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CVMP assessment report
Zuprevo (EMEA/V/C/002009)

International non-proprietary name: Tildipirosin

Assessment Report as adopted by the CVMP with all information
of a commercially confidential nature deleted.

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Summary of the dossier

Zuprevo contains tildipirosin as active substance, which is a semi-synthetic novel macrolide antibiotic.

It is presented as a solution of for injection in two different strengths, 40 mg/ml (pigs) and 180 mg/ml (cattle), and is available in packs/containers of 20 ml, 50 ml, 100 ml and 250 ml.

The target species are pigs and cattle. The route of administration is intramuscular use (pigs) and subcutaneous use (cattle).

The proposed indications are:

- Pigs: The treatment of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Bordetella bronchiseptica* and *Haemophilus parasuis* sensitive to tildipirosin.
- Cattle: The treatment and prevention of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* sensitive to tildipirosin. The presence of the disease in the herd should be established before preventive treatment.

The withdrawal periods for meat and offal are 9 days (pigs) and 47 days (cattle).

Part 2 - Quality

Composition

The finished product is presented as a sterile aqueous solution containing the active ingredient tildipirosin in two different concentrations: Zuprevo 40 mg/ml solution for injection for pigs and Zuprevo 180 mg/ml solution for injection for cattle.
The solution for injection is self-preserving; no additional preservative is added. The excipients contained in the finished product, namely water for injections as the solvent, citric acid monohydrate as the acidifier, and propylene glycol as the co-solvent, are described in the European Pharmacopoeia (Ph. Eur.) and comply with Ph. Eur. requirements.

Container

The product is filled into Type I multidose amber glass vials of 20 ml, 50 ml, 100 ml and 250 ml, which are closed with chlorobutyl rubber stoppers, and sealed with crimp caps.

Development pharmaceutics

The aim of the development studies was a stable, ready to use, and *in vivo* well-tolerated solution for injection. In order to provide the appropriate dose volumes for each target species, two solutions of different concentrations were developed. The development of the finished product dosage form is conclusively and sufficiently described. The choice of excipients has been justified; the vials (made of type I amber glass) and stoppers (chlorobutyl rubber stoppers) have been shown to be suitable. The stoppers should not be punctured more than 20 times; corresponding advice has been included in the SPC.

Method of manufacture

The manufacturing process is a simple process and was sufficiently described. A non-standard method of sterilisation (sterile filtration) will be applied. The manufacturer's experience in sterile filtration was considerably documented. The requirements as stated in Annex II to the note for guidance on process validation (EMEA/CVMP/395/03) can be considered fulfilled. Validation of pilot scale batches indicate that the manufacturing process yields a robust reproducible product. Full production scale process validation will be performed prior to marketing.

Control of starting materials

Active substance

The active substance tildipirosin (20, 23-di-piperidinyl-mycaminosyl-tylonolide; PMT) is a crystalline white to yellowish or beige powder with a molecular weight of 734. It is a mixture of two compounds, PMT and its minor isomer PMT-T. Tildipirosin can exist in several polymorphic forms of which polymorph D is the most stable and therefore selected form. It has a melting point of about 192 °C and is soluble in polar organic solvents (methanol, acetone) and slightly soluble in water. Evidence of structure has been confirmed by several methods.

The synthesis of the active substance is performed in two stages. The starting material used in the synthesis of tildipirosin is tylosin phosphate. The proposed specification for the active substance is appropriate to control the quality of tildipirosin. Information about primary reference material is available. The validations of the analytical methods used for control of the active substance are considered satisfactory and in compliance with relevant VICH guidelines. The studies performed confirm that the analytical methods used are stability indicating.

Appropriate pilot and production scale batch data have been provided.

Stability of the active substance has been supported by results from three pilot scale batches. All these studies have been completed. A re-test period of 24 months at temperatures not exceeding 25°C based on the submitted data is accepted. A stability study on full (commercial) scale batches is ongoing.

Excipients

The excipients used in the manufacture of Zuprevo comply with the current monographs of the European Pharmacopoeia. The microbiological quality of water for injections is routinely controlled. Limits for Total Aerobic Microbial Count (TAMC) and total combined yeast and mould count (TYMC) have been specified for the other excipients.

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

The raw materials and auxiliary materials like lubricants used for manufacture of tildipirosin do not contain and are not derived from material of animal origin. Likewise, no starting materials are used as defined in section 2 of the "Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products" (EMEA/410/01-Rev.2).

Control tests on the finished product

The specification proposed at release is appropriate to control the quality of the finished product.

The shelf-life specification is different from the release specification with regard to the limit for specified impurities, sum of impurities, and the lower PMT/PMT-T content limit. The shelf-life specification is appropriate to control the quality of the finished product during its shelf-life. Batch results of three pilot scale batches per dosage strength, which comply with the specification, have been presented.

Stability

Stability studies in line with the relevant stability guidelines were conducted on pilot scale batches. In the course of these studies a decrease of the PMT/PMT-T, and an increase in the impurity content have been observed. The overall stability of the product is considered acceptable. The stability data justify a shelf-life of 2 years at a temperature up to 25 °C.

The in-use stability was examined in accordance with the CVMP Note for Guidance: In-use stability testing of veterinary medicinal products (EMEA/CVMP/424/01). The proposed in-use shelf life of 28 days is considered acceptable.

Overall conclusions on quality

Overall the documentation on quality is satisfactory and in compliance with current rules and guidelines. Although production scale validation and stability studies (active substance and finished product) are still outstanding at the time of approval, the pilot scale data provided were sufficient to indicate the process yields a robust, reproducible product which is stable for the claimed shelf life.

Part 3 – Safety

Some safety studies were already provided with the respective MRL application dossier for tildipirosin, and detailed descriptions and assessments of the studies are included in the "Tildipirosin European public MRL assessment report" (EPMAR) (EMA/CVMP/709377/2009).

Pharmacodynamics

Primary pharmacodynamics: Please refer to part 4 (efficacy)

Secondary pharmacodynamic effects

Secondary pharmacodynamic effects have been assessed in two safety pharmacology studies in dogs with intramuscular administration of tildipirosin. In consideration of the structural similarities between tildipirosin and tilmicosin, and the known cardiotoxic potential of the latter substance, whether tildipirosin has similar cardiovascular effects as tilmicosin was investigated thoroughly.

First a single-dose tolerability study in dogs with intramuscular administration of tildipirosin was performed to identify appropriate dose levels for the subsequent pivotal cardiovascular safety pharmacology study. This second study was designed as a telemetry study following guideline ICH S7A and conducted in compliance with GLP regulations. It was performed to evaluate possible effects of tildipirosin on blood pressure, heart rate, and electrocardiogram (ECG) lead II. Tilmicosin was used as the reference compound. The cardiotoxic potential of tildipirosin proved to be lower than the known cardiotoxic potential of tilmicosin with a NOEL for cardiovascular effects (small decrease in pulse pressure) of 10 mg/kg bodyweight. Collectively, there is also no evidence for other secondary

pharmacodynamic effects of tildipirosin below toxic dose levels. This is based on the review of literature on the class of macrolide antibiotics and of all studies on tildipirosin safety and effectiveness. However, the CVMP considered it prudent to add a warning in the SPC and product literature that such reactions might be possible in humans. Also, there are data in pigs that show deaths after intravenous injections, with the underlying mechanisms still unknown. Appropriate user warnings have therefore been introduced to the product literature.

Pharmacokinetics

See also part 3B and part 4

Absorption

Absorption of tildipirosin was rapid after oral administration to Wistar rats and Beagle dogs. Blood plasma concentrations of tildipirosin declined with elimination half-lives of 6-17 hours. Tildipirosin plasma concentrations did not differ significantly between genders. The oral bioavailability of tildipirosin was substantially higher in dogs than in rats. No potential for accumulation of tildipirosin was evident by comparison of single and repeated once daily administration.

(Plasma/Tissue) Distribution

After repeated oral administration of ^{14}C-tildipirosin to rats and dogs, the highest radioactivity was detected in colon, followed by liver, kidney, brown fat, and muscle in both species.

Metabolism

Metabolites present in liver, kidney and urine of cattle and swine were also detectable in rats and dogs. Although the fraction of various metabolites varied among species, the metabolite profiles of target and laboratory animals are qualitatively similar.

Excretion

After repeated oral administration of ^{14}C-tildipirosin to rats and dogs, excretion of radioactivity was mainly through faeces (up to 92% in rats and 65% in dogs). The excretion of tildipirosin in urine was very low. Data on biliary excretion were not available and the physiological origin of the major metabolites observed in faeces was therefore unclear.

Toxicology

The toxicological profile of the active ingredient tildipirosin has been already assessed during the MRL procedure, and details can be found in the "Tildipirosin European public MRL assessment report (EPMAR)" (EMA/CVMP/709377/2009).

Single-dose toxicity

Tildipirosin is associated with low levels of acute toxicity in rodents at oral doses of up to 2000 mg/kg bw, and an intermediate level of acute toxicity in mice following intravenous administration ($6.25 < LD_{50} < 12.5$ mg/kg bw).

Repeat-dose toxicity

In rats, following oral administration, NOELs of 25 mg/kg (4 week) and 20 mg/kg (3 months) bw/day were established.

In dogs, the maximum tolerated oral dose was considered 200 mg/kg bw/day. In a 4-week oral toxicity study, a NOEL could not be established (20 mg/kg bw/day was considered to be a LOEL). In a 13-week toxicity study, a NOEL of 6 mg/kg bw/day was established, while in a 55-week repeat-dose toxicity study in dogs, a NOEL was considered at 10 mg/kg bw/day.

Reproductive toxicity

In rats and rabbits, 30 mg/kg bw/day was established as a NOEL for maternal toxicity and for foetal toxicity. No teratogenic potential of tildipirosin was observed in any study at any dose level.

Mutagenicity / Genotoxicity

Tildipirosin was tested in a comprehensive series of mutagenicity test systems. The results indicate that tildipirosin is not genotoxic.

Carcinogenicity

Due to the absence of a chemical relationship to known carcinogens, the negative results of genotoxicity assays and the lack of carcinogenic potential of other macrolide antibiotics, it is assumed that tildipirosin is devoid of a carcinogenic risk.

Studies of other effects

Based on the results of the acute and subchronic toxicology studies in rats and dogs, and target animal safety studies in cattle and pigs, it was concluded that tildipirosin does not exert **neurological effects** at dose levels lower than those that elicit other toxicological effects.

Tildipirosin was **not irritating** nor corrosive to skin and eyes.

The potential of tildipirosin to produce sensitization following topical exposure was evaluated in guinea pigs. Consistent with other macrolides the skin sensitisation with a 20% tildipirosin buffered aqueous solution (200 mg tildipirosin, app. 60 mg citric acid monohydrate, filled up to 1 ml with deionised water) was positive. Tildipirosin has to be classified as a **skin sensitizer**.

The assessment of risks related to **exposure to the human gut flora** from residues in food was performed in compliance with VICH Guideline 36. The information provided included *in vitro* testing of bacteria representing the human gut flora, testing in an *in vivo* rat model to provide excretion and metabolism data for orally administered tildipirosin, a microbiological assay to investigate microbiological activity in colon contents, *in vitro* testing and a literature review on the impact of pH on the microbiological activity of tildipirosin. MICs of tildipirosin were determined according to CLSI standards against 10 isolates from 10 bacterial groups sourced from the faecal microbiota of healthy unmedicated human volunteers. The resulting MIC_{calc} was 5.2 µg/ml and the estimated microbiological ADI was 125 µg/kg or 7.5 mg per person and day. No microbiological ADI with respect to resistance development was calculated.

Information pertinent to the assessment of **risk related to antimicrobial resistance due to exposure of humans to food borne resistant bacteria** and resistance determinants was provided in compliance with VICH Guideline 27.

Macrolides belong to critically important antibiotics in human as well as in veterinary medicine. Although not usually recommended, macrolides may be used for *Salmonella, E. coli* or enterococcus-infections in man and macrolides are in many Member States first choice for treatment of

campylobacter infections. In situations where the likelihood of fluoroquinolone resistance is high there are few evidence based alternative treatment options.

Tildipirosin has activity against *Campylobacter* spp with MICs of the wild type population in the range of 1-16 µg/l (MIC_{90}: 16 µg/ml). Similar *in vitro* activity was also shown for *Salmonella* and *E. coli* with MIC ranges of 4-16 µg/ml and 4- ≥ 64 µg/ml, respectively. The MIC_{90} for both organisms is 16 µg/ml. Tildipirosin seems to be less active than other macrolides against *Campylobacter spp.* and enterococci (MIC range 4- ≥64 µg/ml) but the number and selection of investigated isolates is insufficient for conclusions. Cross-resistance between tildipirosin and macrolides used in veterinary and in human medicine in relevant organisms is expected.

The total concentration of radioactive residues in excreta following administration of the recommended therapeutic dose of radioactively marked tildipirosin was above the 4 mg/l for up to at least 4-6 days. A literature review on cattle and pig colonic pH, *in vitro* testing data on the impact of acidic pH on the microbiological activity of tildipirosin and *in vitro* testing data from a microbiological assay on tildipirosin activity in faecal contents of cattle and pigs have been provided. However, the level and time of exposure of the gastrointestinal flora of food producing animals to tildipirosin and microbiologically active metabolites following parenteral administration is not exactly known. Estimation of exposure of the intestinal flora is, therefore, difficult.

Although information from *in vitro* testing of tildipirosin in *Campylobacter spp.* suggests a low risk for development or transfer of resistance, use of tildipirosin could select for resistant *Campylobacter* and for resistance genes in the intestinal flora of exposed animals. The likelihood depends on the exposure of the intestinal flora and on the number of animals exposed. Both these factors are difficult to estimate.

Food-borne exposure of humans to animal derived macrolide-resistant C*ampylobacter* and to resistance genes with relevance for other potential human pathogens has been shown. However, prevalence of *Campylobacter spp.* in beef and pork at retail, representing food from the target species of tildipirosin, is low, and tildipirosin belongs to an already existing class of antimicrobials with a spectrum of activity that is similar to substances already on the market. The risk that the use of tildipirosin in cattle and pigs selects for antimicrobial-resistant bacteria of human health concern is assumed to be comparable to other macrolides already marketed in Europe. However, due to the long acting characteristics it might constitute a higher risk than short acting injectables, whereas injectables normally have advantages over oral products as the local exposure to the gastrointestinal-tract is lower.

A discussion on possible effects of use of tildipirosin on emergence and spread of MRSA has been provided. Risks related to large animal associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) predominantly represented by ST 398 as an occupational hazard has not been addressed specifically. It can be anticipated that the risk level is comparable to other macrolides in a similar way as for foodborne bacteria. Notably there will be no local exposure to the skin and mucosa in the nostril area (as from oral formulations) with this injectable.

Provided that the overall use of macrolides with similar spectrum is not increased by this new addition, the overall risk from use of macrolides in target species is assumed to be unchanged. Thus, specific risk management measures or Post Marketing Authorisation Resistance Surveillance (PMARS) were not deemed necessary.

User safety

Accidental user exposure may occur in the context of administering the product to the target animal species cattle and pigs. Users are identified as professionals (veterinarians) and, therefore, the likelihood of exposure can be considered to be limited.

Exposure assessment

In rare cases, users could be exposed to Zuprevo during filling the syringe with the product, administering the product to the animal, or at disposing the syringe and needle. The user could be exposed to Zuprevo by accidental self-injection, oral uptake or skin/eye contact.

Parenteral exposure

Accidental human injection represents the worst case user exposure scenario as compared to other routes, because tildipirosin is readily absorbed from the injection site as demonstrated in the target animal species. Assuming a worst case scenario of a maximum single dose of 10 ml of the cattle presentation, a single exposure would result in a dose of 1800 mg per person or 30 mg/kg bw.

Dermal exposure

Another accidental exposure route to the product is considered to be by skin contact. In the worst case the user is assumed to be exposed dermally to 10% of the intended dose of 10 ml of the cattle formulation. A skin penetration of 10% for the external (contacted) dose can be assumed leading to an internal dose of 18 mg tildipirosin per user or 0.3 mg/kg bw for the average 60 kg-person. The figure for the 40 mg/ml solution for injection intended for pigs is 0.033 mg/kg bw.

Oral exposure

Oral ingestion is considered to be negligible when elementary personal hygiene by professional users is maintained. Otherwise, oral exposure may occur when the user is smoking or eating with contaminated hands. Only low exposure volumes are expected in such situations.

Ocular exposure

Single ocular exposure may result from eye contact with contaminated hands or accidental direct splashing. Whereas contamination is considered to be negligible when elementary personal hygiene by professional users is maintained, direct splashing has to be taken into account for qualitative risk characterization, because manipulation of nervous animals can lead to an unexpected movement.

Hazard identification

In mice, the maximum non-lethal dose of the active substance, tildipirosin, after intravenous administration was 6.25 mg/kg bw. Data from single dose tolerability studies in dogs with intramuscular administration of tildipirosin demonstrate a small decrease in pulse pressure at a dose of 20 mg/kg bw. In the target animal species the parenteral administration of tildipirosin at dose levels of 20 mg/kg (swine) and 40 mg/kg (cattle) bw was tolerated with only minor systemic effects. An overall NOEL of 10 mg/kg bw has been established.

The excipients are used as standard solvents or vehicles, and not considered a hazard.

Risk characterisation - Risk management and communication

Potential exposure routes to the product are by skin and eye contact, and by accidental self-injection. Oral exposure is considered to be negligible when elementary personal hygiene by professional users is maintained.

Accidental self-injection represents the worst case user exposure scenario compared to other routes, and might lead to human health effects, involving effects on the cardiovascular system. The margin of exposure is below 100 for both formulations when using the maximum volume as a worst case. Appropriate and adequate information and warning statements are included in the current product literature to ensure the safe and correct use of the products.

Environmental risk assessment

The environmental risk assessment was performed according to the relevant VICH guideline. For both target species, pigs and cattle, at the highest recommended dose the initial Predicted Environmental Concentration in soil is lower than the trigger value of 100 µg/kg. In line with VICH GL6 (CVMP/VICH/592/1998), the environmental risk assessment can therefore stop at Phase I. Based on the data provided, Zuprevo is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

Tildipirosin proved to be of low oral acute toxicity in rodents (LD_{50} < 2000 mg/kg bw). After intravenous injection the acute toxicity was clearly more pronounced, with a maximum non-lethal dose of 6.25 mg/kg bw in mice.

Repeated oral dose toxicity studies in rats and dogs revealed that the liver was the main elimination organ of tildipirosin and sensitive target for toxicity reflected by increased liver enzyme activity with disturbance of lipid metabolism, increased organ weight and morphological changes in rats and dogs as the main toxicological endpoints. An overall NOEL of 10 mg/kg bw has been established in the most sensitive laboratory species (55 week study in dogs).

In oral toxicity studies on reproduction over up to two generations in rats, and on prenatal development in rabbits and rats, no effects of tildipirosin on embryo-foetal development were observed below maternal toxic dose levels. The lowest NOEL was 20 mg/kg bw/day over two generations. No teratogenic potential was observed at any dose level in any study.

Tildipirosin proved not to be genotoxic.

Due to the absence of a chemical relationship to known carcinogens, the negative results of genotoxicity assays and the lack of carcinogenic potential of other macrolide antibiotics it is assumed that tildipirosin is devoid of a carcinogenic risk.

Based on the results of the acute and subchronic toxicology studies in rats and dogs, and target species safety studies in cattle and pigs, it was concluded that tildipirosin does not exert neurological effects at dose levels lower than those that elicit other toxicological effects.

Tildipirosin was not irritating or corrosive to skin and eyes. The potential of tildipirosin to produce sensitisation following topical exposure was evaluated in guinea pigs. Consistent with other macrolides Tildipirosin has to be classified as a skin sensitiser.

Secondary pharmacodynamic effects have been assessed in safety pharmacology studies in dogs with intramuscular administration of tildipirosin. At dose levels lower than those required to elicit toxicity,

there was no evidence that tildipirosin exerts secondary pharmacodynamic activity on organs of concern (renal, gastrointestinal, neurological, cardiovascular, or respiratory system). The NOEL for cardiovascular effects (small decrease in pulse pressure) following intramuscular administration was 10 mg/kg bw.

Information pertinent to the assessment of risk related to antimicrobial resistance due to exposure of humans to food borne resistant bacteria and resistance determinants was provided in compliance with VICH Guideline 27. Tildipirosin has moderate activity against *Campylobacter, Salmonella and E coli.* Information from *in vivo* and *in vitro* testing of tildipirosin in faecal contents of cattle and pigs has been provided. Both, intestinal exposure following treatment and expected incidence of use is difficult to estimate. However, the risk that the use of tildipirosin in cattle and pigs selects for antimicrobial-resistant bacteria of human health concern is assumed to be comparable to macrolides with similar spectrum of activity that are already marketed in Europe. Provided that the overall use of such macrolides is not increased by this new addition, the overall risk in target species populations is assumed to be unchanged. MRSA in food producing animals is an occupational hazard of those in contact with live animals. A discussion on possible effects of use of tildipirosin on emergence and spread of MRSA was provided. The likelihood of spread between animals and ultimately to man is expected to be no other than for other macrolides. A microbiological assessment was performed in compliance with VICH Guideline 36. The estimated microbiological ADI was 125 µg/kg or 7.5 mg per person and day. No microbiological ADI with respect to resistance development was calculated.

Accidental user exposure route to the product is considered to be by skin and eye contact. Oral exposure is considered to be negligible when elementary personal hygiene by professional users is maintained. However, accidental self-injection of a full dose (worst case user exposure scenario) might lead to serious human health effects, involving effects on the cardiovascular system. Appropriate and adequate information and warning statements are included in the current product literature to ensure the safe and correct use of the products.

Zuprevo is not expected to pose a risk for the environment when used according to the SPC

Residues

Some studies were already provided with the respective MRL application dossier for tildipirosin, and detailed descriptions and assessments of the studies are included in the respective "Tildipirosin European public MRL assessment report (EPMAR)" (EMA/CVMP/709377/2009).

Residue studies

Pharmacokinetics - see also part 3A and part 4.

Several kinetic studies of tildipirosin in the target species cattle (ruminant and pre-ruminant) and pig were provided on the pharmacokinetic profile of tildipirosin in blood plasma, bronchial fluid and lung tissue. Single subcutaneous (cattle) and intramuscular (pig) injection of tildipirosin at dose rates of 2, 4 or 6 mg /kg bw, respectively, resulted in rapid absorption from the site of injection in both target species.

To investigate the metabolism of tildipirosin, radio-labelled studies were conducted in cattle and pig after single subcutaneous and intramuscular administration of therapeutic doses of 4 mg/kg bw and 5 mg/kg bw ^{14}C-drug, respectively. The main component found in urine and faeces as well as in all tissues of cattle and pig was unchanged tildipirosin, which was selected as marker residue. Highest tildipirosin residues were detected in kidney, liver and at the injection site. Residue concentrations in fat or fat/skin and muscle were substantially lower.

Depletion of residues

Two GLP compliant tissue depletion studies, one for cattle and one for pig, were designed as marker residue studies to determine tildipirosin concentrations in muscle, liver, kidney, fat or skin/fat and injection site muscle of the target animals and to derive withdrawal periods. The target animals were treated with the final commercial formulation of tildipirosin. The dose and the route of administration were those intended for marketing. A sufficient number of animals (4-6/group) and slaughter time points (4-7) were investigated. The sampling at the injection site (core and surrounding tissue) was according to the relevant CVMP-Guideline on injection site residues (EMEA/CVMP/542/03-Final).

Cattle (298-348 kg bw) were treated with the recommended single subcutaneous injection of 4 mg tildipirosin/kg bw). Tissue residues were determined up to 63 days post dose using a validated SEP-HPLC-MS/MS method (LOQ: 50 µg/kg). At the initial sampling time, highest mean concentrations of tildipirosin were present at the injection site (core tissue: 36693 µg/kg) followed by kidney (8600 µg/kg) and liver (5524 µg/kg) and lowest mean concentrations were present in fat (460 µg/kg) and muscle (324 µg/kg). At day 63 after dosing, mean concentration has declined to 1333 / 2124 µg/kg at the injection site (core/surrounding tissue), around 600 µg/kg in kidney and liver and below the LOQ in fat and muscle.

Pigs (30.1-38.4 kg bw) were treated with the recommended single intramuscular injection of 4 mg tildipirosin/kg bw. Tissue residues were determined up to 16 days post dose using a validated SPE-HPLC-MS/MS method (LOQ: 50 µg/kg). At the initial sampling time, highest mean concentrations of tildipirosin were present in the kidney (11320 µg/kg) followed by the injection site (core tissue: 8649 µg/kg), liver (4145 µg/kg), skin/fat (721 µg/kg) and were lowest in muscle (328 µg/kg). At day 16 after dosing, mean concentrations had declined to 2390 µg/kg in kidney, 1761 µg/kg at the injection site (core tissue), 1928 µg/kg in liver, 184 µg/kg in skin/fat and 68 µg/kg in muscle.

MRLs

Tildipirosin is listed in Table 1 of allowed substances of the Annex of Regulation (EU) No 37/2010 with MRLs for tissues of cattle and pig. For bovine tissues, MRLs are established of 400 µg/kg for muscle, 200 µg/kg for fat, 2000 µg/kg for liver, 3000 µg/kg for kidney with tildipirosin as marker residue, and a marker concentration of 11500 µg/kg as a reference value for the assessment of the injection site residues. There is no MRL established for tildipirosin in milk in bovines.

For pig tissues MRLs are established of 1200 µg/kg for muscle, 800 µg/kg for skin/fat, 5000 µg/kg for liver, 10000 µg/kg for kidney with tildipirosin as marker residue, and a marker concentration of 7500 µg/kg as a reference value for the assessment of the injection site residues.

Other ingredients of the intended product Zuprevo are included in Table 1 of the Annex of Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, or considered as not falling within the scope of Regulation (EC) No 470/2009.

Withdrawal periods

Based on the established MRLs and using the results from the marker residue studies withdrawal periods for the target tissues of cattle and pigs including the injection site were calculated in accordance with the CVMP Note for guidance: Approach towards harmonisation of withdrawal periods (EMEA/CVMP/036/95) and the CVMP Guideline on injection site residues (EMEA/CVMP 542/03).

Using the statistical approach, withdrawal periods were estimated separately for all tissues by linear regression analysis of the residue data and defined as time point when one-sided upper 95% tolerance

limit with 95% confidence falls below respective MRLs or below the reference value for the injection site residues. The longest of these withdrawal periods is proposed as the overall withdrawal period.

The calculated overall withdrawal periods for the edible tissues are 47 days for cattle and 9 days for pigs. According to the SPC the injection volume per site of application is restricted to 10 ml in cattle and 5 ml in pigs.

There is no MRL established for tildipirosin in milk in bovines. The product is therefore not authorised to be used in lactating cattle producing milk for human consumption, and should also not be used in pregnant animals, which are intended to produce milk for human consumption. However, the applicant provided evidence from radiolabelled studies, demonstrating that 60 days post application virtually the entire dose has been excreted, and appreciable residues of tildipirosin do not remain in body compartments of the cow. An estimate based on excretion data and residue depletion data showed that the portion of tildipirosin-related residues excreted via faeces and urine till day 60 post treatment is at least 97% of the administered dose. At the same time, total residues recovered from the major edible tissues (liver, kidney, muscle, fat, injection site) on day 63 comprise approx. 3% of the administered dose. Therefore, it is considered that two months after treatment the fraction of the dose which could theoretically lead to residues in milk is very low (near "zero"). The CVMP accepted these data and concluded that the use in pregnant animals could be acceptable up to 2 months before parturition. The following sentence was therefore added to the SPC and product literature: "Do not use in pregnant animals which are intended to produce milk for human consumption, within 2 months of parturition".

Analytical methods

A fully validated physicochemical analytical method for the determination of tildipirosin in tissues of cattle and pig was applied in the residue depletion studies. Analysis of the marker residue was performed using a SPE—HPLC-MS/MS method with quantification by internal standard calibration with deuterated tildipirosin as internal standard. The limit of quantification was 50 µg/kg for all tissues of the target animals. The method meets the acceptance criteria according to the requirements laid down in Volume 8 of the Rules Governing Medicinal Products in the EU and is considered suitable to determine tildipirosin quantitatively in bovine and porcine edible tissues.

Overall conclusions on the residues documentation

Tildipirosin is rapidly absorbed from its parenteral site of administration. *In vitro* binding of tildipirosin to plasma proteins is limited (approximately 30%), and tildipirosin is distributed to a large extent to therapeutic target areas (bronchial fluid, lung tissue). The plasma pharmacokinetic profile in pre-ruminant calves is similar to that observed in the adult animals.

The metabolism of tildipirosin and the presence and persistence of tildipirosin related residues in both target animal species were assessed by radiometric residue depletion studies, demonstrating the major component as unchanged tildipirosin. Therefore, unchanged tildipirosin was established as marker residue.

For the purpose of calculating withdrawal periods the depletion of the marker residue concentration was investigated in a marker residue study in bovine and porcine tissues muscle, liver, kidney, fat or skin/fat and injection site muscle. Cattle were treated with the recommended single subcutaneous injection of 4 mg tildipirosin/kg bw and tissue residues were determined up to 63 days post application. Pigs were treated with the recommended single intramuscular injection of 4 mg tildipirosin/kg bw and tissue residues were determined up to 16 days post application. A validated analytical method (SPE-HPLC-MS/MS) was available for the determination of tildipirosin in tissues of

both target animals (LOQ: 50 µg/kg in all tissues). The design and conduct of the studies were accurate (GLP compliant) and all current requirements were taken into account.

In cattle and pig highest residue concentrations were detected in kidney, liver and at the injection site. Fat, skin/fat and muscle showed the lowest concentrations of tildipirosin.

Based on the marker residue data and the MRLs established by the CVMP, withdrawal periods for edible tissues of 47 days for cattle and 9 days for pigs were calculated, using the statistical approach according to CVMP guidelines. The injection volume per site of application is restricted to 10 ml in cattle and 5 ml in pigs. The product should not be used in lactating cattle producing milk for human consumption and should also not be used in pregnant animals, which are intended to produce milk for human consumption, within 2 months of expected parturition.

Part 4 – Efficacy

Pharmacodynamics (cattle, pigs) - See also part 3A

Tildipirosin is a semi-synthetic 16-membered ring macrolide antimicrobial agent. The mode of action of tildipirosin is comparable to that of other macrolides. The spectrum of activity of tildipirosin includes Gram-positive and Gram-negative bacteria including common pathogens of the respiratory tract of swine and cattle: *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Haemophilus parasuis, Actinobacillus pleuropneumoniae and Bordetella bronchiseptica*.

MICs of epidemiologically unrelated target pathogens, isolated from pigs (n=349) and cattle (n=*256*) affected with respiratory disease in different European countries (Germany, France, the Netherlands, Hungary, Spain, Belgium, Denmark, Poland and the United Kingdom) during the years 2005 and 2010 were provided and are summarized in the following tables:

Pig:

Species	**Range (µg/ml)**	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**
Actinobacillus pleuropneumoniae (n=100)	2-16	8	8
Bordetella bronchiseptica (n=87)	0.5-8	2	4
Pasteurella multocida (n=99)	0.125-2	0.5	1
Haemophilus parasuis (n=63)	0.032-4	0.5	1

Cattle:

Species	**Range (µg/ml)**	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**
Mannheimia haemolytica (n=88)	0.125-2	0.5	1
Pasteurella multocida (n=105)	0.125-2	0.5	1
Histophilus somni (n=63)	0.5-8	2	4

Data showed mono-modal MIC distribution profiles for target species indicating that no acquired resistance was demonstrated among included isolates. However, in the partial absence of detailed information about e.g. production forms or rearing conditions of the sampled animals it is uncertain to what extent this data set reflects the overall epidemiological situation in Europe.

Results for MIC determinations of swine and bovine target pathogens isolated during clinical studies were generally comparable to those determined in preclinical MIC studies. Since clinical isolates were most likely epidemiologically related, these data were supportive only.

Similar to other macrolides, the *in vitro* antimicrobial activity of tildipirosin is influenced by the pH, serum concentration and incubation with or without 5% CO_2 level.

Like other macrolides tildipirosin acts principally bacteriostatic and time dependant. Bactericidal effects could be demonstrated *in vitro* for the target pathogens *M. haemolytica, H somni, A. pleuropneumoniae and H. parasuis,* but not for *B. bronchiseptica.* Bacteriostatic effects prevailed in *P. multocida*.

Effects such as PAE (post antibiotic effects) and PASME (post antibiotic sub-MIC effect) are not considered clinically relevant for a product with the particular pharmacokinetic spectrum of tildipirosin.

For macrolides, a PK/PD analysis based on conventional PK/PD surrogate markers in plasma is not considered applicable. Also, a PK/PD model provided for tildipirosin using the T>MIC in bronchial fluids/lung tissue as pivotal parameter has limitations and does not allow to draw firm conclusions to predict clinical efficacy.

Development of resistance

Tildipirosin is a ribosome-targeting drug and acts by inhibiting protein synthesis. Tildipirosin most likely binds to the same site at the ribosome as other macrolides and lincosamides. Like other 16-ring-member macrolides and lincosamides, resistance is mediated by methylases encoded by transferrable erm-genes. Mutations in certain positions of the target site may also confer resistance. Tildipirosin is barely exported from the cell by the Msr(A) or Lsa(B) efflux systems, and the *in vitro* activity of tildipirosin is hardly affected by macrolide- or lincosamide-inactivating enzymes.

Data suggest that co- and cross-resistance of tildipirosin will generally be comparable to other 16-membered-ring macrolides. However, due to mono-modal MIC distribution profiles for target species, the issue of co- and cross-resistance in relation to potential resistance among target pathogens was not fully clarified. Thus, complete cross-resistance between all macrolides and lincosamides must be assumed for tildipirosin, and the SPC and product literature include appropriate warnings.

Pharmacokinetics (cattle, pigs)

Cattle

Several pharmacokinetic studies with tildipirosin 180 mg/ml solution for injection in cattle have been provided. Some of these studies had already been discussed during the MRL procedure.

The pivotal pharmacokinetic studies were conducted with the commercial formulation and in compliance with GLP. The pharmacokinetic profile of the substance in plasma and bronchial fluid collected *in vivo* from cattle has been examined after a single subcutaneous injection of the product into the neck of the target animals at doses ranging from 1 to 20 mg/kg bw and, thus, including the recommended treatment dose of 4 mg/kg bw. Blood and bronchial fluid samples have been collected at appropriate intervals over a period of up to 21 days. Lung tissue samples (collected until day 28) have been examined for tildipirosin residues in addition.

Tildipirosin was rapidly absorbed from the subcutaneous injection site (T_{max} of 23 min) with a high bioavailability of 79% when compared to intravenous injection. It reached maximum plasma concentrations (approximately 700 ng/ml) in less than one hour. The mean terminal half life is approximately 9 days. Due to its high distribution volume (approximately 50 l/kg) the compound

rapidly occurred in bronchial fluid and lung tissue. In lung, mean tildipirosin concentrations reached a maximum of 14800 ng/g within 24 hours and slowly declined to 3000 ng/g at 21 days after treatment, indicating rapid absorption of the drug in the target tissue. The lung-to-plasma ratio reached 214 on day 10 and remained above 180 after 21 days. In bronchial fluid (*in vivo)*, tildipirosin levels kept a maximum of 3500 ng/g between 24 and 72 hours after treatment and slowly declined to 1000 ng/g until day 21 after treatment. The mean bronchial fluid-to-plasma ratio reached a maximum of 72 after 10 days.

No PK/PD relationship has been established. It is acknowledged that plasma might not be a relevant compartment for pharmacokinetics but neither is lung homogenate. The relevance of bronchial fluid collected *in vivo* from cattle remains uncertain.

The degree of protein binding rate in bronchial fluid and plasma was 30% and the kinetics were dose-linear in the tested dose range.

To investigate the metabolism of tildipirosin, radio-labelled studies were conducted in cattle and pig after single subcutaneous and intramuscular administration of therapeutic doses of 4 mg/kg bw and 5 mg/kg bw ^{14}C-drug, respectively. Tildipirosin in cattle is metabolised by cleavage of the mycaminose sugar moiety, by reduction and sulphate conjugation with subsequent hydration (or ring opening), by demethylation, by mono- or dihydroxylation with subsequent dehydration and by S-cysteine and S-glutathione conjugation. The main component detected in liver, kidneys, urine and faeces was, however, unchanged tildipirosin.

Pigs

The pivotal pharmacokinetic GLP study provided information about the pharmacokinetic profile of tildipirosin in plasma, bronchial fluid and lung homogenate of pigs, when treated with the final formulation at three different doses (2, 4 and 6 mg/kg bw).

Tildipirosin was rapidly absorbed after single intramuscular administration. After intramuscular administration of the recommended therapeutic dose (RTD) of 4 mg tildipirosin/kg bw T_{max} and C_{max} averaged at 0.38 hours and at 900 ng/ml. At all three dose levels tested, a long persistence of tildipirosin in the animal is demonstrated by a long terminal half life ($T_{1/2}$ > 4 days) and a long mean residence time ($MRT_{(0\text{-}LOQ)}$ > 3.5 days). After administration of the RTD of 4 mg tildipirosin/kg bw, tildipirosin accumulates at the site of respiratory tract infection. Peak tildipirosin concentrations above 14000 ng/g were demonstrated in bronchial fluid (at 5 days after treatment), and above 4000 ng/g in lung homogenate (at 24 hours after treatment) far exceeding those in blood plasma. Tildipirosin concentrations remained above 6000 ng/g in bronchial fluid and 1000 ng/g in lung homogenate for at least 14 days after treatment with concentrations representing a multiple of those in plasma.

No PK/PD relationship has been established. It is acknowledged that plasma might not be a relevant compartment for pharmacokinetics but neither is lung homogenate. The relevance of bronchial fluid remains uncertain and the concentration in bronchial fluids was surprisingly high (even higher than in lung homogenate) indicating that the method used might not appropriately record pharmacologically active concentrations.

Like in cattle, plasma protein binding of tildipirosin proved to be limited (approximately 30%).

To investigate the metabolism of tildipirosin, radio-labelled studies were conducted in cattle and pig after single subcutaneous and intramuscular administration of therapeutic doses of 4 mg/kg bw and 5 mg/kg bw ^{14}C-drug, respectively. Tildipirosin in pigs is metabolised by reduction and sulphate conjugation with subsequent hydration (or ring opening), by demethylation, by dihydroxylation and by

S-cysteine and S-glutathione conjugation. The main component detected in liver, kidneys, urine and faeces was, however, unchanged tildipirosin.

Target animal tolerance

Cattle

Target animal safety studies

The applicant has studied target animal safety with the final formulation in three well conducted pivotal GLP-compliant tolerance studies addressing local and systemic effects. With respect to the claimed indication, the age of the animals ranging from 2 to 9 months was adequately chosen.

Acute tolerance was studied by a single subcutaneous administration to the neck of 10-times the recommended dose to cattle with an observation period lasting until 7 days post treatment. Investigation of blood samples addressing numerous parameters of haematology, coagulation and clinical chemistry and also urinalysis parameters did not reveal any findings of clinical relevance. Mild focal necroses were seen in 3 out of 4 livers in the tildipirosin-group, i.e. in 75% of the treatment animals and in none of the controls; however, this observation (also known as "sawdust liver") is considered to be common at necropsy mainly in feedlot cattle. A clear pathogenesis is not known; focal necrosis is said to occur in many infections and in instances of biliary obstructions. This finding may therefore be interpreted as incidental as there is no sound basis to relate it to the veterinary medicinal product. Apart from these and reactions at the injection sites, the results of the complete gross necropsy and the histopathologic evaluation did not give grounds for treatment related effects.
The injection caused strong signs of distress in tildipirosin-treated animals, and a swelling at the site of injection developed within one hour after administration, and one day after administration pain and heat developed. However, these signs were found to be transient. Necropsy and histophathologic findings showed signs of degeneration, inflammation and probably first signs of regeneration at 7 days post administration. It should be noted that a saline-treated control group with injection volumes equivalent to the treated group did not result in abnormal reactions apart from mild head shaking in 2 animals, and did not reveal any swellings.

The applicant also provided a tolerance study with administration of the recommended (1x = 4 mg tildipirosin/kg bw) and multiples of the recommended dose (3x and 5x), given at multiples of the recommended treatment frequency (3 treatment occasions 7 days apart instead of 1 single administration). The animals were observed until 21 days after start of treatment, i.e. 7 days after the third treatment. As for each treatment occasion a different injection site was chosen, different injection sites could be observed at 7, 14 and 21 days post administration.

At the end of the study no treatment-related or clinically relevant effects were noted concerning systemic tolerance based on haematology, coagulation and clinical chemistry parameters, urinalysis, complete gross necropsy and histopathological evaluation. With respect to the injection sites, animals showed signs of distress and swellings associated with pain in some animals, mostly in the higher dose groups. These swellings which were found to be transient were recorded predominantly in animals injected into the neck. Macro- and microscopically there were no differences in severity and incidence across the groups 7 and 14 days after dosing. The main differences could be seen in the chronology of the reparative process. Considering that nearly no lesions were observed at the oldest injection site (treatment 1) in the 1x dose group, resolution of the reactions can be expected 3 weeks after administration of the recommended dose. However, it has to be taken into consideration that the maximum volume of the 1x dose was 5 ml (range in males and females: 3.5 - 5 ml) in this study.
No alterations were found in the saline-treated group. The CVMP concluded therefore that Zuprevo has

tissue-irritating properties; however, the observed injection site reactions are transient and are considered tolerable.

In a third tolerance study, performed with the final formulation of the product, clinical and local effects following the subcutaneous injection of the maximum intended injection volume of 10 ml per site were investigated up to 14 days after administration.

Injection site swellings developed within 1 to 4 hours post treatment in all animals. The number and size of swellings decreased over time, but were palpable at least until 3 weeks after treatment. Some animals developed noticeable swellings up to about 339 cm^3 one week and up to about 174 cm^3 two weeks after treatment, seeming to not cause pain or heat by palpation, or abnormalities in locomotion and behaviour. However, swelling of such considerable size were the exception and they resolved within a limited period of time.

Necropsy results revealed that 35 and 28 days after treatment administration, subcutaneous tissue still showed some alterations although minimal (e.g. thickened tissue, red or tan foci) while the surface muscles were not affected at these time points. This observation means that recovery of injection sites with the maximum injection volume of 10 ml per site will take about 5 weeks. A respective note was included in the product literature.

Target animal safety in field studies

No systemic signs of intolerance due to treatment were observed in the clinical studies. This observation is in line with the tolerance studies. With respect to local tolerance, it was found that the administration of 180 mg/ml solution for injection resulted in pain on injection and in injection site reactions. These findings are adequately addressed in the product literature.

Tolerance (cattle) - Conclusions

Based on the results of the preclinical and clinical findings, the CVMP concluded that subcutaneous injection of Zuprevo is connected with little or no systemic toxicity in cattle; however, treatment is connected with transient local injection site reactions associated with distress and pain. Appropriate warnings are included in the product literature.

Pigs

Target animal safety studies

Three pivotal GLP target animal safety studies (TAS) in line with the current VICH-TAS guidelines and several non-pivotal studies were carried out between 2005 and 2009 in animals aged 7-19 weeks at study beginning.

Two pivotal GLP- studies were controlled studies with physiological saline solution as negative control. Tildipirosin was injected on 3 treatment occasions, 4 days apart, in doses corresponding to the recommended therapeutic dose (RTD) and multiples thereof (2x RTD, 3x RTD, 5x RTD). The maximum injection volume was 5 ml per site. The pivotal GLP- study on tolerance of the maximum injection volume of 5 ml was run without a control group. In that study the maximum injection volume of 5 ml was administered once. Injection sites were clinically examined *in vivo* over a specified period. At necropsy the injection sites were examined macroscopically. The design of the studies was considered suitable for the safety assessment of the test product at the RTD and at (multiple) overdoses.

Systemic adverse reactions were reported in three studies:
Transient subdued behaviour after the injection occurred in all dose groups tested in the margin of

safety study. In that study, transient tremor in both hind legs was observed after the 3x and 5x RTD, as well as transient body tremor, disability to stand and shock at the 5x RTD.

In the acute tolerance study, administration of the 5x RTD also resulted immediately after the injection in transient tremor in the hind legs, lameness and disability to stand. No other systemic treatment related changes were observed in these studies in respect to blood, urine and faecal analyses. Apart from findings at the injection sites, there were no treatment related gross and histopathologic lesions in these studies.

Similar observations of systemic reactions (transient body tremor, disability to stand and death (possibly shock) were also noted in the study on acute tolerance employing tildipirosin-doses of 6.25 fold RTD and more (37.5 and 50 mg/kg bw). Pathological examinations revealed cardiovascular failure.

The underlying mode and mechanism for the systemic intolerance was not satisfactorily clarified. The applicant argued that signs of intolerance such as subdued behaviour, lameness and muscle tremor would result from stress and discomfort as a consequence of handling and intramuscular injection. However, the CVMP disagreed with these conclusions, as no such systemic reactions were observed in pigs treated with a placebo under the same study conditions. Thus, the cause of these signs of intolerance is not considered fully explained. Therefore, detailed descriptions of these systemic reactions are included in the SPC and product literature.

Following **intravenous administration**, fatalities and severe signs of shock were noted at recommended dose levels. Thus, intravenous administration is listed as a contraindication in the SPC and product literature, and in addition advice is included to strictly inject the product intramuscularly only.

Clinical examination of the injection sites revealed local swellings and pain **at the injection site** after administration of saline, the RTD and overdoses. Macroscopic findings were subcutaneous or intramuscular reddening. Histological findings indicated a gradual progression from necrosis/inflammation to regeneration and fibroplasia in pigs receiving tildipirosin. Swellings persisted for up to 3 days. Pathomorphological injection site reactions resolved within 21 days. These findings are appropriately reflected in the SPC and product literature.

The recommended **maximum injection volume** is 5 ml per injection site. In animals weighing more than 50 kg, the product has to be administered at more than one site. Appropriate information is included in the SPC and product literature.

Target animal safety in the field studies

In the pivotal field study systemic adverse reactions were observed after administration of the RTD in two animals displaying signs of acute intolerance similar to signs observed in the TAS studies after overdoses (shock, convulsion, death in one animal). The study investigator considered these reactions as “possibly related to treatment”. Insufficient scientific data are provided to fully explain the cause(s) of these signs of intolerance. Thus, the serious adverse reactions observed during the clinical field trial are mentioned in the SPC and product literature.

The injection of 40 mg/ml solution for injection resulted in pain on injection and injection site reactions. These findings are also reflected in the product literature.

Tolerance - Conclusions (pigs)

Based on the results of the preclinical and clinical findings, the CVMP concluded that intramuscular injection of Zuprevo in pigs may be connected with systemic reactions (shock), apparent in transient

body tremor, convulsions, and disability to stand, and which might be fatal. In particular, following (accidential) intravenous administration, fatalities and signs of shock were noted at recommended dose levels. Treatment is also connected with transient local injection site reactions associated with distress and pain. Appropriate warnings are included in the product literature.

Since studies were not undertaken in piglets younger than 4 weeks, an appropriate warning was included in the SPC and product literature.

Dose determination / justification

In both cattle and pigs, the exposure following a single injection of Zuprevo is long in comparison with the effect of duration needed for treatment of respiratory disease according to the proposed indications. The applicant provided justification that no potential drug interactions are expected in the case of treatment failure and consequent change to another product would be needed, and the length of exposure is not expected to increase the risks for or severity of reactions or increase the risk for antimicrobial resistance in zoonotic agents or target animal pathogens.

Cattle

For dose determination the applicant provided two laboratory challenge studies undertaken in Germany (2005) using *Mannheimia haemolytica* as challenge strain, and three non-pivotal dose finding studies under US field conditions.

The final product formulation was only used in one of the pivotal studies. The challenge strain had a MIC of 0.25 µg/ml, which is at the lower border of the MIC range of the current field isolates (0.125-2.0 µg/ml). Although the MIC value of the challenge strain cannot be considered representative for the situation in the field, it is acknowledged that the challenge model requires virulence factors which are not necessarily correlated with susceptibility to antimicrobials. No laboratory dose determination studies were provided for the other two claimed pathogens, *Pasteurella multocida* and *Histophilus somni,* because of the absence of validated animal models.

Prevention

In one trial, aiming to derive a preventative dose, 3-4 months old calves were challenged by intratracheal inoculation 5 days after treatment with dose levels of 1, 2, or 4 mg tildipirosin/kg bw. All animals were slaughtered 3 days after challenge. The challenge resulted in a severe course of infection in the untreated control group and in the group dosed at 1 mg/kg bw. Based on clinical, post mortem (lung lesions) and bacteriological findings, a dose of 2 mg tildipirosin/kg bw was found to be the minimum effective dose in the prevention of respiratory disease due to *M. haemolytica*. Looking at the bacteriological results, the 4 mg dose group showed a better performance than the 2 mg dose group.

Treatment

In the other study, aiming to derive a treatment dose, animals were challenged first, treated 20-30 minutes later and slaughtered 10 days after challenge/treatment. The challenge resulted in a mild infection in the unmedicated control animals. Based on clinical, post mortem (lung lesions) and bacteriological findings tildipirosin proved to be effective at all dose levels tested (1.25, 2.5, and 5 mg/kg bw). However, considering that the antibiotic treatment was initiated already at 20 minutes after the experimental infection when the bacteria may still have been circulating and the infection is not yet established, this study gives limited information on treatment of an established *Mannheimia* infection.

None of these two studies allowed a clear conclusion on an optimum effective dose.

In addition, three **non-pivotal dose finding studies** on naturally diseased animals with signs of respiratory disease under US field conditions were provided. Tildipirosin was administered at dose levels of 5 and 10 mg/kg bw, at 1, 2 and 4 mg/kg bw and 2, 4 and 6 mg tildipirosin/kg bw, respectively, in the studies. All studies included a negative and a positive control (tilmicosin or tulathromycin). One of the studies was performed with a developmental formulation while the other two trials were conducted with the final formulation. Pathogens relevant for bovine respiratory disease (BRD) had been isolated in a number of animals before start of treatment; however, inclusion was based on clinical parameters, only. Also the treatment success evaluation was based on clinical endpoints, only.

With respect to two US studies, doses from of 4 mg tildipirosin/kg bw were found to be effective and superior to lower dose levels or saline. However, the result must be considered rather carefully because in one study, the saline group showed relatively high treatment success rates, which were not different to the ones of the groups given 1 mg and 2 mg tildipirosin/kg bw, respectively. In the third dose finding study all tested dose levels, i.e. 2 mg, 4 mg and 6 mg tildipirosin/kg bw were found to be effective. whereas 4 mg tildipirosin/kg bw was numerically more effective than the dose level of 2 mg/kg, and 6 mg tildipirosin/kg bw was not more effective than 4 mg tildipirosin/kg bw.

Consequently, the data indicate that tildipirosin was effective in the treatment of bovine respiratory disease at a dose range between 1.25 and 4 mg/kg bw. The data could not be considered robust enough to clearly conclude on an optimum effective dose. However, data suggest the dose of 4 mg tildipirosin/kg bw as the most appropriate for the treatment and prevention claim to cope with different disease conditions. Although the claimed pathogens, with the exception of *M. haemolytica* in the challenge model, have not been particularly well addressed in the dose finding process and although the dose finding results were not unambiguous, a dose of 4 mg tildipirosin/kg bw was used for the clinical field studies.

Pigs

Prevention

No dose determination studies for the prevention claim were undertaken.

Treatment

Four GLP or GCP compliant laboratory challenge studies conducted 2004-2005 in France and USA were presented employing *A. pleuropneumoniae* and *P. multocida*. Pigs aged 6-7 weeks were intranasally and/or intratracheally challenged, and treated once intramuscularly within a few hours after the challenge. Due to absence of validated animal models, dose-determination studies employing *B. bronchiseptica* (MIC_{90}= 4.0 µg/m) or *H. parasuis* (MIC_{90} = 1.0 µg/ml) were not provided.

A. pleuropneumoniae is the pathogen with the highest MIC_{90} of the pathogens claimed (MIC_{90} = 8.0 µg/ml), *P. multocida* is one of the pathogens with the lowest MIC_{90} of 1 µg/ml. One study used an *A. pleuropneumoniae* strain with a MIC of 8 µg/ml, the MIC of the strain used in the other study was 32 µg/ml. In both studies *P. multocida* strains with a MIC of 0.126 µg/ml were used, which was much lower than the reported MIC_{90} from different EU-areas of 1 µg/ml.

In all studies tildipirosin was tested in a pilot formulation at doses of 2.5, 5 and 10 mg tildipirosin/kg bw. Tulathromycin and untreated pigs served as positive and negative controls. The observation period was 10 days. Primary efficacy parameters were mortality and clinical scores. Secondary parameters included lung lesions weight and total lung lesion score. Taking into account the efficacy parameters

used, the observation period is deemed acceptable to allow conclusions, although tildipirosin is expected to persist in the target tissues/fluids at that point of time.

Studies with *A. pleuropneumoniae*: The studies were considered suitable to deduce an appropriate tildipirosin-dose. A dose of 2.5 mg tildipirosin/kg bw was effective in reducing lung lesion scores. Reduction of lung lesion scores after higher treatment doses were statistically not significantly different from 2.5 mg tildipirosin/kg bw. While mortality rate was significantly reduced with the 2.5 mg tildipirosin/kg bw dose in one study, a dose of 5 mg tildipirosin/kg bw was required to reduce mortality in the other.

Studies with *P. multocida*: One study failed. Validity of the other study was considered questionable. Thus, an effective dose as regards treatment of SRD caused by *P. multocida* with a MIC of 1 µg/ml could not be derived.

It is concluded that based on the data provided a dose higher than 2.5 mg tildipirosin/kg bw but slightly lower than 5 mg tildipirosin/kg bw was considered necessary to treat mixed infections under field conditions associated with *A. pleuropneumoniae, P. multocida, B.bronchiseptica* and *H. parasuis*. Thus, a dose of 4 mg tildipirosin/kg bw was considered to be tested in dose-confirmation studies.

Field trials

Cattle – bovine respiratory disease (BRD)

Two GCP-compliant controlled, multi-centred, randomised and investigator-blinded dose confirmation field studies were performed in Germany in 2007/2008, in support of the treatment, and the prevention claim. Both studies follow current standards, taking into account the relevant guidelines (GCP (CVMP/VICH/595/98-Final), efficacy testing of antimicrobials (EMEA/CVMP/627/01-FINAL) and statistics (EMEA/CVMP/816/00-Final)).

Age of the animals ranged from 1 to 15 months and is adequate with view to the claimed indication. Presence of pathogens relevant for bovine respiratory disease (i.e. *M. haemolytica, P. multocida* and *H. somni*) was confirmed at day 0.

In addition, in order to demonstrate the efficacy of tildipirosin in the treatment and prevention of BRD in Europe, the applicant also performed two field trials each with sites in France, Italy and Germany, which were considered as sufficient to cover the situation in the EU. One field study was designed to cover the treatment claim and the other to cover the prevention claim. Both studies follow current standards and were performed 2008/2009. Age of the animals ranged from 1 to 24 months. The design of the field studies is in principle the same as that for the respective dose confirmation studies performed under field conditions in Germany.

In response to questions, the applicant also provided further GCP-compliant controlled, multi-centred, randomised and blinded dose studies performed in the USA in 2008.

Treatment of BRD

Dose confirmation - Germany

The treatment claim was investigated in a comparative study (tildipirosin versus a positive control group) enrolled from 7 sites. A single subcutaneous injection of tildipirosin (180 mg/ml solution for injection) was given at the recommended dose of 4 mg/kg bw to animals with clinical signs attributable to bovine respiratory disease (rectal temperature ≥ 40 ºC and abnormal respiration and abnormal general attitude). Treatment success was based on defined improvement of respective clinical signs.

Study results demonstrated non-inferiority of tildipirosin compared to the positive control (florfenicol).

The Committee noted that treatment success was based on clinical parameters only, and that investigation of bacteriological cure was not taken into account although recommended by the current CVMP "Guideline on demonstration of efficacy for veterinary medicinal products containing antimicrobial substances" (EMEA/CVMP/627/01). The chosen approach was, however, considered acceptable in view of the multifactorial nature of the BRD complex, and in particular the fact that the isolation rates of the claimed principal pathogens, concomitant viral infections or infections with other bacterial pathogens may be variable. Bacteriological endpoints therefore cannot be considered suitable parameters for success evaluation, and relevant clinical endpoints would be acceptable. Also, the presence of the principal bacterial pathogens had been shown to some extent.

The CVMP expressed, however, some concerns about the study design in regard to the appropriateness of the chosen observation periods for success evaluation on day 14, and relapse evaluation on day 21. These time points were mainly based on PK/PD considerations on concentrations in lung tissue as target organ, and tildipirosin levels in lung homogenates were considerable at the respective time points. Therefore, with relation to the MIC_{90} values of the claimed pathogens, the success and relapse rates may not represent realistic results but may be overestimated with respect to success, and underestimated with respect to failure. This estimation concerns all clinical field studies.

Clinical field study – EU

The multicentre European field study investigated animals treated with tildipirosin or a positive control group using tulathromycin at sites in France, Italy and Germany. Non-inferiority of tildipirosin compared to the positive reference product (tulathromycin) was shown. Treatment success rate on day 14 was approximately 85% for tildipirosin and 80% for tulathromycin, and comparable rates of relapse (8% and 6%, respectively) and success rates on day 21 (78% and 75%, respectively).

However, the aetiology of the clinical signs at inclusion could not clearly be associated with the claimed BRD pathogens due to a considerable rate of concomitant viral respiratory pathogens. The CVMP expressed the same concerns as already for the dose confirmation field study conducted in Germany.

Clinical field study - USA

In-addition to the European field studies, the applicant also provided a GCP-compliant field study conducted under US field conditions in 2008 to support the efficacy of the proposed dose of a single subcutaneous injection of 4 mg tildipirosin/kg bw.

Calves from 15 different US salebarn auctions which met the inclusion criteria were transported to 5 different study sites. Inclusion criteria and criteria for success evaluation were comparable to the ones in the EU studies. The study included the tildipirosin-treatment group, a saline-treated negative control group and a positive control group treated with tulathromycin. Age of the animals ranged from 6 to 12 months. BRD aetiology was confirmed by isolation of the principal bacterial BRD pathogens by pre-treatment nasopharyngeal swab samples. At least 30 isolates per BRD pathogen across all five study sites were required to substantiate therapeutic efficacy.

The treatment success at day 14 was found to be significantly higher in the tildipirosin treated (76%) group compared to the negative control group (32%). Additionally, treatment success rates were not significantly different between tildipirosin and the positive control, tulathromycin (72%). There were no mortalities in the tildipirosin group, 4 deaths in the tulathromycin-treated group and 21 deaths in the saline-treated group. The mortalities were BRD-related.

No systemic adverse reactions attributed to tildipirosin were observed, adverse reactions were restricted to injection site reactions.

The CVMP considered this study a good supplement to the EU field studies, and concluded that overall, the studies would confirm the efficacy of a single subcutaneous dose of 4 mg tildipirosin/kg bw in the treatment of BRD.

Prevention of BRD

Dose confirmation - Germany

To demonstrate efficacy of the preventive use of tildipirosin, animals were enrolled from 4 sites, comparing tildipirosin and a positive control group (ITT population). Treatment of the clinically healthy calves started when at least 10% of pre-study animals sharing the same airspace showed the typical clinical signs of bovine respiratory disease. Tildipirosin was given at the recommended dose of 4 mg/kg bw. Prevention success was based on clinical critera: absence of increased body temperature and respiratory and attitude abnormalities.

Although tildipirosin appeared non-inferior as compared to the positive control (florfenicol), the data showed some statistical shortcomings, and results were not convincing as no negative control group was included. Under such study conditions it was, therefore, not really verifiable whether a BRD outbreak has actually been prevented or not. The number of isolates of principal BRD pathogens from pre-study (sentinel) animals was low, and these animals also showed a high degree of concomitant infections with respiratory viruses and mycoplasma.

Clinical field study – EU

The multicentre European field study compared tildipirosin and a positive control group using tulathromycin at 7 sites in France, Italy and Germany. The CVMP expressed the same concerns as for the dose confirmation study, i.e. an unsuitable study design based on non-inferiority testing, and involvement of only a positive control group.

Clinical field study - USA

In response to questions, the applicant provided a new GCP-compliant study, conducted under US field conditions for proving efficacy of tildipirosin at a single dose of 4 mg/kg bw for the control of BRD in the US. This multi-centred randomised and blinded study included the treatment group, a saline-treated negative control group and also a tulathromycin-treated positive control group. Age of the animals ranged from 6 to 12 months. Inclusion criteria and success evaluation were comparable to the EU studies and based on clinical parameters. From all animals nasopharyngeal swabs were taken to confirm the presence of the claimed bacteria.

However, the study design deviated somehow from current EU requirements, as the presence of known risk factors was considered sufficient to start treatment). The study design included requirements to confirm the BRD aetiology, i.e. 30 isolates per BRD pathogen across all study sites. A further requirement was that 20% of the negative control animals which came from one arrival truckload had to develop BRD, otherwise all animals from this truckload had to be excluded from statistical analysis. As this was the case a number of 39 animals per group had to be excluded from analysis. The severity of the disease may be classified as mild to moderate, there were only 2 deaths in the saline treated group and 1 death in the tildipirosin-treated group.

Success evaluation at day 14 showed that the success rate for tildipirosin treated animals (79%) was significantly higher than the saline treated negative control group (51%). In addition, comparison of all

three treatment groups gave evidence that tildipirosin treatment was as effective as the positive control group (77%). There were no systemic abnormal health observations. Adverse reactions were restricted to injection site reactions like in the EU studies.

As demonstrated by the inclusion of a negative control group, a disease outbreak with typical signs of BRD could be confirmed, supported by the isolation of the principle BRD bacteria. The CVMP therefore considered the data provided sufficient evidence to demonstrate efficacy of a single subcutaneous dose of 4 mg tildipirosin/kg bw in the prevention of BRD.

Taking into consideration the data from all available field studies, the CVMP concluded that a single subcutaneous dose of 4 mg tildipirosin/kg bw is effective in the treatment and also in the prevention of BRD. In addition, with respect to the nature of the BRD complex and taking into account that the prevalence of *Histophilus somni* appears to be low in general, the data were also considered sufficient to accept *H. somni* as target pathogen.

Pigs – swine respiratory disease (SRD)

The applicant provided the results of a number of well conducted GCP-compliant, controlled, multi-centred, randomised and investigator blinded dose-confirmation field studies in Europe and the USA, taking into account the relevant guidelines (GCP (CVMP/VICH/595/98-Final), efficacy testing of antimicrobials (EMEA/CVMP/627/01-FINAL) and statistics (EMEA/CVMP/816/00-Final)). The studies addressed the treatment and the prevention claim.

Treatment

Dose-confirmation field studies - Germany

The applicant conducted two GCP-compliant controlled, multi-centred, randomised and investigator blinded dose-confirmation field studies, both conducted in Germany in 2007/2008, involving a large number of pigs in the tildipirosin and in the positive control groups (florfenicol) at 2 and 4 sites, respectively. The pigs enrolled in the studies were aged 6 up to 14 weeks (8-72 kg bw) representing the target population.

A single intramuscular injection of tildipirosin (40 mg/ml solution for injection) was administered at the proposed dose of 4 mg/kg bw to pigs with clinical signs of SRD (rectal temperature ≥ 40 ºC and abnormal respiration and abnormal attitude). Treatment success was based on defined improvement of respective clinical signs.

Treatment success in one study was poor, and non-inferiority of tildipirosin compared to the positive control could not be shown. Concomitant mycoplasma and virus infections were considered the reason for this failing of antimicrobial therapy.

However, the second study demonstrated an acceptable treatment success, and non-inferiority of tildipirosin compared to the positive control, with an overall treatment success on day 10 of approximately 86% in animals treated with tildipirosin as compared to 81% for the florfenicol group, and similar rates of mortality (2% and 3%, respectively) and relapse (4% and 8%, respectively). Rectal temperatures, respiratory and attitude scores were markedly reduced from D1 in both groups. However, microbiological confirmation of the target pathogens was weak, in particular with regard to *B. bronchiseptica*.

Clinical field trials - Germany and France

An European study conducted in 2008-2009 at 6 sites in Germany and France included a large number of pigs of either sex of the target population aged 4-20 weeks (5-101 kg bw), comparing pigs treated with tildipirosin and pigs treated with a positive control group (tulathromycin). For each study site, the first pigs that had met the enrolment criteria were used to confirm outbreak of SRD in the herd. These pigs were necropsied and examined on the presence of pathogens. *A. pleuropneumoniae* (16%), *P. multocida* (14%), *H. parasuis* (25%) were isolated in animals sent in for necropsy. No *B. bronchiseptica* were isolated in animals sent in for necropsy. At withdrawal, *B. bronchiseptica* (8%) and *H. parasuis* (4%) were isolated (ITT).

Tildipirosin was given intramuscularly at the recommended dose of 4 mg/kg bwbw to animals with clinical signs of SRD (abnormal rectal temperature, respiration and attitude). Treatment success was based on defined improvement of respective clinical signs.

Similar efficacy rates for the tildipirosin and the positive control group were noted in the treatment success rate on day 10 and 17 (93% and 88%, versus 92% and 89%, respectively) and the relapse rate (6% and 3%, respectively). Rectal temperatures, respiratory and attitude scores were markedly reduced from day 1 in both treatment groups, and no statistically significant difference in the daily weight gain was detected between the groups.

The CVMP agreed that the data demonstrated an acceptable treatment success of a single intramuscular dose of 4 mg tildipirosin/kg bw, and non-inferiority of tildipirosin compared to the positive control (tulathromycin), although microbiological confirmation of target pathogens was weak.

Clinical field trial - USA

A clinical field study_conducted in US completed the data to support the treatment claim in *B. bronchiseptica*. Six sites in the US enrolled animals with signs of porcine pneumonia. Animals were treated either with tildipirosin, with a positive control (tulathromycin) or a negative control (saline solution). Efficacy was compared to saline solution. The first pigs at each site were dissected. Animals with a rectal temperature of more than 40°C, altered attitude, and an altered respiratory pattern were eligible for study participation. Animals were checked daily for respiratory pattern and attitude. If the respiratory or attitude scores met the inclusion criteria again or were even worse, rectal temperature was recorded. All animals withdrawn due to SRD and all saline-treated animals were dissected and investigated for target pathogens.

Ten days after treatment, the overall treatment success rate was assessed. Animals with rectal temperature of less than 40.0°C, and just slightly impaired attitude and breathing pattern were considered a treatment success. In 4 out of 6 sites, *B. bronchiseptica* was identified prior to treatment start in up to 3 out of 8 of the animals dissected, with MIC_{90} values of 4.0 μg/ml.

The treatment success rate for tildipirosin (70%) did not differ in comparison to the tulathromycin group (69%). It was also shown that tildipirosin was significantly superior to saline solution (40% success rate).

The CVMP considered that the results from this study confirm the conclusions drawn from European studies in regard to the efficacy of a single intramuscular dose of 4 mg tildipirosin/kg bw in the treatment of SRD.

In addition, the CVMP concluded from this study that *B. bronchiseptica* played a role in the outbreak of SRD. Although the microbiological data are weak, the CVMP considered the data sufficient, taking into account i) that the MIC_{90} of 4 µg/ml is lower than the lead pathogen *A. pleuropneumoniae*, ii) the limitations of field trials as regards the microbiological diagnosis and iii) that the data for

B. bronchispetica are not inferior to those presented in the European field trials for the other claimed pathogens. The CVMP noted also that the success rate involving *B. bronchiseptica* was similar to that of the positive control (tulathromycin); and that *B. bronchiseptica* is as susceptible in the US as it is in the EU.

Prevention

Dose-confirmation field study - Germany

The applicant conducted a GCP-compliant controlled, multi-centred, randomised and investigator blinded dose-confirmation field studies in Germany in 2007/2008.

The study included hybrid female and castrated pigs from 6-14 weeks (7-51 kg bw) sharing the same airspace with pre-study animals of the same age group. Pigs were enrolled at 2 sites, either in the tildipirosin group or in a negative control (saline) group. Treatment of animals with no clinical signs of SRD (rectal temperature below 40°C and normal respiration and attitude) was initiated when at least 10% of pre-study animals sharing the same airspace showed clinical signs of SRD. SRD outbreak was confirmed by clinical signs and the first 10 animals per site were necropsied and examined on the presence of *A. pleuropneumoniae*, *P. multocida, H. parasuis* and *B. bronchiseptica*.

Tildipirosin was given intramuscularly at the recommended dose of 4 mg/kg bw. Prevention success was based on clinical criteria (rectal temperature below 40°C, and normal respiration and attitude). Success was high after preventive treatment with tildipirosin; however, in deviation from the efficacy guidelines, assessment of the response to therapy was not based on microbiological criteria. As treatment success was also high in the negative control group (no superiority of tildipirosin over saline), the CVMP did not consider that these data would support the proposed prevention claim.

Clinical field trials - EU

A positive-controlled, multi-centred, randomized and investigator-blinded European study was conducted in farms with a history of previous outbreaks of bacterial SRD in 2008-2009 at 6 sites in Germany and France. The study included a large number of pigs of either sex, of the target age group 6-21 weeks (from 5-94 kg bw) sharing the same airspace with sentinel pigs of the same age group. Two different treatment groups were compared, tildipirosin and a positive control (tulathromycin) group.

Daily clinical examinations were performed on D0 to D10, again on D17±1 and at any time when requested. Clinical examination included respiratory, attitude and temperature scoring as well as injection site observation. Serum samples were taken from 10% of the included pigs per site on D0 and D17±1 to get information on potential concomitant infections.

Treatment of animals showing no clinical signs of SRD (normal rectal temperature and normal respiration and attitude) started when 3-8% of animals sharing the same airspace showed clinical signs of SRD (abnormal rectal temperature and respiration and attitude). Tildipirosin was given intramuscularly at the recommended dose of 4 mg/kg bw. Prevention success was based on clinical criteria (normal rectal temperature and respiration and attitude).

Success was high after preventive treatment with tildipirosin and with the positive control (tulathromycin). Non-inferiority of tildipirosin compared to the positive control was shown. However, the pre-set level of diseased pigs was not met, and no untreated animals were included in the study. Thus, the risk for disease occurrence during the study period cannot be determined and internal validity of the study is thus not confirmed. Furthermore, microbiological confirmation of SRD in the

herds was weak. Data presented suggest the infectious pressure was low which would lead to erroneous conclusions.

Based on the results of the studies presented, the CVMP did not accept the proposed indication for prevention of swine respiratory disease.

Overall conclusion on efficacy

The spectrum of activity of tildipirosin includes Gram-positive and Gram-negative bacteria including common pathogens of the respiratory tract of swine and cattle: *M. haemolytica, P. multocida, H. somni, H parasuis, A. pleuropneumoniae and B. bronchiseptica*. Against the swine isolates of *H. parasuis, P. multocida* as well as bovine isolates of *M. haemolytica* and *P. multocida* the MIC_{90} was at 1 µg/ml. For swine isolates of *B. bronchiseptica* and bovine isolates of *H. somni* the MIC_{90} was at 4 µg/ml, and for *A. pleuropneumoniae* the MIC_{90} was 8 µg/ml. Like other macrolides tildipirosin acts principally bacteriostatic and time dependant. Bactericidal effects could be demonstrated *in vitro* for the target pathogens *M. haemolytica, H. somni, A. pleuropneumoniae* and *H. parasuis,* but not for *B. bronchiseptica.* Bacteriostatic effects prevailed in *P. multocida*.

Pharmacokinetic properties were demonstrated in both cattle and pigs, characterised by rapid absorption from the injection site, high bioavailability, low plasma protein binding, high volume of distribution, and accumulation at the site of respiratory tract infection. The metabolic fate of tildipirosin had been detailed during the MRL procedure and adequately addressed in the product literature.

Tildipirosin proved to be well tolerated in cattle as shown in a number of well designed and conducted TAS studies. No systemic adverse effects were observed at dose levels up to 5 times the recommended dose. Findings are mainly restricted to local effects at the injection site. Clinical signs like swellings and pain were found to be transient. The maximum injection volume is 10 ml per injection site. Clinical and macroscopic findings at the injection site are adequately addressed in the product literature. Mild to moderate focal liver necroses seen at 10 x overdose were assumed to be incidental and, therefore, corresponding information in the product literature was not considered necessary.

Target animal safety studies in pigs performed at the recommended dose, overdoses and/or prolonged duration of use as recommended in the relevant VICH-TAS guideline, revealed systemic adverse reactions at all doses tested, including the RTD, indicated by subdued behaviour, tremor, disability to stand, and shock. Adequate warnings have been included in the SPC and product literature. The clinical signs appeared transient, but could be potentially life threatening in some cases, particularly in case of accidental intravenous administration. Intravenous injection is therefore contraindicated and additional warnings to strictly inject intramuscularly are included in the product literature.

Commonly, tildipirosin induces local injection site reactions. The local adverse reactions after administration of RTD are adequately described in the product literature. The recommended maximum injection volume is 5 ml per injection site.

<u>Cattle</u>
For **dose determination**, two challenge studies conducted under laboratory conditions with *Mannheimia haemolytica* as challenge strain and three non-pivotal dose finding studies under US field conditions (using clinical endpoints) were provided. No clear optimum effective dose could be derived. However, the data suggested that the optimum effective dose may be between 1.25 mg and 4 mg tildipirosin/kg bw, and a dose of 4 mg tildipirosin/kg bw was chosen for the field efficacy studies.

Treatment of bovine respiratory disease (BRD)

Two European GCP-compliant field efficacy trials addressing the treatment claim, were provided. Both studies follow current standards and were designed as non-inferiority comparison with suitable positive controls (florfenicol and tulathromycin). The results indicate that tildipirosin was non-inferior to the positive controls in the treatment of bovine respiratory disease.

In addition to the EU field studies, a new GCP field efficacy study conducted under US field conditions was provided. The study design was comparable to the EU studies; however, the US study also included a negative control group. The results indicated that efficacy of tildipirosin was significantly higher than the saline treated negative control group, and that tildipirosin was as effective as the positive control group.

The CVMP noted that the study design was not in accordance with the requirements of the current CVMP "Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMEA/CVMP/627/01-Final)", since only clinical endpoints were used for inclusion and efficacy evaluation. However, the CVMP agreed that bacteriological endpoints would not be a suitable target for the purpose of a field study because of the multifactorial nature of the BRD disease complex.

Overall, the CVMP concluded that the data showed sufficient evidence to demonstrate the efficacy of a single subcutaneous dose of 4 mg tildipirosin/kg bwbw in the treatment of BRD.

Prevention of bovine respiratory disease (BRD)

Two European GCP field efficacy trials addressing the prevention claim were provided. Both studies followed current standards and were designed as non-inferiority comparison with suitable positive controls (florfenicol and tulathromycin). Efficacy evaluation was based on clinical endpoints. The results indicated non inferiority of tildipirosin compared to the control products. However, the studies showed some shortcomings.

Therefore, in response to questions, a new US clinical study was provided. This study was considered appropriate to outweigh the deficiencies of the European field studies, mainly because of the inclusion of a negative control group. Outbreak of BRD was confirmed in this control group, by typical clinical signs of disease and by isolation of the relevant bacterial pathogens. Efficacy of tildipirosin-treated animals (and also of the positive control animals treated with tulathromycin) was significantly higher than that of the saline-treated animals.

Consequently, the CVMP agreed that the data showed sufficient evidence to demonstrate the efficacy of a single subcutaneous dose of tildipirosin in the prevention of BRD, given that there is evidence of the disease in the herd.

Pigs

For dose determination (treatment claim), four challenge studies under laboratory conditions using *A. pleuropneumoniae* and *P. multocida* were performed in pigs. While the studies employing *P. multocida* did not result in valid findings, results of studies with *A. pleuropneumoniae* showed that the effective tildipirosin dose should be between 2.5-5 mg/kg bw. Thus, a dose of 4 mg tildipirosin/kg bw was considered to be tested in dose-confirmation studies. No dose determination studies for the prevention claim were undertaken.

Treatment of swine respiratory disease (SRD)

Three well conducted GCP-compliant clinical field studies, two performed in Germany and one performed in the EU were submitted to support the treatment claim. While one study failed to confirm the efficacy of tildipirosin probably due to concomitant mycoplasma and virus infections, the two others

demonstrated an acceptable treatment success and showed non-inferiority of tildipirosin compared to positive control products in the treatment of SRD. However, proof that clinical signs of SRD were associated with the claimed pathogens was poor, in particular as regards *B. bronchiseptica*.

In addition to the European studies, a well conducted GCP-compliant clinical field study from the USA was submitted to complete the data supporting the treatment claim. It was shown that tildipirosin was significantly superior to a negative control, and non-inferior to a positive control.

In addition, the study showed that *B. bronchiseptica* could be isolated in a range not inferior to that in the European field trials for the other claimed pathogens. The claim of *B. bronchiseptica* was therefore supported by the data from the USA.

Prevention of swine respiratory disease (SRD)

Two European GCP compliant field studies were submitted to investigate efficacy of tildipirosin in preventing SRD. In one study including a negative control superiority of tildipirosin in comparison to saline was not demonstrated.
In the other study, tildipirosin proved to be non-inferior in the prevention of SRD compared to a positive control. However, the study showed major shortcomings which precluded a conclusion regarding preventive effects.

Based on the results of the studies presented, the CVMP did not accept the proposed indication for prevention of swine respiratory disease.

Part 5 – Benefit risk assessment

Introduction

Zuprevo contains tildipirosin as active substance, which is a semi-synthetic novel macrolide antibiotic. It is available in two different strengths, 40 mg/ml (pigs) and 180 mg/ml (cattle) and presented as a solution of injection in packs/containers of 20 ml, 50 ml, 100 ml or 250 ml.

The target species are pigs and cattle. The route of administration is intramuscular use (pigs) and subcutaneous use (cattle).

The proposed indications are:

- Pigs: The treatment of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Bordetella bronchiseptica* and *Haemophilus parasuis* sensitive to tildipirosin.
- Cattle: The treatment and prevention of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* sensitive to tildipirosin. The presence of the disease in the herd should be established before preventive treatment.

The withdrawal periods for meat and offal are 9 days (pigs) and 47 days (cattle).

Direct therapeutic benefits

Cattle:

The benefit of tildipirosin is that Bovine Respiratory Disease associated with susceptible *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni* can effectively be treated and prevented with one single subcutaneous injection of 4 mg/kg body weight.

Pigs:

The benefit of tildipirosin is that Swine Respiratory Disease caused by *Actinobacillus pleuropneumoniae*, *Pasteurella multocida, Bordetella bronchiseptica* and *Haemophilus parasuis* susceptible to this substance can effectively be treated with one single intramuscular injection of 4 mg/kg body weight.

Risk assessment

Quality

The overall impression of the quality dossier is positive and part 2 generally is in compliance with current rules and guidelines. The outstanding validation of the manufacturing process at production scale will be conducted on at least three batches per dosage strength and prior to marketing of the product.

The formulation and manufacture of Zuprevo is well-described and adequate specifications have been set. The stability of the product has been shown at 25°C/60% RH, and corresponding storage advice has been included in the SPC and product literature ("Do not store above 25°C").

The rubber stoppers should not be punctured more than 20 times; corresponding advice has been included in the SPC and product literature.

User safety

Tildipirosin is classified as skin sensitiser, and appropriate information and warning statements are included in the product literature to ensure the safe and correct use of the products. As laboratory studies in dogs showed cardiovascular effects (a small decrease in pulse pressure) after intramuscular injection of 20 mg/kg bodyweight, CVMP considered that (accidential) self injection of a full dose of the higher strengths for cattle (worst case user exposure scenario) might lead to human health effects. Appropriate warnings have therefore been included in the SPC and product literature.

Antimicrobial resistance

The risk that the use of tildipirosin in cattle and swine selects for antimicrobial-resistant bacteria of human health concern is considered comparable to macrolides with similar spectrum of activity already marketed in Europe. If the overall use of such macrolides is not increased by this new addition, the overall risk from use of macrolides in target species populations is assumed to be unchanged.
A discussion on possible effects of use of tildipirosin on emergence and spread of MRSA was provided. The likelihood of spread between animals and ultimately to man is estimated at the same level as other injectable macrolides.

Environmental risk

Zuprevo is not expected to pose a risk to the environment when used according to the SPC.

Residues

Based on marker residue data and the MRLs established by CVMP, withdrawal periods for edible tissues of 47 days for cattle and 9 days for pigs have been calculated using the statistical approach according to CVMP guideline. The maximum injection volume at injection site should not exceed 10 ml in cattle and 5 ml in pigs. Zuprevo is not authorised for use in lactating cattle producing milk for human

consumption and should also not be used in pregnant animals, which are intended to produce milk for human consumption, within 2 months of expected parturition.

Target animal safety

As for any other veterinary medicinal product, Zuprevo is contraindicated for animals with hypersensitivity to the active ingredient (or other macrolide antibiotics) or any of the excipients.

The safety of tildipirosin during pregnancy and lactation has not been established in the target species. The applicant proposes that under these conditions the responsible veterinarian should decide on the use of this product according to a benefit/risk assessment. This is acceptable as there is no evidence for any developmental or reproductive effects in any of the laboratory studies.

Adequate information as regards susceptibility testing and official/ local policy on the use of antibiotics is included in the SPC.

Cattle:

Target animal safety studies and clinical studies have not shown any systemic adverse reactions, neither at 5x overdose levels nor at the recommended dose levels. Therefore, based on the provided data, no serious risk can be identified with respect to animal welfare. At a 10x overdose, however, focal liver necroses were seen in 3 out of 4 of the treated animals and in none of the controls. Based on the available data this finding is considered incidental.

However, transient injection site reactions like swellings and pain on injection and on palpation were very commonly observed. The local findings at the injection sites studied are considered tolerable. These findings and the pain at injection are adequately reflected in the product literature.

Pigs:

Preclinical and clinical data revealed signs of systemic intolerance shortly after injection.

In pre-clinical studies mild systemic adverse reactions like subdued behaviour were reported at all dose groups tested including the recommended treatment dose (RTD). Tremor in hind legs, body tremor and impossibility to stand and shock/death were reported after multiples of the RTD.

During clinical trials, treatment caused shock symptoms in 2 out of 1048 animals. These symptoms quickly resolved in one animal but led to death in the other animal.

The definite cause(s) of these signs of intolerance are not known. However, systemic signs of intolerance after administration of the RTD and of multiple overdoses are highlighted in the SPC and product literature. The adverse reactions appear to be particularly serious in case of intravenous administration since fatalities and signs of shock are then noted at recommended dose levels. Thus, intravenous administration is contraindicated, and advice is included in the product literature to strictly inject the product intramuscularly. The safety has not been established in piglets younger than 4 weeks, and a warning has been included in the SPC and product literature.

Pain on injection and injection site reactions after administration of the RTD were very commonly reported. These findings are considered adequately addressed in the SPC and product literature.

Risk mitigation measures

Appropriate warnings have been placed in the SPC to warn of the potential risks to the target animals and user.

Concerning any environmental risks, the standard advice for disposal of any unused product or waste material is included in the product literature.

Evaluation of the benefit risk balance

Overall, the benefit-risk balance is considered positive for Zuprevo.

Zuprevo 180 mg/ml solution for injection for cattle proved to be efficacious in the treatment and prevention of bovine respiratory disease, although microbiological confirmation of the target pathogens (*M. haemolytica, P. multocida and H. somni)* was weak. The product proved to be well tolerated in cattle when administered according to the instructions for use. Adverse reactions are restricted to pain and swellings at the injection site.

Zuprevo 40 mg/ml solution for injection proved to be efficacious in the treatment of swine respiratory disease, in principle, although microbiological confirmation of the target pathogens *(Actinobacillus pleuropneumoniae*, *Pasteurella multocida, Bordetella bronchiseptica* and *Haemophilus parasuis*) was weak. As major concerns remained in relation to the efficacy of Zuprevo in the proposed indication for prevention of swine respiratory disease, this indication was not accepted by the CVMP. The product proved to be well tolerated in pigs when administered according to the instructions for use. Systemic adverse reactions may be serious. They are adequately addressed in the product literature. Local adverse reactions are restricted to pain and swellings at the injection site.

User and consumer safety, and risks for the environment have been adequately considered with suitable warnings in the SPC and product literature.

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of Zuprevo were considered to be in accordance with the requirements of Directive 2001/82/EC, as amended, and that the benefit-risk balance was favourable.