin capsules. The Guidelines (Evaluation of the safety of veterinary medicinal products for the target animals) state that the product to be evaluated must be identical to the product to be marketed i.e. same chemical, same particle size and same formulation. As the “plain” tablets and capsules used in the tolerance studies were demonstrated to be bioequivalent to the final product to be marketed, that is coated tablets, the concentrations of active ingredient in plasma, organs and tissues will be similar.

As useful information regarding the safety of the active ingredient in the target species was also provided, it was concluded that sufficient reassurance on tolerance was provided. Furthermore, as the products are already marketed in some EU Member States pharmacovigilance data could be examined. No suspected adverse reactions were reported from approximately 150,000 treated animals.

However, there were several areas of concern regarding difloxacin hydrochloride in dogs. Those of greatest concern included the occurrence of joint abnormalities in young dogs, vomiting and convulsions, ataxia and tremors. Renal and hepatic toxicity (including the occurrence of biliary crystalline deposits) appear to only occur at very high doses. Retinal lesions were noted at higher doses (4-5 X the recommended dosage, up to 90 days) from electro-retinographic examinations.

It was noted that the CNS related signs (i.e. convulsions, ataxia and tremors) only seemed to occur at doses greatly in excess of the recommended dose. Facial swelling was reported in some dogs but the data appeared to demonstrate that this was not directly related to the administration of the product, and the low incidence of this justified the absence of a warning on the product literature.

Joint problems were seen in dogs given the recommended dose. All of the target species tolerance studies were performed in Beagle dogs, which are not representative of large or giant breeds in which the rapid growth phase may be considerably longer. The SPC therefore, contained a contra-indication for the use of this product during the rapid growth phase: in small and medium breeds up to and including 8 months of age, in large breeds up to one year of age and in giant breeds up to 18 months.

Vomiting and some loss of appetite at the recommended dose level were noted. In the SPC, therefore, it also stated that clinical signs recorded in the field trials included inappetence, emesis, diarrhoea and anal irritation, although the signs were self-limiting within one or two days and did not require additional treatment.

As no data were provided on the safety of this product in pregnant or lactating bitches or in male stud dogs, a contra-indication was included under section 5.6 of the SPC for the use of this product in pregnant or lactating bitches and in male stud dogs.

Resistance

The mechanism of resistance of the fluoroquinolones was discussed and some studies in which the mechanism of resistance was investigated were presented. However, these studies were considered to provide insufficient information due to shortcomings in the data such as: data on human isolates, a different fluoroquinolone was used, there were an unknown number of isolates, the studies were too old.

These products were marketed in several Member States (via the Decentralised Procedure), and additional study data were provided on the difloxacin sensitivities of recent canine isolates. These data demonstrated that in general the resistance of canine isolates was low (less than 5 %). *Streptococcus spp*. showed intermediate susceptibility (64-70 % susceptible). Additional susceptibility data were then provided from more contemporary studies and these demonstrated that no substantial increase in resistance of relevant pathogens had taken place from the early 1990s.

The Committee took note of published information on increasing problems caused by fluoroquinolone resistance in human and veterinary medicine but concluded that no other restrictions were necessary other than the inclusion of the following statement in the SPC "*Dicural Coated Tablets should only be used based on susceptibility testing.*"

Clinical Studies

Dicural 15 mg, 50 mg, 100 mg, and 150 mg tablets contain difloxacin (as hydrochloride) and are intended for oral administration to dogs. The proposed dosage was 5.0 mg/kg bodyweight per day for at least 5 days, up to a maximum of 21 days. Treatment was indicated for the following clinical conditions:

- Acute uncomplicated urinary tract infections caused by *Escherichia coli* or *Staphylococcus spp*.
- Superficial pyoderma caused by *Staphylococcus intermedius.*

<u>Urinary Tract Infections</u>

Dose Confirmation Study:

The therapeutic dose of 5 mg difloxacin/kg per day on 7 consecutive days was investigated in 36 Beagle dogs (1.5 – 3 years) in a blinded, GLP compliant study in the US. Dogs were infected artificially with *E. coli* (canine cystitis strain from American Type Culture Collection, Rockville, MD) infused into the bladder via a urinary catheter. Treatment was initiated 8 days after infection with dogs receiving difloxacin HCl as tablets (n=12) or capsules (n=12) or placebo tablets.

The clinical status of the dogs was examined daily and urine and blood samples were investigated on days 0 (prior to inoculation), 7, 12, 15 and 19.

Two animals in Group 1 (difloxacin tablets) and one in Group 2 (difloxacin capsules) died or were euthanased on humane grounds prior to the initiation of treatment. There were no statistically significant differences in *E. coli* urinary levels among the three groups 7 days after inoculation. The geometric mean *E. coli* count was significantly lower in both the tablet and capsule groups as compared to the control group on days 12 and 15 (= treatment days 5 and 7) but continued to be lower only in the tablet group on day 19 (= 4 days after the last treatment). The geometric mean *E. coli* count was significantly lower in the tablet group than in the capsule group on day 19. On day 15 (= last day of treatment), 0 of 10, 1 of 11 and 7 of 12 dogs were positive for *E. coli* in the urine in the tablet, capsule and placebo groups, respectively. On day 19, 0 of 10, 4 of 11 and 3 of 12 in the tablet, capsule and placebo groups respectively were positive.

Although the antibacterial efficacy of both tablets and capsules was demonstrated, the clinical significance was not evaluated and the effectiveness of treatment as opposed to no treatment was not conclusively demonstrated. However, the Committee was of the opinion that low levels of bacteria after treatment would not necessarily be indicative of therapeutic failure and that both tablets and capsules would be likely to have significant therapeutic effects when compared to no treatment. However, it could be argued that the 4 out of the 11 dogs treated with the capsule formulation which were positive for *E. coli* in the urine on day 19 were relapsed cases and that the claim for efficacy in the treatment of cystitis has not been adequately substantiated.

Clinical Field Trials

Field trials have been performed in the United States and two countries in the European Union.

US Clinical Field Trial:

A randomised, blinded multicentre study was performed in 1992, in 18 veterinary practices, in 31 dogs of various breeds and age groups (9 months - 16 years) with lower urinary tract infections. Nineteen dogs were treated with difloxacin (5 mg/kg, once daily) and 19 dogs (=positive control) received enrofloxacin (2.5 mg/kg, twice daily). Depending on the severity of the symptoms, animals were treated for 5 to 10 days. Urine samples were obtained for bacterial culture and sensitivity testing pre-treatment and after 5 days of treatment. Treatment continued for a maximum of 10 days, if the second culture obtained was positive or clinical signs persisted. After the last day of treatment another bacterial culture was taken to determine if pathogens had been eliminated. Clinical evaluation was based on bacterial and clinical status (resolved – not resolved – relapsed).

All clinical cases in the enrofloxacin group (n=12) and 18 out of 19 cases of the difloxacin group (95%) were resolved. However, the results of this study were considered unsatisfactory as only a small number of cases were included and the number of bacterial isolates derived from this trial was insufficient to justify the claims for efficacy in respect of the individual pathogens.

European Clinical Field Trial:

A multicentre study (non-blinded, uncontrolled) was performed in 1995, in three university veterinary clinics in three different EU member states (n=1, n=9 and n=18 respectively) and in one veterinary clinic in a fourth European Union member state (n=1). A total of twenty nine dogs of various breeds and age groups (10 months - 14 years) with lower urinary tract infections were included.

All dogs were treated with difloxacin (5 mg/kg, once daily) for 5 to 14 days depending on the severity of the symptoms. Dogs were investigated at the beginning of treatment (clinical parameters; radiography, haematology) and 5 days after treatment. Urine samples were obtained for bacterial culture and sensitivity testing pre-treatment and after 5-7 days of treatment. 3-5 days after the last treatment another urine sample was taken to determine if pathogens had been eliminated.

All dogs investigated in three of the four EU member states (n=20) were cured (100% total clinical cure) while the total cure rate of dogs investigated in another (n=9) was 66.7 % (partial cure: 22.2%). The results of this study were considered unsatisfactory as the number of clinical cases in the study (n = 29) was too few and the levels of efficacy which were achieved were inconsistent (total cure rate of only 66.7 % at one trial site).

As the data did not support the indications satisfactorily, the Applicant provided additional trial data from two EU member states.

EU Member state code ‘A’ field trial:

In the field trial in EU member state ‘A’, the efficacy of combined use of difloxacin as injectable solution and tablets in urinary tract infections was investigated. However, for clinical purposes the injection may be regarded as bio-equivalent to the tablet formulation. Enrofloxacin injectable solution and tablets were used as a positive control. There was no negative control group. In cases of uncomplicated urinary tract infections, 7 dogs were treated with difloxacin and 8 with enrofloxacin (5 mg/kg bw/day). Duration of treatment was at least 5 days, but not more than 10 days. A case was considered as cured once clinical signs had resolved and bacteruria was absent. Cure rates were 85.7 % for difloxacin and 87.5 % for enrofloxacin. *E. coli* was the predominant pathogen (8 out of 14 cases).

The outcome of the study indicated that cure rates were likely to be comparable for both difloxacin and enrofloxacin. Only acute cases were included. Treated animals were predominantly males. Except for acute cases, urinary tract infections in males tend to be more complicated. Within this limitation, this study offers a serious hint (but no unequivocal, independent proof) of efficacy in acute cases.

EU Member state code 'B' field trial:

The trial in EU member state 'B' had a similar experimental design as the one in EU member state 'A'. The major difference was the reference product used; a combination product of amoxicillin and clavulanic acid instead of enrofloxacin. The dosage of amoxicillin/clavulanic acid was 12.5 mg/kg body weight every 12 hours. Another important difference was that only tablets were used and treatment was not started with an injection.

34 dogs were treated with Dicural Tablets (5 mg/kg bw/day). 7 animals were withdrawn during the experiment. Most clinical cases were acute infections (cystitis, prostatitis) and occasionally a case of chronic cystitis was also treated. Other types of urinary tract infection did not occur in this trial. Inclusion criteria were clinical symptoms, confirmed by bacteriological assay. The causative pathogens isolated during the trial were: *E. coli* (51 %), *Staphylococcus spp*. (18 %), *Pseudomonas spp.* (12 %) and *Streptococcus spp.* (9 %). The average treatment duration was 18 days. Efficacy was assessed on the basis of clinical scores and bacterial assay of the urine.

In both groups the clinical score decreased strongly during treatment and was virtually normal at the end of treatment. On the basis of clinical scores the cure rates were 89 % for Dicural and 73 % for amoxicillin/clavulanic acid. In addition, in both groups 7 % of the animals were designated as "highly improved". On the basis of bacteriological assay cure rates were 72 % and 53 %, respectively.

The outcome of the study indicated that cure rates were likely to be comparable for both difloxacin and amoxicillin/clavulanic acid. Mostly acute cases were included in the trial. Unfortunately, in 40 % of the animals no bacteria could be isolated from the urine at the start of the trial, and so these animals did not meet the inclusion criteria. Furthermore, the results from these animals were not analysed separately from those of the animals in which urinary tract infection was confirmed by bacterial assay. Similarly, the results from chronic cases were not presented separately from those of acute cases. Moreover, the number of chronic cases was low (7 out of 27). However, despite these drawbacks, the results of the acute cases, that were confirmed by bacterial assays, offered a serious hint (but no unequivocal, independent proof) of efficacy of difloxacin (similar or even better efficacy than amoxicillin/clavulanic acid) in acute infections caused by *E. coli* and *Staphylococcus spp*.

As these results were further supported by the pre-clinical data (high concentrations of difloxacin in e.g. the kidneys and urine and the relatively high susceptibility of the causative pathogens), it was concluded that, overall, sufficient data were provided to substantiate the claim.

Superficial Pyoderma

Dose Titration Study:

The dose range of difloxacin was investigated in a randomised, blinded study in the US in 1990. Dogs (n=48) of various breeds were infected artificially with *E. coli* and *K. pneumoniae* by subcutaneous injection. Treatment with difloxacin HCl as tablets was initiated 8 hours after infection in 4 groups of dogs (n=12) at a dosage of 2.5 mg/kg, 5 mg/kg, 7.5 mg/kg or with placebo tablets.

Blood samples were taken one day prior to treatment and on days 1, 3, 6, 9, and 13 of treatment. Aspirations from artificially-created skin lesions were taken on days 1, 2, 3, 6 and 9. A post mortem was undertaken on day 13. Evaluation parameters included scoring of wound infection and clinical appearance as well as haematological and bacterial investigations (including MIC determination).

The cultural results showed a statistically significant improvement in Groups 3 (5 mg/kg) and 4 (7.5 mg/kg) compared with Groups 1 (placebo) and 2 (2.5 mg/kg). There were no statistical differences between Groups 3 and 4. At post mortem, Groups 3 and 4 were significantly less affected by infection than Groups 1 and 2.

However, the suitability of this model for the general evaluation of antibacterial activity is questionable. The clinical evolution of the lesions did not differ markedly between the treated and untreated control groups. The strain of *K. pneumoniae* which was used in the study was more sensitive to difloxacin than field strains (MIC 0.13 μg/ml v. 8 μg/ml).

Dose Confirmation Study:

A dose confirmation study was carried out using a canine skin infection model and protocol similar to that described above in the dose titration study. Twenty four dogs of mixed breeds were used and randomly allocated to two treatment groups receiving either 5 mg/kg difloxacin or a placebo. The treatments were blinded.

Analysis of the clinical responses on days 5 and 6 post treatment demonstrated that a dosage of 5 mg difloxacin/kg bodyweight significantly reduced clinical scores at these days. The bacterial contamination of the lesions in the group receiving difloxacin was significantly less than in the placebo group.

Since contaminated wounds are at a critical equilibrium at the 5th and 6th days, the approach adopted by the Applicant in respect of the confirmation of dose was regarded as reasonable.

Clinical Field Trials

US Clinical Field Trial:

A randomised, blinded multicentre study was performed in 1992, in 18 veterinary practices, in 56 dogs of various breeds and ages (9 months - 16 years) with soft tissue infections and skin lesions. Thirty dogs were treated with difloxacin (5 mg/kg, once daily) and 26 dogs (= positive control) received enrofloxacin (2.5 mg/kg, twice daily).

Dogs were treated for 5 days and then another examination was performed. If lesions had not regressed, a new sample for bacteriology was taken and treatment continued for another 5 days. Clinical evaluation was based on bacterial and clinical status (resolved – partial response – no response (after 5 days) and after 10 days (resolution, - incomplete resolution - relapse). Bacteriological investigation included pathogens eliminated from dermal wound infections based on the second or, if necessary, final examinations.

Twenty-seven out of 30 clinical cases (90%) in the difloxacin group and 25 out of 26 (96%) in the enrofloxacin group were resolved. However, the results of this study were considered unsatisfactory as only a small number (n=30) of cases were tested and the number of bacterial isolates derived from this trial was insufficient to justify the claims for efficacy in respect of two of the pathogens listed in the SPC: *E. coli* and *Klebsiella pneumoniae*. Furthermore, the level of efficacy which difloxacin achieved against one of the pathogens listed in the SPC, i.e. *Staphylococcus intermedius* was equivalent to that achieved by enrofloxacin. It was considered that this evidence provided limited support for this indication.

As both dose titration studies investigated infected wounds and not superficial pyoderma and the field trials provided limited evidence of efficacy, additional data were then provided by the Applicant, from the field trials in EU member states ‘A’ and ‘B’.

EU Member state code ‘A’ field trial:

In a field trial in EU member state ‘A’, the efficacy of combined use of Dicural 5 % Injectable Solution and Dicural tablets in bacterial skin infections was investigated. However, for clinical purposes the injection may be regarded as bio-equivalent to the tablet formulation. Enrofloxacin (injectable solution and tablets) were used as a positive control. There was no negative control group.

In the case of bacterial skin diseases, referred to as 'pyoderma, diagnosed on general clinical disease symptoms', 16 dogs were treated with difloxacin and 16 dogs with enrofloxacin. Duration of treatment was at least 10 days, but not more than 20 days. A case was considered as cured if clinical signs and skin lesions had resolved. Cure rates were 75 % for both difloxacin and enrofloxacin. *Staphylococcus spp.* was the predominant pathogen (22 out of 36 isolates).

Regarding the scores for clinical symptoms on Day 1 of the trial, the number of animals really suffering from a skin disease was considered to be low. Moreover, the inclusion criteria were broad and cases were not classified as either superficial or deep. However, treatment did reduce the number of positive bacterial isolates. The outcome of the study indicated that cure rates were comparable for both antibacterial products.

EU Member state code 'B' field trial:

A trial in EU member state 'B' had a similar experimental design as the one in EU member state 'A'. The major difference was the reference product used: a combination of amoxicillin and clavulanic acid (12.5 mg/kg body weight every 12 hours), instead of enrofloxacin. Another important difference was that only tablets were used and the treatment was not started with an injection.

Twenty six dogs were treated with Dicural Tablets and 31 with amoxicillin/clavulanic acid. The inclusion criteria were clinical symptoms confirmed by bacterial assay. The main criterion of efficacy was the lesion score (presence/absence of lesions, size of lesions). Animals were treated for superficial or deep pyodermatitis. The primary indication was "secondary folliculitis"; a type of superficial pyodermatitis. There were various other types of skin and soft tissue but their numbers were low. For Dicural the average treatment duration was 28 days. In 86 % of the animals *Staphylococcus spp.* were isolated. In both groups there was a gradual but steady decline of the lesion score and the lesion size. On the basis of lesion scores the cure rates for superficial pyoderma were 85.3 % for Dicural and 64.7 % for amoxicillin/clavulanic acid ("cured" = no lesions at the end of treatment).

At the beginning of treatment the average lesion score was 8-10 on a scale of 0 to 24, meaning that, on average, the lesions were not very extensive in size and number (mild to moderate lesions). Given this fact, the treatment duration may be regarded as rather long (28 days, on average). On the other hand, cure rates were high, and efficacy of Dicural was similar to, or even better than, that of amoxicillin/clavulanic acid.

It was therefore concluded that the clinical data from the trial in EU member state 'B' provided adequate proof of efficacy in cases of superficial pyoderma caused by *Staphylococcus spp.* and that the data from the trial in EU member state 'A' provides further supportive evidence.

RISK-BENEFIT ASSESSMENT AND CONCLUSION

Based on the original and supplementary data presented, the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were in accordance with the requirements of Council Directive 81/852/EEC and supported the claims for simple uncomplicated urinary tract infections caused by *Escherichia coli* and *Staphylococcus spp.*, and superficial pyoderma caused by *Staphylococcus intermedius*.

IV DICURAL 50 MG/ML SOLUTION FOR INJECTION FOR CATTLE AND DOGS

QUALITY ASSESSMENT

Dicural 50 mg/ml solution for injection for cattle and dogs contains difloxacin hydrochloride as the active substance. It is a clear yellowish solution containing 50 mg/ml difloxacin for subcutaneous administration. The solution is packaged in amber coloured glass vials of 50 ml (cattle and dogs), 100 ml (cattle only) or 250 ml (cattle only), fitted with a bromobutyl rubber stopper and an aluminium overseal.

Composition of the veterinary medicinal product

Dicural 50 mg/ml solution for injection for cattle and dogs is presented in packs/containers of 50 ml, 100 ml and 250 ml. The composition of the product is detailed below:

Labelled composition per ml:	**Quantity**	**Unit:**	**Reference standards**[**)]
Active ingredient			
Difloxacin (as hydrochloride)	50.0[*)]	mg	M.S.
Excipients			
Benzyl alcohol	50.0	mg	Ph. Eur.
Other Excipients			
Water for injections	q.s. ad 1.0	ml	Ph. Eur.
[*)] Difloxacin HCl is used in the formulation, equivalent to 50.0 mg difloxacin base, taking into account water content and salt form.			
[**)] M.S: Manufacturers Specification ; Ph. Eur: European Pharmacopoeia			

Container

The product is to be packed in amber coloured glass vials of 50, 100 or 250 ml with bromobutyl rubber stoppers and aluminium overseals. The vials are packed in a carton outer box. The suitability of the stoppers, in terms of leaching, fragmentation and self-sealability characteristics, using suitably sensitive techniques was not addressed adequately in the original dossier. Therefore a commitment from the Applicant to repeat the experiments on leaching using Dicural 50 mg/ml Solution for Injection for cattle and dogs was requested. Fragmentation and self-sealability data were presented for a type of stopper which is closely related to the proposed stopper. The data demonstrated that this stopper could withstand 15 punctures using a 21-gauge needle. The Applicant has now committed to present similar data for the proposed stopper. *Post-authorisation note: These data have since been provided and assessed as satisfactory.*

Taking into account the recommended dosage regimen, it has been estimated that for dog, the 100 and 250 ml vial will allow the treatment of a considerable number of animals. In order therefore to reduce the number of punctures per stopper for dogs, the only vial to be used should be the 50 ml one. The applicant has been advised to develop a more appropriate vial size, e.g. 10 ml for dogs.

Clinical trial formulations

The composition of the batches used in the clinical trial is identical to the above-mentioned composition.

Product Development Studies

Pre-formulation studies with difloxacin HCl have shown that the solubility of difloxacin HCl is pH-dependent. Lowering the pH results in a decrease of the solubility (at pH 5.5 the solubility is 0.8 mg/ml; at pH 9.7 this is 11.6 mg/ml).
Three of the excipients are used as co-solvents. The quantities have been chosen in a way that difloxacin is completely dissolved at a pH as low as possible, with minimum tissue irritation and no separation. The pH of this formulation is between 9.2 - 10.0. This is above the physiological pH and because of the buffering capacity this product could theoretically cause tissue irritation. However, according to the data submitted, it seems that the product is not really harmful to the tissue at the injection site.

Benzyl alcohol in this formulation also acts as preservative. The preservative efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs was determined in accordance with the test on "efficacy of anti-microbial preservation" in the Ph.Eur. III, 5.1.3. The results indicate that Dicural 50 mg/ml solution for injection for cattle and dogs meets the A-requirements for bacteria as well as fungi and yeast for parenteral preparations.

Amber coloured type I glass vials are used to package the solution since difloxacin HCl is sensitive to light.

Since difloxacin HCl and the other ingredients are not sensitive to heat the solution is sterilised in the final container by autoclaving (20 min. 121°C; saturated steam). Batch results show that no degradation occurs as a consequence of this sterilisation method.

DESCRIPTION OF METHOD OF PREPARATION

Difloxacin is used in the formulation equivalent to 5 kg base per 100 litre, taking into account moisture and salt form. The actual batch size of Dicural 50 mg/ml solution for injection for cattle and dogs will be multiples or sub-multiples of 100 L. The batch size will vary between 500 and 5000 L.

Difloxacin HCl and one of the excipients are initially suspended in 80% of the required water for injections. Thereafter, the other ingredients are subsequently added while stirring. After complete dissolution the pH is checked and adjusted if necessary with hydrochloric acid or potassium hydroxide. The solution is then made up to volume with water for injections. The solution is filtered through a 0.2 µm PVDF filter before filling into vials. The vials are closed with bromobutyl rubber stoppers and aluminium overseals. Finally the product is autoclaved (121°C, 20 min.). These conditions conform to Ph.Eur.

During the production process a batch record is kept of each production batch. This contains a detailed description of each production step. The following in-process controls are performed:
- Control of weighing process;
- Control of the formation of a homogeneous suspension;
- Control of the dissolution process;
- Control of the pH and density;
- Control of filling volume;
- Control of the sterilisation process.

Requirements are stated. The product is a true solution. Therefore, visual inspection on the homogeneity is considered appropriate. Filter integrity has been tested appropriately by the bubble point testing method.

The applicant has adapted the in-process control for filling volume to +0 to +4 % of the declared volume.

Validation of the process

The ruggedness of the production process is investigated on pilot production batches of 200 and 600 L scale. During pilot production the critical steps in the production process are investigated such as: dissolution profile of difloxacin, pH and absorption to the filter. Stirring for 60 minutes is sufficient to dissolve difloxacin. The pH is within the required range and after filtration of 3 L no absorption of difloxacin or alcohol onto the filter occurs. During the production process parameters such as temperature and stirring rate were varied. It was found that these variations had no significant influence on the final product. Therefore the production process is considered to be robust in the batch size range from 200 to 600 L. It has not been demonstrated that the manufacture of a batch of 5000 L (maximum proposed batch size) is robust. The applicant has committed to submit validation data on full-scale batches in due time post-approval. *Post-authorisation note: These data have since been provided and assessed as satisfactory.*

CONTROL OF STARTING MATERIALS

Active substance

The Applicant initially referred to two suppliers for Difloxacin HCl. During the assessment, one of these suppliers was withdrawn. Therefore, in this report, reference is made only to the supplier finally retained by the applicant, i.e. Profarmaco. The applicant's part of the Drug Master File (DMF) is submitted in the dossier.

Details of the identity, manufacturing site, synthesis and control of the active ingredient are provided. A flow chart, details of the batch size and full details of the specifications and control methods for the starting material, reagents, catalysts and solvents are provided, with certificates of analysis. Specifications and control methods are also provided for the intermediate. Evidence of structure data are provided from a variety of techniques and the physico-chemical properties of difloxacin hydrochloride are described.

The difloxacin hydrochloride purity is determined by HPLC and a description of the method is given. This is validated for linearity, precision, specificity and the stability indicating nature of the assay.

Appropriate validation data are provided for all the other assay methods used in the active ingredient specification, and details of the reference standards are provided.

The results of three batch analyses are given. The batches comply with the specifications.

Other Ingredients

All excipients comply with the requirements described in the current versions of the respective monographs.
Certificates of analysis for one batch of each of the excipients and auxiliary materials are provided.

Packaging material

The glass vials (type I) and the bromobutyl rubber stoppers are in compliance with the monograph in the Ph.Eur. III, 3.2.1. and 3.2.9.
Drawings of the vials, rubber stoppers and the aluminium overseal are provided. The applicant has committed to provide Certificates of Analysis for the glass vials, including results on light transmittance. *Post-authorisation note: These data have since been provided and assessed as satisfactory.*

CONTROL TESTS CARRIED OUT AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

Not applicable.

CONTROL TESTS OF THE FINISHED PRODUCT

Specification and routine testing

The Finished Product Specifications (FPSs) agreed include Release and Check (shelf-life) limits which are considered sufficient to assure consistent quality of the finished product.

Identity:
Identity Tests for difloxacin (2), benzyl alcohol and other relevant excipients are provided.

Assays:
Validated methods for the assay of difloxacin, benzyl alcohol and the other relevant excipients have been provided

Purity Tests:
Also for checking the purity the HPLC-method used for the assay of Difloxacin is used. The selectivity of this method for the potentially occurring impurities at release or during shelf life is demonstrated. A representative HPLC-chromatogram is provided.

Microbiological Tests:
Sterility: sterile.
For sterility testing the membrane filtration method according to Ph.Eur (III, 2.6.1) is used with modifications with regard to the amount of test sample (5 ml/test), the filter (0.45 Duropore filter), the rinsing and dilution fluid (Na_4EDTA and increased volume) and the speed of filtration. The Applicant has demonstrated that in terms of the detection of bacteria these modifications are necessary in order to optimise, as far as is practical, the test sensitivity.

Pharmaceutical Technical Tests:
Density and pH are tested according to the test methods of the Ph.Eur.
Fill volume is tested according to the method of the USP monograph on “deliverable volume”

Scientific data

Three pilot batches of the product with Profarmaco Difloxacin-HCl were manufactured at Fort Dodge Animal Health, Weesp. The batch size was 200 (2x) and 600 litre. The batch results indicated that all three batches complied with the finished product specifications**.** Batch results of batches manufactured at the Vall de Bianya site were missing. The applicant has committed to provide those in due time post-approval.

During stability studies a test for Bacterial Endotoxins is performed. Given the route of administration (subcutaneous) such a limit is not necessary.

STABILITY

Stability studies on active substance

For the stability data the applicant refers to the DMF. *Claim:* 5 years

Studies
Samples from a total of 6 batches manufactured by Profarmaco have been stored at ambient temperature/ ambient humidity, 30°C/ ambient humidity, 40°C/ ambient humidity and 40°C/75% RH. The samples have been stored for a period of time up to 18 months. The study at ambient temperature has been continued up to 5 years for 4 batches and 3 years for 2 batches.

Parameters tested, methods used
Content difloxacin, content impurities, moisture and colour. Specifications and control methods as described in Control of Starting Materials/Active Substance, above.

Discussion of results
The results of the manufacturer are all within the proposed specifications. Although the stability study is performed for only 18 months under controlled conditions, the proposed re-test period of 5 years could be accepted because the results of the long term stability study under uncontrolled conditions show no significant changes in the physical and chemical character.

Stability tests on the finished product

Claim: 2 years at temperatures below 25°C

Studies
Three pilot production batches have been placed on stability. The batches were manufactured at Solvay Pharmaceutical facility in Weesp (now: Fort Dodge Animal Health Holland) with active substance manufactured by Profarmaco.

Table. Information on relevant batches in the stability trials.

batch no.	strength	man. site	% full scale	ASM	Packag-ing	conditions	storage time
DF00101	50 mg/ml	SolvayPharm Weesp	4-40 %	Profarmaco	A	a & b	12 & 6
DF00200			4-40%			a & b	12 & 6
DF00300			12-120%			a & b	12 & 6

A = amber coloured type I glass multiple dose vials (50, 100 and 250 ml), bromobutyl rubber stopper and aluminium overseal
a = 25°C/60% RH
b = 40°C/75%RH

Parameters tested, methods and validation and shelf-life specifications
Same as finished product. Additionally tests on Bacterial Endotoxins, the degree of coloration (NMCT BY2) and a preservative efficacy (Ph.Eur.) are performed in the stability study. A limit and test method for Bacterial Endotoxins is lacking. However on basis of the route of administration (subcutaneous) this parameter is not necessary.

Results and discussion
After 24 months at 25°C and 6 months at 40°C all parameters comply with the proposed requirements. Results for the sterility, bacterial endotoxins and the preservative efficacy test are missing. The preservative efficacy and the sterility at the end of the 3-years storage period should be demonstrated.

As the vials of one batch are stored upright as well as upside down and no differences in results were observed between these vials, there appears to be no interaction between the difloxacin solution for injection and the rubber stopper. Based on the results the limit for the sum of impurities has been tightened to 1.0%. The requirement for the content of difloxacin has been tightened to 95-105% as release specification because no degradation appears. However leaching of ingredients from the stoppers should still be examined during storage.

The applicant has committed to conduct a study to demonstrate that the formulation in contact with the product will not give rise to unacceptable levels of extractives being present in the product. *Post-authorisation note: These data have since been provided and assessed as satisfactory.*

The claimed shelf life is acceptable on the basis of the submitted stability study.

According to the stability results for visual appearance of the reference samples stored at 5°C, omission of the label claim "do not refrigerate" is justified. As no stability studies have been performed at freezer conditions, an additional label claim "do not freeze" is stated. Stability results of three production batches should be provided after authorisation.

In-use stability
An in-use stability study was performed on 2 pilot production batches. The two batches were manufactured at the Solvay Pharmaceuticals facility in Weesp and packed in 50 ml, 100 ml and 250 ml vials from which 25% and 75% of the volume was removed. After that, the vials were placed in the stability study for 3 years. The difloxacin solution for injection was stored under the following condition: 25°C/60% RH. The stability was investigated by determination of the same parameters as stated above.

Claim: 13 weeks at 25°C and ambient humidity. According to the Note for Guidance on in-use stability the in-use shelf life should normally be no longer than 28 days.

Results
Only interim results of the 13 weeks time point are available. After 13 weeks all parameters comply with the requirements, but since the applicant did not provide any antimicrobial preservative efficacy data in broached vials, let alone the special repeat challenge antimicrobial studies which would be required if an in-use shelf-life beyond 28 days was considered, the in-use shelf life is now restricted to 28 days.

SAFETY ASSESSMENT

Pharmacodynamics

The main issues addressed in this section are the potential adverse effects of difloxacin. A large number of study reports have been included in the dossier of Dicural coated tablets for dogs (EMEA/V/031/02-05/0).

The effects of difloxacin on spontaneous motor activity, the cardiovascular system (heart rate, blood pressure) and on blood clotting have been investigated in several animal species (e.g. mice, rats, and monkeys). Difloxacin was administered by various routes (intravenous, intraduodenal, oral).

In monkeys a single, intraduodenal dose of 20 mg/kg bw produced no significant changes in heart rate and blood pressure. Similarly, single oral doses of difloxacin from 10 to 1000 mg/kg bw had no significant effect on blood pressure of rats; however doses from 100 to 1000 mg/kg bw increased the heart rate of these animals whilst doses of 10 and 30 mg/kg bw had no effect on heart rate.

There were no effects on blood coagulation in monkeys, rats, rabbits and other rodents, cats and dogs.

Effects on dogs have been investigated in four studies. When a single dose of 6.25 mg/kg bw was administered intravenously to dogs, 15-30 min after administration minor effects on blood pressure heart rate and vascular resistance were noticed. The dose administered led to concentrations of 3-4 µg difloxacin per ml plasma. A dose of 31.25 mg/kg bw gave rise to a significant decrease of blood pressure and vascular resistance. Single and multiple oral doses from 10 to 600 mg difloxacin per kg bw did not significantly affect prothrombin time in Beagles.

It was concluded that, at the concentrations expected to be achieved in the clinical situation, no adverse effects of difloxacin on blood pressure, heart rate and blood coagulation were noted in various species. Potential side-effects have been identified and investigated sufficiently.

Pharmacokinetics

The relative bioavailability of Dicural coated tablets and Dicural 50 mg/ml solution for injection for cattle and dogs has been investigated in dogs. A single dose of each product (5 mg/kg bw) was administered orally or subcutaneously, depending on the product formulation, to 6 male and 6 female Beagles following a crossover study design. Based on AUCs, the bioavailability of the solution for injection appeared to be about 20% higher than the bioavailability of the tablet. However, C_{max} (1313 ng/ml for the solution for injection, 1342 ng/ml for the tablets), T_{max} (3.1 hours for the solution and 3.7 hours for the tablet) and $t_{1/2}$ (5.8 hours) of both formulations were not significantly different. At 24 hours after administration the concentration of difloxacin in plasma was 210 and 123 ng/ml for the solution for injection in males and females and 205 and 102 ng/ml for the tablets in males and females. A slight amount of accumulation is expected to occur after repeated administration; this accumulation is not expected to cause any adverse effects. Although the bioavailability of the solution for injection was higher than that of the tablet, the plasma profiles were very similar. For safety and efficacy purposes both formulations may be regarded as bioequivalent. No information on concentrations of difloxacin in tissues and organs was provided.

A summary report on the data has also been provided concerning the kinetics of difloxacin in general and of the final product in cattle. In cattle the same trends appear as in dogs, illustrated by significant bioavailability (nearly 90%) and volume of distribution of ±2.5 l/kg. Also, a slight amount of accumulation of active substance is noted. However, given the proposed duration of treatment (for 5 days) this is not expected to produce a risk for the animals involved. No information on concentrations of difloxacin in tissues and organs is provided.

Single dose toxicity

The dossier of Dicural 50 mg/ml solution for injection for cattle and dogs contains no data on acute toxicity of difloxacin, but a reference is made to the safety expert report of the dossier of Dicural coated tablets (EMEA/V/V/031/02-05/0) for dogs in which data on the acute toxicity after oral administration to rats and mice are discussed.

The toxicological studies with difloxacin hydrochloride were also reviewed in the Assessment Report for the MRL application for difloxacin in poultry and are referenced in the original application for Dicural Oral Solution in poultry. The main findings are summarised below.

The acute toxicity of difloxacin hydrochloride administered as a suspension in 0.2% hydroxypropylmethylcellulose was investigated as follows:

Species	Strain	Sex	Acute oral LD50 (mg/kg bw)
Mouse	ICR	male	1380
		female	1600
Rat	Sprague-Dawley	male	5510
		female	6270

Signs of toxicity included reduced activity, ataxia, strabismus, dyspnoea, tremors and decreased bodyweight.

Difloxacin hydrochloride was tested in New Zealand White rabbits for acute percutaneous toxicity, primary skin irritation and ocular irritation. The acute dermal LD50 was greater than 2000 mg/kg bw, the maximum dose applied. The primary irritation score was zero after 500 g was applied to the skin for a 4-hour exposure period. After instillation of 100 mg into the conjunctival sac, transient mild conjunctivitis was observed at 24 hours and had completely resolved by 48 hours.
Symptoms of intoxication have been identified and a safety margin in relation to these symptoms can be estimated. These data, in combination with the results of the target animal tolerance studies were considered to be sufficient to support the safe use of difloxacin in cattle and dogs.

Repeated dose toxicity

The dossier of Dicural 50 mg/ml solution for injection for cattle and dogs contains no data on repeated dose toxicity. For the data on (sub)chronic toxicity reference is made to the Dicural coated tablets dossier.

Repeated-dose oral toxicity studies were carried out in rats and dogs.

Several studies were performed in rats, including one month and three month studies. It is concluded that the oral NOEL in rats for difloxacin hydrochloride is at least 50 mg/kg bw/day. Specific target organs for the drug are difficult to indicate; relatively high concentrations were found in the liver. Observed side-effects at lower dosages were vomiting, tremors, decreased activity, ptosis, salivation, loss of body weight and sialodacryoadenitis, and at dosages above 225 mg/kg bw/day convulsions and ataxia.

For dogs, a dose-range study in young dogs of 11 months old in which difloxacin was administered during 14 consecutive days, demonstrated a NOEL of 20 mg/kg bw/day for this species claimed by the applicant. However, since at this dosage some toxic effects still remained (in particular bilirubinemia), the NOEL is considered less than 20 mg/kg bw/day. In a 28-day study in young adult dogs a NOEL of 25 mg/kg bw/day was demonstrated, whereas in a 90-day study in young adult dogs no toxic effect occurred at 20 mg/kg bw/day. As in the rat, no specific target organs could be identified.

In a 90-day study, 3 groups of 4 male and 4 female Beagle dogs were administered doses of 0, 0.1, 1 or 3 mg/kg bw/day. In this study, marginal effects were observed at the 3 mg/kg bw dose level and consequently, a toxicologically derived ADI of 10 µg/kg bw per day was calculated by applying a safety factor of 100 to the NOEL of 1 mg/kg bw per day, which was established based on the observed effects on articular cartilage in immature dogs.

For dogs the potential side-effects have been identified, those most frequently observed include vomiting and deformation of the cartilage of the carpal joint (puppies). A safety margin can be estimated from the above data. Furthermore, from the pharmacokinetic section of the dossier it is known that for Dicural the oral route and the parenteral route of administration may be regarded as bioequivalent in dogs and sufficient tolerance data on the target species haven been provided. Therefore, it is concluded that sufficient data on the toxicity of difloxacin after repeated administration were provided.

Tolerance in the target species

Cattle:

Systemic tolerance:

One study has been submitted to investigate the systemic tolerance in five groups of calves aged from 6 to 9 months.

Group number	Dose (mg/kg/day)	Route	Duration of treatment	Day of necropsy
1	0 (saline)	i.m.	15 days	D16
2	5	i.m.	15 days	D16
3	15	i.v.	15 days	D16
4	25	i.v.	15 days	D16
5	50	i.v.	5 days	D6

At the recommended dosage the use of Dicural 50 mg/ml solution for injection for cattle and dogs is not likely to cause any serious systemic side-effects in calves. Some unsteadiness may occur, especially after prolonged treatment. In most cases minor injection site reactions were observed, but occasionally more severe reactions have been seen. Examination of the knee joint showed some swelling and oedema, without any abnormalities of the cartilage.
At overdose CNS symptoms, such as ataxia, unsteadiness, shaking, tremors, twitching occur.

Approximately 200 calves aged between 2 and 4 weeks old at the start of treatment have been treated. There was no indication of arthropathy. This is the most reassurance that can be given, although the joints were not specifically monitored.

Local tolerance:

Dicural 50 mg/ml solution for injection for cattle and dogs was injected intramuscularly or subcutaneously for 5 consecutive days. During the treatment the usual clinical parameters were assessed (body temperature, heart rate, respiratory rate, growth, food consumption blood and urine composition, etc.). Animals were killed 1 or 32 days after the last injection. At necropsy the injection sites were investigated.

There were no significant, adverse effects as measured by the clinical parameters. The main side-effects were transient injection site reactions. Severity and size were dependent on the actual site (i.e. deep intramuscular injection caused more severe reactions than injection in the neck). In the period from D6 to D33 recovery of muscular tissue took place; scar tissue remained present. Generally, the size of the injection site reactions was small (2x2x2 cm), but occasionally larger ones occurred (50-400 cm3). Also, at overdoses the injection site reactions were more severe. Finally, swelling and oedema formation in the knee joints were also observed in calves.

Dogs:

Systemic tolerance:

Reference is made to 4 toxicity studies of difloxacin after repeated administration to dogs. Although informative, they do not contain information on the tolerance of dogs towards the product. In addition, two references concerning the tolerance of dogs towards difloxacin tablets have been provided. From these studies it can be concluded that the maximum tolerated dosage of tablets in mature dogs lies between 25 and 50 mg/kg bw/day.

In general, Dicural at the proposed dose level is well tolerated. The main side-effect is the occurrence of joint lesions in immature dogs (reported in the previous section - repeated dose toxicity). The lesions may even occur at the proposed dosage. The use of Dicural during the rapid growth phase of dogs is therefore contra-indicated.

Occasionally vomiting may occur, but mainly at higher doses (3-5 times the proposed dose). At very high overdoses adverse reactions of the CNS have been noticed (ataxia, tremors, convulsions, etc.). At recommended dosage these CNS symptoms are not likely to occur.

Remarks on bio-equivalence of the various tablets, capsules and solution for injection:

Based on the conclusion of the assessment of the dossier "Dicural coated tablets", the plain tablets, the capsules and the tablets (Dicural coated tablets) may be regarded as bio-equivalent. Similarly, it was shown that for the evaluation of safety and efficacy Dicural coated tablets and Dicural 50 mg/ml solution for injection for cattle and dogs may be regarded as bio-equivalent. Therefore, the results of the toxicity studies using plain tablets (tolerance) and capsules (repeated dose toxicity) are also applicable to Dicural 50 mg/ml solution for injection for cattle and dogs. Similarly, the results of the repeated dose toxicity studies and the tolerance studies may be also regarded as applicable to Dicural 50 mg/ml solution for injection for cattle and dogs. Local effects of oral and parenteral formulations will not be the same.

Local tolerance:

The applicant has provided a recent study on the local tolerance of dogs towards Dicural 50 mg/ml solution for injection for cattle and dogs. Daily doses of difloxacin were administered subcutaneously to Beagles (10 months old, 10 kg bw). Dosage: 0 (saline), 5 or 15 mg/kg bw/day during 5 consecutive days. For each dosage 2 male and 2 female dogs were used. One or 8 days after treatment 1 male and 1 female animal of each dose group were killed and necropsied. Both macroscopic and microscopic post-mortem examinations were performed. During treatment all animals were observed for clinical signs of intolerance (behaviour, appetite, injection site reactions, etc.); body weights and food consumption were recorded regularly.

No changes in the clinical parameters were observed. Growth and food uptake of treated animals were similar to those of animals in the control group. However, in 100% of the difloxacin-treated animals there were injection site reactions. Generally, these reactions were transient, and disappeared within 2 days after administration of 5 mg/kg bw. The size of the largest injection site reaction was 10 x 50 cm. The extent (haemorrhage, inflammation, oedema, necrosis) and the size of the injection site reactions appeared to be dose-dependent. Microscopic examination of injection sites revealed that recovery of the tissue took about 12 days.

In conclusion, although the numbers of animals were small, local side-effects will occur when the product is used as recommended in the SPC; the main effect being the occurrence of injection site reactions. Macroscopic swelling, sometimes in combination with pruritic reactions, will occur. Microscopic haemorrhage, inflammation, oedema and necrosis may also be observed. However, the injection site reactions are transient. The results of this study are confirmed by the results of the monitoring of adverse reactions in the clinical efficacy trials.

Reproductive effects, including teratogenicity

The dossier of Dicural 50 mg/ml solution for injection for cattle and dogs contains no data on this subject. The Dicural coated tablets dossier contains several reports on maternal and foetal toxicity of difloxacin.

Reproductive toxicity studies were carried out in rats using oral administration. In a fertility and general reproductive performance study and a peri/post-natal study, no significant effects were found on reproductive performance, physical development, reflex responses or behaviour of the pups. There were adverse effects on maternal body weight gain and food consumption and foetal weight and pup body weight gain at 45 and/or 150 mg/kg bw. In a 3-generation study of reproductive performance in rats, fertility was reduced at 100 mg/kg bw but not at 50 mg/kg bw.

Difloxacin was not teratogenic in rats or rabbits. In rats, administration of oral doses of 65 mg/kg bw to the dams caused reduced foetal weight and foetal delayed ossification. The NOEL for foetoxicity was 15 mg/kg bw/day. Oral administration of 75 mg/kg bw per day to pregnant rabbits caused severe maternal toxicity and consequent foetotoxicity. No side-effects were seen at an oral dose of 35 mg/kg bw/day.

Although considerable data concerning reproductive toxicity, including teratogenicity, was provided, it was not possible to come to a final assessment of this issue and therefore a warning that the safety in pregnant, breeding or lactating animals has not been established will be included in the SPC, labelling and package inserts.

Mutagenicity

Difloxacin is considered to be a non-genotoxic substance.

Carcinogenicity

Difloxacin was not carcinogenic in 2-year studies in rats and mice.

Immunotoxicity

No specific data concerning the immunotoxicity of difloxacin were provided. The results of the repeated-dose studies in dogs and rodents revealed no haematological or histopathological changes indicative of an immunotoxic effect.

In a Magnusson and Kligman maximisation test in guinea pigs, difloxacin did not induce delayed contact hypersensitivity.

Observations in humans

Difloxacin is not authorised for administration to humans. Oral administration of difloxacin to male human volunteers at (single) doses ranging from 100 - 600 mg resulted in a low incidence of adverse reactions including headache, dizziness, disturbed sleep, nausea, vomiting and upset stomach. Some volunteers reported visual disturbances. Prothrombin time was significantly increased in comparison with controls, but not by more than 2 seconds above the normal range.

Microbiological studies

In vitro MIC data were provided for a range of micro-organisms which were representative of those found in the human gut. Based on these data, a microbiological ADI of 40.6 µg/kg bw per day was established. Because the microbiologically-derived ADI was higher than the toxicologically-derived ADI, the latter was used for the derivation of MRLs for difloxacin in cattle.

User Safety

The active ingredient difloxacin hydrochloride demonstrated slight to moderate acute oral and percutaneous toxicity. It was not an eye or skin irritant and did not induce delayed contact hypersensitivity. It was not teratogenic. It was considered to be a non-genotoxic substance and was not carcinogenic.

A number of fluoroquinolones (but not difloxacin) have been authorised for human use. These are contraindicated for the use in children due to the risk of arthropathy and in patients who have shown hypersensitivity to the quinolones.

Other relevant product characteristics:
- Flammability: there is no risk of flammability from the formulated product.
- pH of the product: The product Dicural 50 mg/ml solution for injection for cattle and dogs contains L-arginine base as a buffer. The pH of the final solution is 9.2-10.0. This is needed to ensure a sufficient solubility of the active ingredient difloxacin.

Exposure to the user and other humans

Dicural 50 mg/ml solution for injection for cattle and dogs is a solution intended for subcutaneous administration by a veterinary surgeon for animals under his/her supervision. The human exposure is limited to accidental dermal exposure to the solution during transfer of the liquid from the bottle to the hypodermic syringe or leakage of the hypodermic syringe during injection, or accidental self injection. The likelihood of exposure is low since this product is intended for the individual treatment by an experienced person.

Dermal exposure

The active ingredient difloxacin is not irritating to the skin. No information was provided on the acute dermal toxicity, the irritating effects to the skin or the sensitising potential of the excipients However, having considered the acute oral toxicity, the percentage difloxacin in Dicural 50 mg/ml solution for injection for cattle and dogs and the excipients, the CVMP considers that Dicural 50 mg/ml solution for injection for cattle and dogs is unlikely to be harmful following acute dermal exposure.

Accidental self injection

No observations in humans with the product Dicural 50 mg/ml solution for injection for cattle and dogs were recorded. There were however some data with respect to effects in humans after oral exposure to the active ingredient difloxacin. A small increase in prothrombin time was found in human volunteers after administration of 100 to 600 mg of the active ingredient difloxacin. Assuming comparable bioavailability after oral and parenteral administration, an amount of 2 ml needs to be injected in a 60 kg person to reach this effect. Accidental self-injection of this amount is unlikely but cannot be ruled out completely. This risk is considered acceptable because of the low likelihood of self-injection of this amount and the nature of the effect.

Acute toxicity after parenteral administration was not investigated. Acute toxic effects (mortality) were seen in mice after oral administration of the active ingredient difloxacin with an LD_{50} of approximately 1.5 m/kg bw (this study was included in the dossier and evaluated, but not reported in this assessment report, as the oral route is considered not relevant for the person administering the product). Assuming comparable bio-availability after oral and parenteral administration, an amount of more than one litre needs to be injected in a 60 kg person to reach a lethal dose. No information was provided on the acute parental toxicity of the excipients. Necrosis and acute inflammatory reaction were induced at the site of injection in target animals after intramuscular injection of 5 to 10 ml of the product Dicural 50 mg/ml solution for injection for cattle and dogs. A similar response can be expected after accidental self-injection. The severity of the reaction after accidental self-injection will probably be lower because the volume introduced after accidental injection will probably be lower than 5 ml. The local reaction is likely to heal but this was not shown. Nevertheless, this risk is considered acceptable because of the low likelihood of self-injection and the expected reversibility of the effect.

Risk management proposals

The following phrase is included under section 5.12 of the Summary of Product Characteristics: *"Persons with known hypersensitivity to quinolones should avoid any contact with the product"*. It is considered that no other precautions are warranted.

Ecotoxicity

Cattle

An environmental risk assessment has been provided by the applicant, where it is stated that according to the Phase I decision tree for environmental risk assessment, Dicural 50 mg/ml solution for injection for cattle and dogs has to be exempted from further testing since the product is intended for "individual treatment for a small number of animals", and that therefore a further environmental risk assessment is not required.

The opinion of the Committee was that a phase II environmental risk assessment would be required if the PEC trigger values for soil or groundwater are exceeded, unless adequate justification can be provided that the product is for use in individual animals only.

Phase I Calculations have been made with a worst case scenario and lead to the following results:[1]

Table i.: calculated maximal concentrations.

	PIECslurry [$mg_c.kg_{wwt}^{-1}$]	PIECsoil arable land [$\mu g_c.kg_{soil}^{-1}$]	PIECsoil grassland [$\mu g_c.kg_{soil}^{-1}$]	PIECgw arable land [$\mu g_c.l^{-1}$]	PIECgw grassland [$\mu g_c.l^{-1}$]
dairy cow	**0.73**	**19**	**-**	**0.63**	**-**
suckler cow	**0.90**	**19**	**-**	**0.63**	**-**
beef cattle	**1.6**	**32**	**-**	**1.1**	**-**
veal calf	**2.9**	**35**	**180**	**1.2**	**6.1**
fattening pig	**3.1**	**20**	**65**	**0.67**	**2.2**
breeding sow	**2.8**	**13**	**33**	**0.42**	**1.1**
trigger value:	***0.1***	***10***	***10***	***0.1***	***0.1***

Table ii. Calculated maximal concentrations for grazing animals.

	PECdung [$mg_c.kg_{wwt}^{-1}$]	PIECsoil grazing [$\mu g_c.kg_{soil}^{-1}$]	PIECgw grazing [$\mu g_c.l^{-1}$]
beef cattle	**1600**	4.8	**0.16**
suckler cow	**1600**	4.8	**0.16**
dairy cow	**4100**	7.1	**0.24**
trigger value:	***0.01***	*10*	***0.1***

The tabulated figures are based on the assumption that all animals are treated. If it is assumed that for respiratory diseases 30-50% of the animals are treated, then a reduction of the values in the table by a factor of 2-3 is reached. The resulting figures are then well within the range established for chickens and turkeys during the assessment of Dicural oral solution and for which a phase II assessment was already done. For this product the environmental impact was considered acceptable (given the commitment of the applicant to submit additional data in relation to the effects on seedling growth, upon renewal of the registration of Dicural Oral Solution). The same commitment is applicable for Dicural 50 mg/ml solution for injection for cattle and dogs. Post-authorisation note: These data have

[1] As laid down in EMEA/CVMP/055/96, Note for Guidance: Environmental risk assessment for veterinary medicinal products other than GMO containing and immunological products.

since been provided and assessed as satisfactory.

Dogs

According to the Phase I decision tree[1] for environmental risk assessment, no environmental assessment is needed for companion animals.

RESIDUES

Pharmacokinetic studies - *Cattle*

A cross-over evaluation of the pharmacokinetics of Dicural 50 mg/ml solution for injection for cattle and dogs and oral bolus following intravenous, intramuscular, subcutaneous and oral administration to cattle was submitted with the MRL application for difloxacin HCl.

Four groups of two male and two female cattle (cross-bred, approximately 4-8 months of age, bodyweight 230-270 kg) were given Dicural 50 mg/ml solution for injection for cattle and dogs (Dicural, containing 50 mg difloxacin/ml) by intravenous (iv) injection in the right jugular vein, subcutaneous (sc) injection under the skin of the left side of the neck, intramuscular (i.m) injection in the right side of the neck, and an oral bolus containing 1.7 g difloxacin/bolus, all at a single dose of 5 mg difloxacin/kg bw.

The washout period between the four sessions was two weeks. Plasma samples were taken from the jugular vein at a number of time points up to 36 h (iv), 48 h (i.m, sc) and 72 h (oral) post administration. Difloxacin concentrations in plasma were determined using an HPLC method.
Following intravenous administration, difloxacin was rapidly eliminated from plasma (monoexponentional). The mean plasma concentration declined from 12182 µg/L at 2 minutes post dose via 3761 µg/L at 1 hour to 56 µg/L at 36 hours post dose. $T_{1/2\ el}$ was estimated to approximately 6.5 h. Subcutaneous administration of difloxacin resulted in a mean measured peak plasma level of 1417 µg/L at 6 hours post administration, declining via 81.2 µg/L at 36 hours to 45.6 µg/L at 48 h post dose. $T_{1/2\ el}$ was estimated to approximately 7.65 hours. The bioavailability, as calculated from $AUC_{0-\infty}$ comparisons, was 66% after oral administration, 88% after subcutaneous administration, and 95% after intramuscular administration.

Another study describing the pharmacokinetics of difloxacin in cattle following intramuscular and subcutaneous administration of Dicural 50 mg/ml solution for injection for cattle and dogs once daily for five consecutive days was submitted. Eighteen healthy cattle (cross-bred, 9 males, 9 females, approximately 4 months of age, bodyweight 150-185 kg) received Dicural 50 mg/ml solution for injection for cattle and dogs (Dicural, containing 50.1 mg/difloxacin/ml) at a dose of 5 mg/kg bw, on each of five consecutive days by either the intramuscular route (n=6) or the subcutaneous route (n=12) of administration in alternate sides of the neck (left-right-left-etc.). Blood samples were collected from the jugular vein on a number of time points during the treatment period up to 48 hours after the last administration. Difloxacin concentrations were determined in plasma using an HPLC method. Swelling of the subcutaneous injection sites was observed in 10 animals after the final dose. Difloxacin was rapidly absorbed from the intramuscular injection site and quickly eliminated from plasma after both the subcutaneous and intramuscular route. A slight accumulation was observed over a dosing period of five days. Using the subcutaneous route, C_{max} after the first injection was 1397 [illegible] and 1951 µg/L after the last one. After 5 days (7.7-8.2 h at day 5 and 6.3-6.6 h at day 1).

The dose proportionality of Difloxacin 50mg/ml solution for injection in cattle following intravenous administration was also investigated. In a three-way cross-over study, groups of 2 male and 2 female cattle (approximately 4 months of age, bodyweight 160-190 kg) received difloxacin 50 mg/ml solution for injection (Dicural, containing 50.1 mg difloxacin/ml) by intravenous injection at doses of 2.5, 5 and 10 mg difloxacin/kg bw. The washout period between the treatments was one week. Blood samples were collected from the jugular vein at several time points up to 36 hours post administration. Difloxacin concentrations in plasma were determined using an HPLC method.

From the pharmacokinetic parameters, the degree of proportionality (expressed as β; β=1: dose proportionality; β=0: no dose proportionality) was quantified. The plasma concentrations of difloxacin showed a dose-response relationship, as indicated by the pharmacokinetic parameters. The data revealed that the plasma kinetics in cattle were proportional with the dose.

Estimate for dose-proportionality for difloxacin after intravenous administration to cattle at a dose of 2.5, 5 and 10 mg/kg bw :

Parameter	β	Lower 95% confidence limit for β	Upper 95% confidence limit for β
$AUC_{0-\infty}$	1.01	0.93	1.09
AUC_{0-24}	1.00	0.93	1.07
C_{max}	0.97	0.78	1.17

Residue depletion studies - *Cattle*

The total drug related residues, excretion and metabolic profiles after subcutaneous and intramuscular administration were investigated in a radiolabel study in which cattle were dosed with [^{14}C]-difloxacin formulated as the commercial solution at the recommended dose of 5 mg/kg bw/day for 5 consecutive days. More than 80% of the radioactivity was excreted in the faeces and less than 10 % was excreted in the urine within 14 days after both routes of administration.

Highest residues were found at the injection site. Residues at the injection site following subcutaneous dosing were somewhat higher than after intramuscular dosing and were still 5 to 6 mg/kg at 28 days after treatment. The total radioactive residues in the other tissues were comparable after intramuscular and subcutaneous administration, being highest in the liver, followed by kidney. Lowest levels were found in muscle and fat. At day 3 after treatment the levels of total radioactivity in liver, kidney, muscle and fat were (intramuscular/subcutaneous route) 1.19/1.06, 0.53/0.44, 0.27/0.130, and 0.10/0.09 mg/kg, respectively, whereas for the parent compound values of 0.84/0.73, 0.42/0.35, 0.25/0.13 and 0.06/0.12 mg/kg, respectively, were found.

Determinations of the parent compound revealed that the approximate ratio parent:total residues in liver was declining from 90% at 12 hours to 50% at 14 days. In kidney, the ratio also declined from 90% at 12 hours to 70% at 14 days. In fat the ratio was around 60% (although one exceptional value of 138% was found) without an apparent decline. In muscle (including injection sites) all radioactivity was attributed to the parent compound. At three days after treatment the parent compound represented 70, 80, 100 and 60% of the total residues in liver, kidney, muscle and fat, respectively.

The metabolite sarafloxacin was identified in some liver and kidney samples, as well as in urine. The N-oxide of difloxacin was identified in urine and exceptionally in fat. Relatively low levels of unknown metabolites were found in urine, liver, kidney, muscle, injection sites and fat.

The residue depletion of the parent compound difloxacin was studied in cattle in two cold residue studies using both proposed routes of administration. The commercial formulation was administered at the recommended dose of 5 mg difloxacin/kg bw/day for 5 consecutive days. The results of the determinations of the residue concentrations in edible tissues were consistent and comparable with those obtained in the radiometric studies.

However, it was noted that the administration of the product in these studies did not reflect the situation in practice. The last dose was given at a site remote from the first four injections, and the last dose was given in two administrations at two different sites (residues were studied in samples of tissue that was injected with only half of the final dose volume).

The radiolabel study showed that much higher residues were found at the site where the first four injections were given, compared to the residues at the final injection site, indicating that multiple injection sites contain higher residue levels than single injection sites. It is therefore concluded that the residue concentrations in injection sites measured in this study are much lower than the residues that will occur in practice.

For the routine determination of difloxacin in edible tissues of cattle) an HPLC method is proposed. This method has been well described in accordance with ISO standard 78/2. The validated LOQ's are 25 µg/kg for bovine liver and muscle, and 104 µg/kg in bovine kidney and fat.

Elaboration of the MRL

For difloxacin a toxicological ADI of 10 µg/kg bw (600 µg for a 60 kg person) was established, which was calculated from the overall NOEL of 1 mg/kg bw/day (based on effects on articular cartilage in immature dogs) and a safety factor of 100. The toxicological ADI was lower than the microbiological ADI based on effects on the human gut flora, which was set at 40.6 µg/kg bw.

The Committee recommended the inclusion of difloxacin for bovine and porcine in Annex III 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
Difloxacin	Difloxacin	Bovine	400 µg/kg 100 µg/kg 1400 µg/kg 800 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.1.2001 Not for use in animals from which milk is produced for human consumption
		Porcine	400 µg/kg 100 µg/kg 800 µg/kg 800 µg/kg	Muscle Skin + fat Liver Kidney	Provisional MRLs expire on 1.1.2001

The MRLs are considered provisional because the proposed routine analytical method was not fully validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community, with respect to the specificity. The Committee concluded that additional information on possible interference by other substances is required.

Withdrawal period

Cattle, subcutaneous administration:

Considering the cold residue studies in cattle, it is clear that the concentrations of difloxacin in edible tissues were below the MRLs at 7 days after the final injection. However, the residues in the injection sites remained above the MRL for muscle for quite a long period after treatment. (The residue concentrations are still above the MRL for muscle of 400 µg/kg at 28 days after treatment). Therefore, according to the current approach of the EU, the withdrawal period has to be based on the residue depletion data in injection site samples, using the MRL for muscle.

Taken into account that the SPC recommends that injections should be given at different sites, results obtained in the cold residue depletion have been considered. Using the statistical approach recommended by the CVMP, the withdrawal time has been established to 46 days. The proposed withdrawal period of 46 days is also compatible with the results of the depletion of residues at the first four (left) injection sites in the radiometric study.

The SPC also indicates that the maximum injection volume should not exceed 7 ml per injection site, which is compatible with the volumes used in the residue studies.

Routine analytical method for the detection of residues

Difloxacin was analysed by high performance liquid chromatography with fluorescence detection. The analytical method for the detection of residue has been validated in cattle liver, muscle, kidney and fat. All tissues are homogenised in NaOH. Then acetonitrile, TCA (to neutralise) and citrate buffer are added. To fat samples hexane is added. All other tissues are centrifuged twice. Finally all tissues are centrifuged prior to HPLC analysis. Samples are analysed with reversed phase HPLC coupled with fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 440 nm). For liver tissues gradient elution is applied.

Validation of the method

Bovine tissues:

specificity: Specificity is insufficiently guaranteed. In the report it is shown that peaks of other quinolones (danofloxacin, enrofloxacin, orbifloxacin, marbofloxacin and sarafloxacin) and some metabolites (N-oxide difloxacin, N-acetyl difloxacin and 3-oxo difloxacin) do not interfere with the peak of difloxacin. However the chromatographic conditions of this assay are not given. Moreover, the conditions for liver are different from the conditions of other tissues.
The applicant has to take into account the different chromatographic conditions for liver and all other tissues.

accuracy: Determined as relative recovery out of 6 measurements (samples) per concentration level in the range 25 (- 104 - 1040) - 3400 µg/kg:
liver: between 98% and 92%
muscle: between 92% and 83%
kidney: between 114%* and 88%
fat: between 114%* and 104%
* At 25 µg/kg the accuracy for kidney and fat is too high (114%). Therefore the lowest concentration in these matrices at which accuracy is acceptable, is 104 µg/kg.

precision: For all tissues in the range 25 (- 104 - 1040) - 3400 µg/kg: < 13%

limit of quantitation: Considering accuracy and precision: 25 µ g/kg in liver and muscle; and 104 µg/kg in kidney and fat.

limit of detection: Determined from 20 blanks: 10 µg/kg for all tissues.

practicability: The method is practicable, since conventional commercially available chemicals and equipment are used and the method can be performed safely by trained analysts.

applicability: The linearity of the method is demonstrated for all tissues with coefficients of correlation> 0.998.

susceptibility: Representative chromatograms (blanks and spiked samples) are shown.
The stability of storage of samples during the assay is determined and the change in difloxacin concentration turned out to be < 4%.

Conclusions on the analytical method:
This method has been well described in accordance with ISO standard 78/2. The validated limits of quantitation are 25 µg/kg for bovine liver and muscle, and 104 µg/kg in bovine kidney and fat.

EFFICACY ASSESSMENT

Pharmacodynamics

Difloxacin inhibits the enzyme DNA gyrase.

Many studies containing susceptibility data, both from the USA and from various European countries are presented in the dossier. In the following paragraphs for each target species an overview of susceptibility of relevant pathogens will be presented.

General remarks:
MIC values are in µg/ml; criteria for susceptibility are:

MIC ≤ 1 µg/ml:	susceptible
MIC=1-2 µg/ml:	intermediate
MIC ≤ 4 µg/ml:	resistant

Dogs

Pathogen	MIC_{90} (USA)	MIC_{90} (EU)	
Staph. intermedius	0.50	0.25	susceptible
	1.0		
E. coli	0.12	0.125	susceptible
	0.25		
Klebsiella pneumoniae	0.25	8.0	susceptible
	0.5		
Proteus spp.	2.0	1.0	(intermediate)
	1.0		susceptible
Pseudomonas spp.	0.25	2.0	(intermediate)
			susceptible

Remarks

- The MIC_{90} of European isolates of *Klebsilella pneumoniae* is 8.0 µg/ml. Of the 5 isolates 4 were susceptible to difloxacin; only one had a MIC of 8 µg/ml (MIC_{50}=0.125 µg/ml). Given this fact and given the USA results (MIC_{90}= 0.25-0.5 µg/ml), it may be assumed that this particular pathogen is generally susceptible to difloxacin.
- The data on susceptibility of *Pseudomonas* spp. are very limited. The European MIC_{90} is based on 14 replicates of one single isolate. The USA MIC_{90} is based on 5 isolates. Therefore a firm conclusion on the susceptibility of *Pseudomonas* spp. is not possible. From the very few results in the dossier it seems that this pathogen is intermediately susceptible.
- There was no significant difference in susceptibility of isolates of one pathogenic species from various sites of the body (e.g. *E. coli* isolated from the skin, respiratory system and urinary tract).
- There was no significant difference in susceptibility patterns between various geographical locations in the USA.
- Results of USA susceptibility studies and European studies are comparable (with reference to order of magnitude of MICs and to trends in the development of susceptibility).

New data in relation to the susceptibility to Difloxacin of *Pseudomonas spp,* and *Klebsiella spp,* relevant from canine tissues, have been submitted later on.

The most recent data can be summarised as follows:

	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Escherichia coli	0,062	0,125
Klebsiella spp.	**0,125**	**0,125**
Pasteurella spp.	0,031	0,031
Proteus spp.	0,5	8*
Pseudomonas spp.	0,5	0,5
Staphylococcus intermedius	***0,25***	***0,25***
Streptococcus spp.	***1,0***	***1,0***

*85% of the isolates had a MIC ≤0,5 µg/ml. (i.e. were susceptible to difloxacin)
The 142 isolates originated from skin, ear, and wound infections (34%), from urinary tract infections (42%), from genital infections (7%), and from respiratory tract infections (17%) in dogs.

These MIC values were compared with those obtained between 1992 and 1994 in Europe (52 isolates) MIC_{50} and MIC_{90} values of 1998 are very similar to those of 1992-1994, suggesting that no change in susceptibility of relevant pathogens has taken place.

The applicant also referred to the Dicural coated tablets dossier, which contains additional susceptibility data of 548 isolates (1996-97-98). These isolates were sampled from diseased dogs (and cats) from a country in the EU. The pathogens were isolated from dogs (ca. 85%) and cats (ca. 15%) affected by skin and wound infections, and infections of the uro-genital and respiratory tract. The susceptibility of the isolates was determined by Disk Agar Diffusion Test (DADT). It was demonstrated that from 1996 until 1999 there was no fundamental change in the susceptibility of the pathogens investigated (*E. coli*, *Pasteurella* spp., *Streptococcus* spp., *Staphylococcus intermedius* and *Proteus* spp.). Over the years 84-93% of the isolates were susceptible to difloxacin and 2-5% appeared to be resistant, whereas 4-13% were intermediately susceptible. Susceptibility of *Pasteurella* spp. and *Staphylococcus intermedius* was 100%, both in 1996, 1997 and 1998. Overall, 93% of *E. coli* and 82% of *Proteus* spp. were susceptible to difloxacin, whereas resistance was 5% and 8%, respectively. Sixty-four (64) percent of *Streptococcus* spp. were susceptible to difloxacin, 35% were intermediately susceptible, and 1% were resistant.

In summary, over a three years period susceptibility patterns have not changed substantially, and generally, the most relevant dog pathogens are still susceptible to difloxacin. Only a large proportion (35%) of *Streptococcus* spp. is intermediately susceptible. Resistance is low.

The complete picture confirms that the relevant canine pathogens are (still) susceptible to difloxacin; even more so, since the indications for Dicural 50 mg/ml solution for injection for cattle and dogs have been limited to acute, uncomplicated urinary tract infections (caused by *Escherichia coli* and *Staphylococcus spp*.) and superficial pyoderma (caused by *Staphylococcus intermedius*) in dogs. At the present moment *E. coli*, *Klebsiella spp*., *Pasteurella spp*. and *Staphylococcus intermedius* are (almost) completely susceptible to difloxacin (95-100% susceptibility). *Proteus spp*., *Pseudomonas spp.* and *Streptococcus spp.* appear to be slightly less susceptible, but 85% -100% of the strains may be defined as being susceptible or intermediately susceptible. In general, resistance of canine isolates is low (≤5%); resistance of *Proteus spp.* is however 5-15%. Thus, lack of efficacy of Dicural will not be due to lack of susceptibility of the pathogens to the active ingredient. The pre-clinical data can be used to make an estimate of the efficacy to be expected.

Post-antibiotic effect (PAE)

An *in-vitro* method to assess the PAE has been developed. Isolates from dogs with skin, respiratory and urine tract infections were incubated with various concentrations of difloxacin (1-8x MIC). After 1 hour the difloxacin was washed away and bacterial growth of the different samples was determined at various points of time after washing. The PAE is dependent on active substance, bacterial species, concentration of the drug, and time of exposure to the drug. In this particular experiment a situation was simulated in which each pathogen was exposed for 1 hour to various concentrations of difloxacin. The results indicate that a PAE of difloxacin on relevant dog pathogens (*Staph. intermedius*, *E. coli*, *Klebsiella pneumoniae*, and *Proteus* spp.) is possible, except for *Pseudomonas* spp. (Even at concentrations 8x MIC the PAE for *Pseudomonas* is nil.) At concentrations equivalent to the MIC the PAE is nil, except for *Staph. intermedius* (PAE=1.9 hours) and *Proteus* spp. (PAE= 0.9 hour). At 2x MIC the PAE is 0.1-2.1 hours; at 4x MIC the PAE is 0.2-2.8 hours, and at 8x MIC the PAE is 0.2-3.0 hours, depending on the pathogen. At concentrations up to 2x MIC there is only a significant PAE for *Staphylococcus intermedius* (i.e. ± 2 hours). Given the concentrations established in the pharmacokinetic experiments, it is not expected that the PAE will contribute much to the efficacy of Dicural. This study was considered appropriate to prove that a PAE may occur in dogs. However, given the attainable concentrations of difloxacin in the body, this effect will be marginal under practical circumstances.

Bactericidal activity

It has been shown that a bactericidal effect of difloxacin on (urinary tract) pathogens isolated from dogs is possible, that this effect is concentration-dependent, and that pH is an important influence on the bactericidal activity. The optimum pH is 7.1. The results also indicate that bactericidal activity only occurs when rather high concentrations of difloxacin (1-4x MIC) are maintained for a longer period (24 hours). In view of the results of the pharmacokinetic studies, bactericidal activity will not contribute significantly to the efficacy of difloxacin.

In one study, isolates from dogs with skin, respiratory and urinary tract infections were incubated with various concentrations of difloxacin (1-8x MIC). After 1 hour the difloxacin was washed away and bacterial growth of the different samples was determined. The results after 1 hour of exposure were compared with control values. The results indicated that, depending on the particular bacterial pathogen, the in vitro bacterial killing rate would be sufficient at difloxacin concentrations varying from 1xMIC to 8xMIC (e.g. bacterial killing rate of *Klebsiella pneumoniae* is 99.6% at 1xMIC, whereas the killing rate of *Streptococcus canis* is 92% at 8xMIC).

The pharmacokinetic results indicate that the concentrations of difloxacin would be above the MIC of the pathogens relevant to this application (*E. coli* and *Staphylococcus intermedius*) for a sufficiently long period: the concentration in plasma will be similar to or higher than the MIC of relevant pathogens for at least 24 hours.

MIC_{90} (*Staph. intermedius*) = 0.25 μg/ml.
MIC_{90} (*E. coli*) = 0.125 μg/ml.
C_{max} (plasma) = 1.36 μg/ml; C_{12h} = 0.25 μg/ml; C_{24h} = 0.20 μg/ml

From distribution studies in dogs, it is known that, during the first 24 hours after administration, the levels of difloxacin in the urinary tract are much higher than plasma levels, and that the concentrations of difloxacin in the skin are 1-2 times the plasma concentration.

The results of susceptibility testing and of pharmacokinetic experiments demonstrate that the concentrations of difloxacin will be sufficiently high, both in plasma and in relevant tissues, for a sufficiently long period to at least guarantee sufficient inhibitory action. In some cases the product will also have considerable bactericidal activity.

The results demonstrate that –at least on the basis of the pre-clinical results- it is expected that Dicural 50 mg/ml solution for injection for cattle and dogs will have sufficient antibacterial efficacy when used for the indications proposed by the applicant.

Cattle

Pathogen:	MIC_{90} (USA)	MIC_{90} (EU)	
Past. haemolytica	0.06	1.0	susceptible
Past. multocida	0.06	0.031	susceptible
Haemophilus somnus	1.0	0.125	susceptible
Mycoplasma spp.	- - -	0.05-0.5	susceptible

Remarks:

- MIC values from the USA are based on hundreds of isolates from various locations and over a number of years. The relevant pathogens appear to be susceptible for several consecutive years. There are no apparent differences in susceptibility between isolates from different geographical locations.
- MIC values from Europe are based on testing of tens of isolates from various countries.
- In Europe the susceptibility of *Pasteurella haemolytica* is dependent on the geographical location of the isolate. For instance, isolates from two EU member states are generally less susceptible than those from a third EU member state (MIC_{90}=2 µg/ml vs. MIC_{90}=0,125 µg/ml, respectively).
- Similarly, the susceptibility of *Haemophilus somnus* is location-dependent: in two EU member states this bacterial species is much more susceptible to difloxacin than in another EU member state (MIC_{90} = 0.031-0.062 µg/ml vs. MIC_{90} = 8.0 µg/ml, respectively).
- The European *Mycoplasma* strains originate from three different geographical locations. At every one of the three locations, all of the tested *Mycoplasma* strains are susceptible to difloxacin.

A PAE effect is not unlikely in cattle, but the duration of the PAE for each relevant pathogen at various concentrations of difloxacin still needs to be established.

In view of the relatively good susceptibility of *Pasteurella multocida* and *Mycoplasma* spp. to difloxacin, and the data summarised above, it is expected that Dicural in a dosage of 2.5-5 mg/kg bw/day for 3-5 days may be efficacious in the treatment of respiratory tract infections, caused by these two pathogens, in calves.

A general and unambiguous conclusion on the efficacy against infections caused by *Haemophilus somnus*, is not possible. Efficacy may be dependent on geographical location. Susceptible strains of *Haemophilus somnus* may be treated effectively with Dicural at the recommended dosage.

The efficacy against *Pasteurella haemolytica* is questionable. With MICs of 1-2 µg/ml Dicural at a dose of 2.5 mg/kg bw/day is not likely to be efficacious. Even the efficacy of a dose of 5 mg/kg bw/day is doubtful.

A dosage of 2.5 mg/kg/day for 3-5 d. may not be efficacious against <u>all</u> susceptible (MIC≤1 µg/ml) pathogens; only the most susceptible may be treated effectively.

<u>Secondary pharmacodynamic effects</u>

Studies investigating the effects of difloxacin on the cardiopulmonary system and on blood coagulation were performed, as effects on these systems have been reported in man, mice and rats. An intravenous dose of 6.25mg/kg in anaesthetised dogs induced a minor, compensated vasodilation whereas a dose of 31.25mg/kg induced an uncompensated vasodilation. It is concluded that the recommended oral dose of 5mg/kg was unlikely to induce any significant cardiopulmonary effects.

Studies conducted with doses higher than the recommended dose, on both normal and Factor VII deficient dogs revealed no adverse effects. Secondary pharmacological effects are, therefore, unlikely.

A relatively new aspect that is discussed in this part of the dossier is the combination of difloxacin with NSAIDs. It is assumed that difloxacin inhibits GABA receptors and that NSAIDs reinforce the inhibition by difloxacin. In the section of the SPC on interactions the applicant has included the warning that the combination of fluoroquinolones and NSAIDs may cause seizures in some animals.

Pharmacokinetics

Some studies on the kinetics of difloxacin after administration (mainly oral) to various animal species have been provided. Informative as these studies may be, they do not support the efficacy of the product Dicural 50 mg/ml solution for injection for cattle and dogs in the target species.

In addition a study was done to demonstrate bio-equivalence between Dicural 50 mg/ml solution for injection for cattle and dogs and Dicural coated tablets. Although the results show that, in a strict sense (e.g. according to EU guidelines), both Dicural formulations do not completely comply with the requirements of bio-equivalence, the differences are so small that they may be regarded as negligible. For the purpose of evaluation of efficacy both Dicural formulations may be treated as if they were equivalent.

A study on dose proportionality of Dicural 50 mg/ml solution for injection for cattle and dogs is presented.
Another study contains some information on the metabolism of difloxacin after oral administration to dogs. The main route of excretion is via the bile. About 80% of the dose was recovered from the faeces; 14% was found in the urine. Difloxacin is excreted largely unchanged (65%). The main metabolites are desmethyl difloxacin (±12%) and difloxacin glucuronide (±13%). In addition, protein binding of difloxacin was discussed. *In-vitro* plasma protein binding in rats, rabbits, dogs and humans was about 40-55%. Furthermore, in residue studies it was found that at the recommended dosage the tissue binding of difloxacin in cattle was low (±12%). These results indicate that after absorption from the injection site, the availability of difloxacin will not be largely impaired by binding to plasma proteins, tissues and organs.

In the following paragraphs the kinetics and metabolism of difloxacin in the target species are reviewed in more detail.

Dogs

The data on kinetics of the product in dogs have been limited to data on kinetics after oral administration. The references on this subject have been derived from the Dicural coated tablets dossier.

The only product–related data are the results of the investigations on bio-equivalence between Dicural coated tablets and Dicural 50 mg/ml solution for injection for cattle and dogs. After a single dose of 5 mg/kg bw (oral or subcutaneous) to 6 male + 6 female Beagles the following results were obtained:

Parameter	**Dicural coated tablets**	**Dicural 50 mg/ml Sol. for injection**
C_{max} (µg/ml plasma)	1.42	1.36
T_{max} (hours)	3.7	3.1
$AUC_{0\text{-}24\ h.}$ (µg.h/ml)	10.3	12.3

The tablets and the solution are not strictly bio-equivalent, but the differences are so small that, for the purpose of assessment of efficacy, they may be regarded as equivalent. The results indicate a large bioavailability. At a dose rate of once a day a small amount of accumulation of difloxacin in plasma may occur. The quantities of accumulating difloxacin are small, however, and difloxacin is a relatively safe drug; thus it is highly unlikely that this will lead to intoxications.

It is concluded that the efficacy of the injection is at least as good as the efficacy of the tablets. In addition, the local safety of the parenteral formulation has been shown. The applicant further states that the advantage of the successive use of the solution for injection and the tablets is that treatment may start by injection immediately after the diagnosis, and may be continued with the tablets (earlier treatment start and more convenient for the veterinarian). Consequently, the proposed dosage schedule and treatment interval must be the same as for the tablets.

Conclusion:

On the basis of the following results:

- the relevant pathogens are susceptible to difloxacin (MIC≤1 µg/ml; most pathogens have a MIC <<1 µg/ml),
- C_{max} = 1.4 µg/ml,
- the tissue/plasma ratio is at least 1 (but often >>1),
- Dicural 50 mg/ml solution for injection for cattle and dogs is equivalent to Dicural coated tablets,

It may be expected that Dicural, at the recommended dosage will be efficacious in the treatment of respiratory and urinary tract infections and skin infections, caused by *E. coli, Klebsiella pneumoniae* or *Staphylococcus intermedius*.

<u>*Cattle*</u>

Two pharmacokinetic studies using Dicural 50 mg/ml solution for injection for cattle and dogs have been provided. Data corresponding to the intramuscular route are not provided here, since this route of administration has been withdrawn from the claims submitted by the Applicant.

It has been shown that difloxacin is widely distributed within the body (V_d=±2.5 l/kg). Bioavailability after subcutaneous administration of Dicural is large (≈90%). Only a small part of the dose is bound to proteins/tissue. At a dose of 5 mg/kg bw administered by subcutaneous route, C_{max} is 1.7 µg/ml in plasma; $C_{12\ hours}$ is 1.1 µg/ml. The lung/plasma ratio might be 1.0 (the conclusions are based on results of one or two animals). Data on concentrations after administration of a dose of 2.5 mg/kg bw/day are lacking.

In addition to these two pivotal studies the applicant has provided a study in which it is demonstrated that after intravenous administration of Dicural to calves the C_{max} and AUC are linearly related to the dose. V_d, clearance and $t_{1/2,\ elim.}$ are not dose-dependent. It is not clear if the same results will be obtained when Dicural is administered subcutaneously or intramuscularly.

Investigations on metabolism, tissue distribution and protein binding have also been presented. According to the results of the residues and metabolism study after repeated (5 d.) subcutaneous and intramuscular administration of difloxacin to cattle, it was established that difloxacin is excreted mainly via the faeces (80%) and urine (8%). The largest part of the 'metabolites' is unchanged difloxacin (60-80%). Only 10% of the residues is bound to protein/tissue. Unfortunately, this study contains no information on concentrations of difloxacin in the respiratory tract.

Tolerance in the target species

Most aspects of target species tolerance have been discussed under Safety Assessment. In this section additional information on the tolerance towards the product is discussed.

Cattle

The main side-effects are transient injection site reactions observed after subcutaneous injection (macroscopically: transient swelling, microscopically: inflammation, haemorrhage, hyperaemia, occasionally necrosis). Average diameter of the injection site reactions: ±25 mm (maximum: 55mm). Injection site reactions were reversible.

Since all the field trials were performed by injecting cattle subcutaneously, the local tolerance by intramuscular injection has been judged insufficiently demonstrated, and this route of administration has therefore been withdrawn from the claims.

Additional information on the tolerance in cattle is also provided. Dicural (5% and 10%) was compared with other antibacterial preparations, including enrofloxacin. Again, the main side-effects were (reversible) injection site reactions. Injection site reactions after administration of Dicural were comparable to those after administration of other antibacterials. The maximum size of the injection 14 days after administration of Dicural 50 mg/ml was 4 x 4 x 3 cm.

Dogs

For dogs, in addition to the fact that, based on the pharmacokinetic parameters, the solution for injection is expected to be at least as efficacious as the tablet, and that the systemic safety of both formulations will be similar, the applicant summarised further advantages of the availability of the injectable product:

- The treatment can be initiated immediately (beneficial in acute infections).
- The solution for injection is a convenient, safe and accurate means of administration for the veterinarian.
- The solution for injection allows treatment of dogs that cannot be treated orally (e.g. shortly after anaesthesia).
- No general adverse drug reactions have been observed with the solution for injection, even at doses up to 4 times the recommended dosage.

The Committee considered that the local tolerance of dogs to the solution of injection has been described accurately. Local reactions at the injection sites may occur, but are transient and not very severe. By contrast, the use of the solution for injection appears to have several advantages. Balancing the potential local reactions (frequency, severity) against the advantages of the solution, the use of the solution for injection in dogs is justified.

Resistance

Several references on the possibility of induction of resistance to quinolones have been provided. There appear to be two major mechanisms of induction of resistance: mutations in the gyrase gene of bacteria (so that inhibition of gyrase by quinolones can not take place), and changes in cell permeability towards quinolones. It has been shown that resistance can be induced by a transferable plasmid.

The applicant's investigations revealed that there was no evident difference in susceptibility between pathogens isolated from dogs in 1996 and those isolated in 1997. Resistance had not increased. Relevant bacterial species (*Staph. intermedius, E. coli, Proteus* spp.) were still susceptible to difloxacin.

By contrast, data from one EU member state indicates that, for instance, resistance of *E. coli* (isolated from cattle) towards enrofloxacin had increased significantly (from 0% to ±15%) between 1990 and 1994. The changes in resistance probably reflect the use of quinolones in cattle in that particular EU member state. For an extensive overview of susceptibility and resistance one is referred to the data and information in the Pharmacodynamics section above.

In conclusion, it has been shown that resistance against quinolones can develop, and has developed to some degree. The mechanism of induction of resistance has been made plausible by investigations presented in the dossier.

Clinical studies

Dogs

Administration of the solution for injection and the tablet yields comparable difloxacin levels in plasma. However, the solution for injection produces a higher peak level, but in view of the persistence in levels, and the similarity of the further progress of difloxacin concentrations in plasma, this is considered less important with regard to efficacy. Hence, this treatment regimen, commonly used in practice when active ingredients are available as both injectable and tablets, is considered to be valid for Dicural too.

Most efficacy data on dogs are derived from the Dicural coated tablets dossier. In addition, three efficacy studies with the combined use Dicural 50 mg/ml solution for injection for cattle and dogs and Dicural coated tablets have been provided.

The applicant has furnished three new studies involving the efficacy of combined use of Dicural 50 mg/ml solution for injection for cattle and dogs and Dicural coated tablets in urinary tract infections, bacterial skin infections and respiratory tract infections, respectively. In all 3 studies enrofloxacin injectable solution and enrofloxacin tablets were used as a positive control. There was no negative control group. The study investigating respiratory tract infections will not be discussed further as there was insufficient evidence for efficacy and the claim was not retained.

In case of uncomplicated urinary tract infection, referred to as 'general clinical disease symptoms, including abnormal voiding and positive bacteriology of urine' 7 dogs were treated with difloxacin and 8 with enrofloxacin (5 mg/kg bw/day). Duration of treatment was at least 5 days, but not more than 10 days. A case was considered as cured if clinical signs had resolved and bacterinuria was absent. Cure rates were 85.7% for Dicural and 87.5% for enrofloxacin. *E. coli* was the predominant pathogen (8 out of 14 cases).

From the CVMP's point of view, the outcome of the study indicates that cure rates are likely to be comparable for both Dicural and enrofloxacin. Only acute cases were included. It was noted that treated animals were predominantly males. Except for acute cases, urinary tract infections in males tend to be more complicated. Within this limitation, this study offers a serious hint (but no unequivocal, independent proof) of efficacy in acute cases.

In case of bacterial skin diseases, referred to as 'pyoderma, diagnosed on general clinical disease symptoms', 16 dogs were treated with Dicural and 16 dogs with Enrofloxacin. Duration of treatment was at least 10 days, but not more than 20 days. A case was considered as cured if clinical signs and skin lesions had resolved. Cure rates were 75% for both Dicural and Enrofloxacin. *Staphylococcus* spp. was the predominant pathogen (22 out of 36 isolates). Regarding the scores for clinical symptoms at Day 1 of the trial, the number of animals, really suffering from a skin disease, is considered to be low. Moreover, the inclusion criteria are broad. However, treatment did reduce the number of positive bacterial isolates. The outcome of the study indicates that cure rates are likely to be comparable for both antibacterial products. However, an unambiguous conclusion on the true level of efficacy cannot be drawn.

The applicant referred to the Dicural Coated tablets dossier. The efficacy of Dicural Solution for Injection is based primarily on its assumed bio-equivalence to Dicural Coated Tablets, and, consequently, on the data in the coated tablet dossier, including the data from the field trials. The Dicural Coated Tablets have been granted a marketing authorisation. The same indications are now proposed for Dicural Solution for Injection. Thus, the Dicural Solution for Injection may be assumed to be efficacious when used for the indications mentioned in the SPC of Dicural Solution for Injection.

Conclusions on the clinical studies in dogs:

None of the clinical studies was completely blinded. In addition, clinical symptoms were assessed by using a scoring system with several subjective elements. At the beginning of the treatment the clinical scores for the individual animals were rather low, indicating that, on average, the treated animals were not very ill. Although pre-clinical information has been submitted that suggests sufficient efficacy in the case of respiratory and urinary tract infections and bacterial skin diseases, there is no unequivocal clinical confirmation of the expected efficacy. However, the efficacy of Dicural Solution for Injection is based primarily on its bio-equivalence to Dicural Coated Tablets, and, consequently, on the data in the coated tablet dossier, including the data of the field trials. Thus, Dicural Solution for Injection may be assumed to be efficacious in the treatment of acute uncomplicated urinary tract infections caused by *Escherichia coli* and *Staphylococcus spp.* and superficial pyoderma caused by *Staphylococcus intermedius*.

Cattle

Experimental studies:

In a dose-confirmation study, Dicural 50 mg/ml solution for injection for cattle and dogs in dosages of 2.5 and 5 mg difloxacin/kg bw/day was compared with Enrofloxacin injectable at a dosage of 5 mg enrofloxacin/kg bw/day and a placebo (saline). Four groups of 12 animals (139-210 kg bw) were challenged with Bovine Herpes Virus Type 1 (BHV1) and 4 days later with *P. haemolytica* (MIC 0.062 µg/ml). Treatment was started 1 day after the last challenge and lasted 5 days. Mortality and clinical symptoms were observed. Necropsy was performed at D15. Treatment reduced mortality, clinical symptoms and lung lesions, compared with the negative control.

Treatment was started at a fixed time point, regardless of the individual clinical symptoms. In view of the experimental model and the low clinical scores at day 1 the outcome of the study was firstly regarded to be relevant for group treatment of shipping fever, meaning that treatment was aimed at the prevention of mortality in the first place and not at individual cure. Mortality is reduced significantly.
It is observed that after treatment had stopped a considerable number of animals relapsed and final cure rates were as low as 8.3% for 2.5 mg difloxacin/kg bw and 16.7% for difloxacin and enrofloxacin at 5 mg/kg bw.

In an *in vivo* experimental study, 7x12 calves were infected with a difloxacin-susceptible *P. haemolytica* field strain. When the clinical symptoms became apparent (one or two days after challenge), groups of animals (71-79 kg bw) were treated for 5 days with 5% and 10% Dicural Injectable formulations and a Enrofloxacin 5% injectable formulation. Dosages were 2.5 and 5 mg/kg bw/day for all Dicural formulations and 5 mg/kg bw/day for Enrofloxacin. A saline control was included. Animals were observed for another 10 days after the last treatment. Cure was considered to be present when scores had returned to normal at D6/7 and remained low afterwards. Necropsy was performed at the end of the study (D16/D17). Treatment resulted in prevention of mortality and reduction of clinical symptoms and lung lesion scores, without differences between dosages or products. *P. haemolytica* could still be isolated afterwards, but was reduced considerably. The study was blinded. Treatment was started in the acute phase of the infection. Results indicate that Dicural injectable formulation - at the recommended dosage level of 2.5-5 mg/kg bw/day- can be effective when used as a group treatment.

In a dose finding study, the efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs in dosages of 1, 2.5, 5 and 7.5 mg/kg bw/day (3-5 days) was compared with that of ceftiofur (2.2 mg/kg bw/day) and a placebo, in spontaneously occurring cases of Bovine Respiratory Disease (BRD). The study included 6x32 animals (av. bw: 227 kg; age: 6-8 months). Animals were included in the experiments if body temperature was ≥40°C, depression / poor appetite was present and respiratory rate was ≥45/min. Animals were regarded as cured if body temperature and clinical scores had returned to normal. Necropsies were performed at the end of the study. Compared to the placebo treatment animals responded fairly well to Dicural treatment and differences in efficacy between the dosages could not be detected. Ceftiofur performed best with the highest success rate and the lowest relapse rate; however, average weight gain was lower. It was concluded that difloxacin administered at 5 mg/kg bw/day was suitable for treatment. The investigators mentioned that this study in fact failed to demonstrate the optimal dosage for Dicural. Dosing with a 5% injectable formulation at 5 mg/kg bw/day was considered to be inconvenient because of the large volumes to be injected. Durable cure rate for Dicural (≤65%) was lower than the minimum standard used by the US Cattle Industry for BRD (>70%).

In another study, the efficacy of Dicural 50 mg/ml injectable, dosed at 2.5, 5 and 7.5 mg difloxacin/kg bw/day (3-5 days), was compared to that of Enrofloxacin 5% injectable at 5 mg enrofloxacin/kg bw/day and Ceftiofur at 2.2 mg ceftiofur/kg bw/day in case of natural occurring BRD. A placebo-treated group was included. Animals (190-260 kg bw; 6-10 months old) were allotted to treatment after showing disease symptoms. Medication was administered for 3 or 5 days. Animals were observed for 15 days and necropsied at the end of this period. The results of all treatment groups differed from those of the placebo group. There were no differences between treatment groups. Average durable cure rate was 70% for the placebo treatment, so the severity of the infection is questionable. Treatment was started 1-2 days after shipment. This could have been in favour of the results, since it is felt that a difloxacin (and enrofloxacin) treatment gives better results when performed in the very early stage of infection. A dosage of 2.5 mg difloxacin/kg bw/day was preferred by the investigators, since they considered the needed volume of difloxacin 5% at a dose of 5 mg/kg bw to be larger than acceptable.

The efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs, dosed at 1, 2.5 and 5 mg difloxacin/kg bw/day, was also compared to that of Ceftiofur at 2.2 mg ceftiofur/kg bw/day in cases of natural occurring BRD. A placebo-treated group was included. Animals (180-322 kg bw) were allotted to treatment after showing disease symptoms. Medication was administered for 3 or 5 days. Animals were observed for 15 days and necropsied at the end of this period. Compared to the placebo treatment animals responded well to Dicural treatment and differences between treatment groups could not be detected. Ceftiofur performed best with the highest success rate and durable cure rate, and the lowest relapse rate. Average durable cure rate was 58% for the placebo treatment, so the severity of the infection is questionable.

Conclusions on the experimental studies in cattle:

Studies indicate that the treatment effect of Dicural can vary considerably. Success rate appears to depend on severity of infection and on timing of treatment rather than on dosage. The efficacy of Dicural appears to be hampered by the presence of inflammation and this may add to the absence of differences in effects for the various dosages. The efficacy of Dicural did not compare favourably to that of Ceftiofur and the differences between Dicural and a placebo were smaller than expected. The absence of differences between effects of the different dosages raises doubts about the reproducibility of treatment effects. The fact that Dicural compared favourably with Enrofloxacin also raises doubts about the efficacy of Enrofloxacin, and it appears that the quinolones are clinically less effective than would be expected on the basis of pre-clinical information.

In all studies the experimental unit was the pen and all animals in a group were treated simultaneously. The condition described as 'shipping fever' is not a major cause of the *Pasteurella*-associated BRD observed in Europe, whereas bronchopneumonia in calves is closely associated with BRD. Animals affected by BRD are usually treated individually after showing disease symptoms (curative treatment). The submitted data indicated that Dicural will not be very effective in case of a curative treatment of BRD in individual cases. However, efficacy of Dicural in case of bronchopneumonia, when used as group treatment, is plausible.

The results of the dose titration studies and the pre-clinical do not allow an unambiguous conclusion on the preferred dosage of Dicural 50 mg/ml solution for injection for cattle and dogs when used in calves for the indications proposed. The data presented make it plausible that a dosage of 2.5 mg /kg per day for 3 days may cure calves affected by the respiratory diseases described in the 'indications section' of the SPC. For the more obstinate or complicated diseases, or for intermediately susceptible pathogens prolonged treatment (5 days in total) or a dose of 5 mg/kg per day may be beneficial.

Field trials:

In the first field trial, the efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs at 5 mg difloxacin/kg bw/day was compared to that of Enrofloxacin 5% Injectable Solution in calves, 2-4 weeks of age (40-68 kg bw). Animals were included on the basis of a clinical score and treatment was given for 5 days. From the clinical scores at the start of the trial it appears that the severity of the infection is questionable. The study was blinded. Dicural performed better than Enrofloxacin, resulting in a higher cure rate and a lower relapse rate. Notably, treatment did reduce body temperature rapidly. According to the CVMP, the study design only allows conclusions on relative efficacy of the two products. Conclusions on the true, intrinsic efficacy are not possible. In view of the relapse rate of 18% the curative effect of Dicural appears to be rather low and improvement of scores was likely to be due to the fact that the whole group of animals was treated.

In the second field trial, efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs was compared for dosages of 2.5 and 5 mg/kg bw/day and administered for 3 or 5 days, using calves (44-69 kg bw) suffering from respiratory disease. A positive control group was treated with Enrofloxacin (5 mg/kg bw/day, 3-5 d.). Animals were included on the basis of a clinical score. The study was blinded. Mean cure rates were 71% for all groups. Average duration of treatment was 4.4 days. According to the CVMP, results were in favour of a 5-day treatment regimen. Severity of infection was low and the product was used as a group treatment in the early stage of the infection.

In the third field trial, the efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs at 5 mg difloxacin/kg bw/day was compared to that of Enrofloxacin 5% Injectable Solution in calves, 2-10 weeks of age, suffering from respiratory disease. Animals were included on the basis of a clinical score and treatment was given for 5 days. Both products were 100% effective. Compared to the previous studies the reported cure rate, as observed in this study, is rather unusual. The investigators consider the results to be in agreement with the literature. However, lower cure rates from previous studies were also considered to be in agreement with (the same) literature.

In the fourth field trial, the efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs at 5 mg difloxacin/kg bw/day was compared to that of Enrofloxacin 5% Injectable Solution in calves, 1.5-6 weeks of age, suffering from respiratory disease. Animals were included on the basis of a clinical score and treatment was given for 5 days. Dicural performed better than Enrofloxacin, with success rates of 83% and 72 %, respectively. The lower success rate of Enrofloxacin was due to a higher relapse rate (24%). The relapse rate for Dicural was 11%. These relapse rates indicate a poor performance of both products in case of curative treatment. Hence, observed effects did depend on the improvement of animals that were treated in the early stage of the (acute) infection.

In the fifth blinded comparative randomised trial, the efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs at 5 mg difloxacin/kg bw/day was compared to that of Enrofloxacin 5% Injectable Solution in calves, 2.6-5.4 weeks of age, suffering from respiratory disease. Animals were included on the basis of a clinical score and treatment was given for 3 or 5 days, depending on the clinical score on D3/4. Dicural performed slightly better than Enrofloxacin but differences are not considered to be significant.

In the sixth field trial, which was blinded, comparative and randomised, the efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs at 5 mg difloxacin/kg bw/day was compared to that of Enrofloxacin 5% Injectable Solution in calves, 1-5.5 months of age, suffering from respiratory disease. Animals were included on the basis of a clinical score and treatment was given for 3 or 5 days. Cure rates were comparable for both products (about 72%). Relapse rates were 14%. In view of the clinical scores the severity of infection was low. Results confirm the observations form the previous studies, viz. that the curative performance of Dicural is rather poor and that effects are mainly caused by the improvement of slightly diseased animals.

In the last field trial, 92 calves of a population of 98 (5-10 months old; 160-320 kg body weight) were treated with Dicural 50 mg/ml solution for injection for cattle and dogs or with Enrofloxacin (active component: enrofloxacin). The remaining 6 animals conformed to the exclusion criteria. Dosages were the same for both products: 5 mg/kg bw/d. for 3 or 5 days (subcutaneous). Inclusion and exclusion criteria were the same as in previous trials, although the inclusion criterion for respiratory rate appears to be mitigated (previously, animals with a respiratory rate >50 min^{-1} were included, here a respiratory >40 min^{-1} did suffice). Cases of chronic infection were excluded. Clinical scores were given on the first day of treatment (D1), one day after the last injection (i.e. D4 or D6), D10 and D15. Scoring system and efficacy criteria were the same as in previous trials. However, in this trial the results were subdivided in the results in animals treated for 3 days and those treated for 5 days. Overall, the qualification 'cured' was attributed to 82% of the Dicural-treated and 72% of the Enrofloxacin-treated animals; relapse rates were 7% and 19%, respectively, and failures were 11% and 9%. Of the Dicural-treated animals 71% were treated for 3 days; 29% were treated for 5 days. (For Enrofloxacin these percentages were 77% and 23%, respectively.) Efficacy rates of Dicural in these two groups are very different: 94% in the animals treated for 3 days, and 54% in the animals treated for 5 days. (For Enrofloxacin these rates are 86% and 27%, respectively). In >80% of the cases *Pasteurella* spp. were isolated; *Haemophilus somnus* and *Mycoplasma* spp. were not isolated.

An overall statistical analysis of all field trials has also been presented. The overall number of cured animals was significantly higher in the Dicural-treated group compared to the Enrofloxacin-treated group (84% vs. 78%). Similarly, the cure rates per sickness category at treatment start (initial sickness scores: 16-20: high; 12-15: moderate; <12: low; maximum sickness score =20) have been analysed. For each category the cure rates of Dicural were always >75% (Enrofloxacin: >70%); in the category with a highest sickness scores (>16) the overall cure rate for Dicural was 82% (Enrofloxacin: 80%).

The statistical analysis of all results taken together shows that the initial state of health of the animals had no influence on the cure rate. Irrespective of the initial sickness score (whether high, moderate or low), the eventual cure rate is similar for each category of sickness. Thus, the severity of the disease does not appear to influence the outcome of treatment. In general, the cure rates for Dicural are similar to or higher than those for Enrofloxacin. In addition, it is demonstrated that there is no distinct relationship between the initial sickness score and the treatment duration (i.e. that the sickest animals will not necessarily have to be treated for 5 days). Considering the conclusion that severity of disease ('initial sickness score') does not appear to influence the outcome of treatment, one may conclude that the observed cure rates are also representing the cure rate of seriously ill animals. This being the case, the overall results of the dose titration studies indicate that 2.5 mg/kg per day is the optimal dosage, no matter how good or bad the initial state of health of the animals is. In general, a higher dose does not have a positive effect in treatment outcome. The success rate appears to depend on severity of infection and on timing of treatment rather than on dosage.

Regarding the exact definition of the indications for calves, it was noted that in most of the field trials *Pasteurella spp*. were isolated; occasionally *Mycoplasma spp*. were also present. *Haemophilus somnus*, however, was rarely found. This pathogen has therefore been deleted from the indications: it was not demonstrated that this particular pathogen plays a substantial role in bovine respiratory disease, and, even if it does, it is not proven that Dicural will effectively counteract its harmful effects. A general and unambiguous conclusion on the efficacy against infections caused by *Haemophilus somnus*, appeared to be impossible. Efficacy apparently depended on geographical location. Susceptible strains of *Haemophilus somnus* might be treated effectively with Dicural at the recommended dosage. The efficacy against *Pasteurella haemolytica* is questionable. With MICs of 1-2 µg/ml Dicural at a dose of 2.5 mg/kg bw/day is not likely to be efficacious. Even the efficacy of a dose of 5 mg/kg bw/day is doubtful. Thus, the pre-clinical data indicate that a dosage of 2.5 mg/kg/day for 3-5 d. may not be efficacious against all susceptible (MIC≤1 µg/ml) pathogens; only the most susceptible may be treated effectively.

A major point of concern regarding the efficacy of difloxacin was the discrepancy between the outcome expected on the basis of pre-clinical data, and the actual outcome observed in clinical trials. On the one hand, the model studies, the dose–titration studies and the individual field trials do not unambiguously support the curative claims. On the basis of individual studies it may be concluded that it is doubtful if clinically ill animals will be cured sufficiently, whereas use of Dicural in the early stage of an infection may be efficacious. On the other hand, analysis of the overall results suggests that fairly ill animals may be cured as well. Absolute proof for either one of these possibilities is lacking.

Conclusions on field trials in cattle:

All field trials were dealing with *Pasteurella*-associated bronchopneumonia in calves. In all the studies, animals were treated as a group and clinical scores were rather low. Animals were included that were not very ill or were still in the early stage of infection. The relatively high relapse rates indicate that in a number of animals infection was not cured, but only stopped until difloxacin had disappeared from the tissues. It was observed that changes in clinical scores were associated with a reduction of body temperature. In view of its sharp decline, reduction in body temperature is considered to be a CNS-mediated effect, which is also observed for other quinolones. However, reduction in body temperature will support the cure e.g. by stimulating appetite, especially in slightly diseased animals.

It is concluded that Dicural can be of benefit in case of respiratory disease in calves, provided it is used in the early stage of an acute infection, in animals not yet affected or only mildly affected and for group treatment only. An optimal dosage could not be established, but the recommended dosage will be efficacious, when given for 3-5 consecutive days.

Overall conclusions on clinical studies in cattle:

Based on the comparative results with Enrofloxacin injectable solution, the conclusion is that Dicural 50 mg/ml solution for injection for cattle and dogs undoubtedly will have a positive effect when used for the proposed indication. It was noted, however, that the studies on efficacy did not contain sufficient evidence that the product is effective in the individual treatment of bovine respiratory disease, since the seriously ill animals are not cured in sufficiently high numbers. Submitted clinical data indicate that difloxacin can be used in case of *Pasteurella*-associated respiratory disease in cattle, when used in the early stage of infection, when animals do not or begin to show the clinical symptoms of the disease.

Given the description of the posology (with the possibility to use a higher dose of 5 mg/kg, and a prolonged treatment duration of 5 instead of 3 days) and of the indications for use (now including the prerequisite that the product is ‘to be used only based on susceptibility testing’, as well as the statement that fluoroquinolones should not be used as first line treatment and should be reserved as second or third line when there was resistance to other antibiotics) there is sufficient evidence to allow the claim for bovine respiratory disease.

Conclusions on Efficacy

The efficacy of Dicural 50 mg/ml solution for injection for cattle and dogs has been substantiated for the indications and dosages as indicated in the approved SPC. Generally, use of the product is confined to treatment of acute infections. In dogs efficacy has been substantiated in the treatment of uncomplicated urinary tract infections caused by *Escherichia coli* and *Staphylococcus spp* and superficial pyoderma caused by *Staphylococcus intermedius*. In cattle the use of Dicural is indicated for the treatment of acute respiratory disease, caused by *Pasteurella* spp. and/or *Mycoplasma* spp., in calves and young cattle.

RISK-BENEFIT ASSESSMENT AND CONCLUSION

The suitability of the specification for the active ingredient, the method of manufacture of the product and the validity of the test methods applied to the product have been satisfactorily presented in the Quality Part of the application. The quality data submitted confirm the acceptability of the proposed formulation.

In dogs, the product is to be administered once at 5 mg/kg body weight. Adverse effects such as pruritis and/or local swelling and pain reaction have been reported in the SPC. Symptoms of overdose have been observed at 10 times the recommended dose by oral administration showing mild adverse clinical signs such as orange/yellowing discoloration of the faeces, emesis and hypersalivation. Since signs of arthrogenic potential (induction of cartilage lesions) of difloxacin were observed in studies with immature dogs even at the recommended dose, the use of difloxacin in skeletally immature dogs is contraindicated.

The use of Dicural 50 mg/ml solution for injection is not likely to cause any serious systemic side-effects in cattle (calves and young cattle). However, incidental unsteadiness may occur especially at prolonged treatment. In most cases minor injection site reactions were observed, but occasionally more severe reactions have been found. At overdoses CNS symptoms, such as ataxia, unsteadiness shaking, tremors, twitching will occur. Also, at overdoses the injection site reactions are more severe. Finally, swelling and oedema formation in the knee joints have also been observed in calves, but without cartilage abnormalities.

Effects on pregnancy and lactation have not been investigated for Dicural 50 mg/ml solution for injection for cattle and dogs as it is stated in the SPC.

The use of difloxacin may result in an increase in prevalence of resistant strains. The SPC includes the statement that “heavy reliance on a single class antibiotic may result in the induction of resistance in a bacterial population”. It is prudent to reserve the fluoroquinolones for the treatment of clinical conditions which have responded poorly, or are expected to respond poorly, to other classes of antibiotic. Dicural 50 mg/ml solution for injection for cattle and dogs should only be used based on susceptibility testing due to known problems with increasing fluoroquinolone resistance. Given these limitations, the risk of an increase in prevalence of resistant strains is considered to be very limited within the target species for which the product is intended.

Due to the fact that the use of fluoroquinolones like difloxacin in combination with non steroidal anti-inflammatory drugs may cause seizures in dogs, a statement was included in the section 5.6 of the SPC: Interactions with other medicaments and other forms of interactions.

In dogs, it was concluded that Dicural 50 mg/ml solution for injection for cattle and dogs could be considered as bioequivalent from efficacy point of view to the Dicural coated tablets. Systemic tolerance also can be considered as similar with both products.

In view of the relatively good susceptibility of *Pasteurella multocida* and *Mycoplasma* spp. to difloxacin, and the pharmacokinetic data, it is expected that Dicural 50 mg/ml solution for injection for cattle and dogs in a dosage of 2.5-5 mg/kg bw/day for 3-5 days may be efficacious in the treatment of respiratory tract infections, caused by these two pathogens, in calves.

Based on preclinical results, the efficacy against *Pasteurella haemolytica* is questionable. With MICs of 1-2 µg/ml Dicural at a dose of 2.5 mg/kg bw/day is not likely to be efficacious. Even the efficacy of a dose of 5 mg/kg bw/day is doubtful. However, based on the results from model studies and field trials, it was concluded that Dicural may also be efficacious against this pathogen.

The submitted clinical trials were considered to support the following claims:
Dogs :

Treatment of superficial pyoderma caused by *Staphylococcus intermedius*,
Treatment of acute, uncomplicated urinary tract infections, caused by *Staphylococcus spp.* and *E. coli.*

Cattle (calves and young cattle) :

Treatment of bovine respiratory disease (shipping fever, calf pneumonia) caused by single or mixed infections with *Pasteurella haemolytica*, *Pasteurella multocida* and/or *Mycoplasma spp.*

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