SCIENTIFIC DISCUSSION

1. SUMMARY OF THE DOSSIER

The active substance of Zactran is gamithromycin, a semi-synthetic macrolide medicinal product, (ATC vet code: QJ01FA95). Gamithromycin has both bacteriostatic and bactericidal action, mediated through the disruption of bacterial protein synthesis. The broad spectrum antimicrobial activity of gamithromycin includes *Mannheimia haemolytica, Pasteurella multocida and Histophilus somni*, the bacterial pathogens most commonly associated with Bovine Respiratory Disease (BRD).

The benefits of Zactran are the efficacy of a single dose of 1 ml/25 kg for the treatment and prevention of BRD, demonstrated by the improvement of the clinical signs of the disease. Also, the efficacy of gamithromycin was demonstrated as comparable to that of an already approved veterinary medicinal product containing tulathromycin. The most common side effect is a transient local swelling at the injection site consistent with the macrolide class of antimicrobials.

The approved indication is:

Therapeutic and preventive treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*.
The presence of the disease in the herd should be established before preventive treatment.

2. QUALITY ASSESSMENT

Composition

The product contains 150mg/ml gamithromycin as active substance in a buffer solution. The active ingredient complies with Merial's in-house specification. All excipients are in compliance with relevant specifications (Ph.Eur, USP/NF or in-house).

Qualitative Composition	Quantitative composition	Reference to analytical quality
Active Substance(s)		
Gamithromycin	15.0% (w/v) 150 mg/ml	In-house
Other ingredients		
Succinic acid Monothioglycerol Stabilised glycerol formal Nitrogen gas		

Container

The primary packaging materials are Type I colourless glass bottles of 100-mL, 250-mL & 500-mL sizes, with chlorobutyl rubber stoppers. The stopper will be held in place by an aluminium seal.

Clinical Trial Formula(e)

The clinical trial formulation is the same as for the final product.

Development Pharmaceutics

The product is presented in an injectable formulation packaged in 100, 250 and 500 ml, type I clear glass bottles.

Active substance: gamithromycin is the active substance, which is a white to beige powder and is insoluble in water. Gamithromycin is crystalline with one stable polymorphic form which is generally stable under elevated temperatures and not light sensitive.

Choice of excipients: The selection of excipients was thoroughly described. To enhance the solubility of gamithromycin in glycerol formal, the addition of an acid (succinic acid) to the formulation was evaluated.
An antioxidant, monothioglycerol, is also included in the formulation to reduce oxidative degradation and maintain good product appearance.

The final formulation does not include any antimicrobial preservatives, due to self-preserving properties, compliant with the Ph. Eur. 5.1.3 Efficacy of Antimicrobial Preservation.

Packaging material: Based on the results of photostability study the product is considered photostable when stored in either glass, High Density Polyethylene (HDPE) or Polypropylene (PP) bottles. Results from tests regarding container closure integrity and fragmentation/self sealability were found to be acceptable.

The development work was performed in accordance with the EU guidelines *Development pharmaceutics for veterinary medicinal products* and *Annex: Development pharmaceutics for veterinary medicinal products (EMEA/CVMP/315/98*): *Decision trees for the selection of sterilisation methods.*

Method of manufacture

Manufacturing Process and In-process Controls
The manufacturing process for gamithromycin solution for injection 15% (w/v) involves compounding, pre-filtration, sterile filtration, filling and packaging. All ingredients are added in rapid succession and mixing continued until a clear solution was obtained. The drug product is then sterile filtered and aseptically filled into 100, 250 or 500 ml type I glass bottles. Detailed descriptions of the manufacturing steps and in process controls were provided.

Validation of Manufacturing Process
The validation of the manufacturing process for gamithromycin solution for injection was described adequately. Key parameters identified prior to the validation exercise were: mixing time, filtration, holding time and fill volume. However, as the finished product is sterilised by aseptic filling it is normally considered as a non-standard method and full-scale batches were, therefore, used in the validation studies. The suitability of the method was confirmed by batch results. They showed that the manufacturing process leads to production of a consistent product. The Applicant provided validation reports for the 500ml, 100ml and 250ml bottles which were considered acceptable.

Control of starting materials

Active substance
Full documentation on the active substance was presented by the Applicant.
Gamithromycin is part of a new class of azalide antibiotics and is not detailed in any pharmacopoeia. It differs structurally from erythromycin A, by the insertion of a n-propyl substituted nitrogen at the N position in the lactone ring to create a 15-membered macrolide.

Specifications for the active substance were provided and included stability-indicating parameters such as appearance, water content; colour and clarity of solution, assay and related substances are monitored by appropriate tests.

Scientific data
Nomenclature:
INN Name: gamithromycin
Chemical (IUPAC) Name: 1-Oxa-7-azacyclopentadecan-15-one,13-[(2,6-dideoxy-3-C-methyl-3-O-methyl-.alpha.-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy 3,5,8,10,12,14-hexamethyl-7-propyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-.beta.-D-xylo-hexopyranosyl]oxy]-, (2R,3S,4R,5S,8R,10R,11R,12S,13S,14R)-
Merial Code: ML-1,709,460 or ML-460
Chemical Abstracts Service (CAS) registry number: 145435-72-9

Description: gamithromycin drug substance is a white to beige fine powder with antimicrobial properties. Only one stable polymorph form has been identified. It is an azalide antibiotic with a molecular formula of $C_{40}H_{76}N_2O_{12}$ and molecular weight of 777.04 g/mole.

Manufacture: gamithromycin is derived from the starting material erythromycin A E-oxime, which is obtained by chemical conversion of erythromycin A, a well-known antibiotic. The starting material is a well-characterised chemical entity that provides a significant structural element to gamithromycin and is commercially available from multiple manufacturers to a well-defined specification. The purity of the starting material, the water content and total impurities are adequately controlled. The flowchart of the process was provided.
Samples are taken for in-process controls (IPC's) in various steps to assure the quality of the intermediate prior to continuing to the next step.

Specifications for the starting material, solvents and reagents were provided.

The proposed limits for impurities in the starting material specifications were acceptable and are in compliance to EU/VICH guideline GL10.

Development Chemistry
Physico-chemical characteristics: Adequate information was provided on the following physico-chemical properties of gamithromycin: solubility, appearance, melting point, particle size, hygroscopicity, chirality, isomers, polymorphism and lack of sensitivity to light. Gamithromycin is insoluble in water, reversibly hygroscopic and not sensitive to light. It is also a chiral molecule and has 18 chiral centers. The manufacturing crystallisation processes for gamithromycin leads only to the one stable polymorph form of gamithromycin. A description of the spectral evidence for the structure of gamithromycin was given.
Residual solvents used in the process are controlled in line with Ph Eur requirements.
A complete list of organic solvents used was provided.
Water is controlled as gamithromycin is hygroscopic.
Satisfactorily validations of analytical testing methods were performed in accordance with EU/VICH guideline.
Batch analysis data from nine batches were provided. The results were found to conform to the specifications.

Packaging of the active substance: The gamithromycin drug substance is packaged in a double plastic liner made of low-density polyethylene. The inner bag is 100% low-density polyethylene while the outer bag is aluminium laminated. The inner bag is tied by means of a tag. The specifications for the packaging materials were provided.

Stability Tests on the Active Substance
Retest period: 4 years with no storage restrictions.

Stress studies:
On the basis of results from stress studies the following conclusions were made:

- Stable when exposed to ICH light conditions. No degradation impurity was observed.
- In aqueous/solvent solutions at different pH degradation are observed.

Batches tested
Six of the batches were stored at long term and accelerated VICH conditions. Three batches were stored for 48 months and three for 12 months. No significant changes were observed at any of the tested conditions.

Excipients

Excipients described in a Pharmacopoeia:
Monothioglycerol and succinic acid are described in USP/NF.

Excipient(s) not described in a Pharmacopoeia:
Glycerol formal, stabilised is used in the manufacture of gamithromycin solution for injection and is a well-known excipient already as is used in other veterinary medicinal products. In-house methods and acceptance criteria are used to control the quality of the raw material.

All excipients used in the manufacturer of gamithromycin solution for injection were in compliance with the EU/VICH guideline GL18 "Impurities: Residual Solvents in New Veterinary Medicinal Products, Active Substances and Excipients".

Packaging

The primary packaging materials for gamithromycin solution for injection are Type I glass bottles of 100-ml, 250-ml & 500-ml sizes, with chlorobutyl rubber stoppers (4588/40 gray), 20mm, 30mm, and 30mm, respectively. All packaging materials comply with the relevant Ph. Eur. requirements. The stopper is held in place by an aluminium seal.
The specifications for the bottles and stoppers were provided. The glass bottle and rubber stoppers are in contact with the product while the aluminium seal has no product contact.

The components that come into contact with the injection were tested for compatibility with the formulation and the results were provided. Accelerated and long-term stability studies showed that the formulation was stable when packaged in the vials and no unexpected impurities or degradation products were seen. The packaging materials were considered suitable.

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

A TSE statement was provided stating that all starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.
No raw materials of animal origin are used either in the manufacture of the starting material or in the manufacture of the active substance gamithromycin. The material used for strain preservation during the fermentation process of the starting material Bacto-Tryptone is metabolised by the microorganisms and is not contained in the end product. Bacto-Tryptone is derived from bovine milk, which is sourced in the same manner as milk collected for human consumption in compliance with the Note for Guidance, (EMEA/410/01 Rev. 2 – October 2003) on *Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products*. A declaration of compliance from the manufacturer was also provided regarding the use of calcium stearate, used in the manufacture of stoppers.

The finished product manufacturer uses Trypticase Soy Broth in their media fill experiments. A declaration of compliance from the supplier was provided.

Control tests during production

Not applicable.

Control tests on the finished product

Product Specification and Routine Tests:
The release specification includes tests for the following parameters: Appearance, Clarity, Colour, Specific Gravity (25°C), Delivered Volume, assay of gamithromycin, Antioxidant and Microbiological Tests. The specifications and routine tests are regarded as sufficient to ensure adequate and consistent quality of the finished product.

Control methods
Tests procedure for identification and quantitative determination for the active substance:
Identification is performed by two independent procedures, TLC and HPLC. The assay is determined by the same isocratic reverse-phase HPLC used for identification and is capable of separating gamithromycin from potential degradation products.

Identification and determination of excipients:
Monothioglycerol is identified and assayed by a gradient HPLC with UV detection.

Safety tests
Impurities are determined as specified, unspecified and total impurities as described in the VICH guidelines. Sterility is performed according to Ph Eur methodology. The bacterial endotoxin test is performed according to Ph Eur methodology with a gel-clot method or a kinetic chromogenic method.

Analytical validation of methods
The TLC method used for identification is validated with respect to specificity, repeatability and robustness. The HPLC method used for identification, assay and degradation products is validated with respect to specificity, linearity; accuracy; precision; robustness; low-level linearity/accuracy/precision, LOD and LOQ.
The sterility test and endotoxin test are validated according to Ph. Eur. requirements.

Justifications for the choice of analytical methods were provided and found acceptable.

Batch analyses: Three production batches were tested. Analytical results were reported and were found acceptable.

STABILITY

Stability Tests on the Finished Product

Product Specification and Routine Tests for shelf life: Three production batches were tested for the following parameters: Appearance, Assay of gamithromycin (by HPLC), Related Substances, Monothioglycerol, Sterility, Endotoxins. The identity tests and colour change test are also included in the shelf-life specification.

Widening of the limits for assay content and antioxidant content was justified by the stability data presented. The proposed shelf life limit for monothioglycerol was justified with respect to its functionality.

Stability Tests
Batches tested: The three production batches were stored in vials of 100 ml, 250 ml and 500 ml at VICH conditions for 24 months at 25°C/60% RH, 30°C/60% RH and at 40°C/75% RH for 6 months. A biobatch was stored at the same conditions. At the 24 months time point, inverted as well as upright oriented samples were tested.

Further investigations were performed regarding VICH photostability, temperature cycle (-20°C to 40°C/75% RH every 48 hours at each temperature for 2 complete cycles) and in-use tests.

Results: The same trends were observed for all batches and were found acceptable. No differences were observed when vials were stored upright or inverted. Changes increased linearly with the increase of temperature. No changes were observed in the temperature cycle and in the photostability. All results were within acceptable levels.

Ongoing stability studies: The Applicant committed to perform stability studies on the first three commercial batches. At least one batch per year will be selected.

Shelf-life /storage conditions: A shelf-life of 3 years and in-use shelf life of 28 days, with no specific storage precautions, were adequately justified.

In-use Stability Tests

The results from one month in-use stability study performed on 36 month old samples were presented and were in compliance with specifications. The data supported an in-use shelf life of 28 days with no specific storage precautions.

OVERALL CONCLUSION ON QUALITY

The quality part of the dossier was acceptable in general and in compliance with current rules and guidelines.

The Applicant committed to the following points:

1) A retest period for the active substance of 4 years with no storage restrictions is acceptable. It is a precondition that the initiated drug substance stability studies will be continued and EMEA will be informed if any unexpected events appear during the testing period. When finalised the results from the studies will be submitted.

2) The first three batches manufactured in vials with the 32 mm stoppers will be placed on stability and any out-of-specification results will be reported to the EMEA

3) A shelf-life of 3 years with no specific precautions for storage is acceptable. A precondition is that the initiated finished product stability studies will be continued and EMEA will be informed if any unexpected events appear during the testing period. When finalised the results from the studies will be submitted.

3. SAFETY ASSESSMENT AND RESIDUES

Pharmacokinetics

Distribution and absorption
The pharmacokinetics of gamithromycin were determined after intravenous (IV) (3 mg/kg) and subcutaneous (SC) (3, 6 and 9 mg/kg) administration to cattle weighing 182-260 kg in a GLP study. Blood plasma was analysed by HPLC- MS/MS. The Limit of Quantification (LOQ) was set at 2 ng/ml. The volume of distribution was large indicating a high tissue distribution. Both after IV and SC administration the half-life was long and the body clearance was relatively low. Absorption after SC administration was approximately 100 % and the kinetics were linear in the tested dose range.

It was concluded that Zactran administered subcutaneously into the neck of cattle (182-260 kg) at a single dose of 6 mg/kg body weight, resulted in rapid absorption with peak plasma concentrations observed between 30 and 60 minutes post administration and with a long plasma half-life (51 h). The Area Under the Curve $(AUC)_{last}$ was 9253 h.ng/ml. The bioavailability of the drug was >98% with no gender differences. The volume of distribution at steady-state was 25 L/kg. In the lungs gamithromycin reached maximum levels in less than 24 hr, with lung-to-plasma ratio of >264 indicating that the drug was absorbed rapidly into the target tissue for BRD. Gamithromycin accumulates rapidly and persists in lung tissue at concentrations at or above the MIC_{90} of the target pathogens through at least Day 10. Biliary excretion of the unchanged drug was the major route of elimination.

Pharmacokinetics were also investigated in 12 non-ruminating calves aged from 35 to 42 days. They received a dose of 6 mg/kg SC and plasma drug levels were assessed by HPLC-MS/MS. The time of maximum concentration (Tmax) varied greatly between animals – from 0.5 to 6 hours and maximum concentration (Cmax) values were also very variable (169 – 843ng/ml). Local reactions with swelling were observed at the injection site in 5 animals. After 20 days the swelling was resolved in 3 animals, but remained in 2 animals.

Protein binding
The protein binding of gamithromycin was determined in rats, dogs, cattle and swine. Plasma samples from non-treated animals were incubated for 30 min at 35°C with 0.1, 0.3, 1.0 or 3.0 µg/mL of tritiated gamithromycin. The mean plasma-protein binding was 26.0 % in cattle plasma, 23.1% in swine plasma, 21.8% in rat plasma and 21.5% in dog plasma and remained constant over the concentration range studied.

Metabolic profile of gamithromycin:
The metabolic profile of gamithromycin was investigated in 14 cattle which received approximately 6 mg/kg of the tritiated compound and were sacrificed on Days 21, 49 and 70 after dosing. Total radioactive residues were determined and the selected tissues, fluids and excreta were analysed to monitor gamithromycin and its metabolites (in liver, kidneys, lungs and injection site). Total radioactivity levels were highest at the injection site, then in the liver, followed by lung, kidney, fat and muscle. The parent compound and the metabolites Declad (loss of a cladinose, sugar moiety) and M2 (N-dealkylated-declad) were the major residues in the selected tissues, fluids and excreta.

The metabolites of gamithromycin were further studied in rats and dogs and although the relative fraction of each metabolite may differ between species, gamithromycin was found to be metabolised in a similar manner across the species.

Excretion
Most of the excreted radioactivity for gamithromycin was found to be primarily eliminated in the faeces (42.5 to 58.5%) and secondarily in the urine (14 to 17.8%) of cattle by 2 weeks following administration (average 66.4% of total recovery). It was also found that by day 14 following subcutaneous administration of 6mg/kg the mean plasma concentration of gamithromycin was below detectable level. The elimination half life of gamithromycin for adult cattle administered (SC) at a

dose rate of 6mg/kg was found to be similar to those of non ruminating cattle (51h and 56.4h respectively).

Overall the pharmacokinetic activity of this product was described adequately.

Toxicology

Single dose toxicity
In a study in which male and female rats were administered drug orally using methylcellulose as a vehicle it was concluded that gamithromycin possesses low acute toxicity.

The Applicant also provided a study on acute dermal toxicity in rabbits. No mortality or signs of clinical toxicity were observed in rabbits exposed dermally to gamithromycin at 2000 mg/kg.

Repeated dose toxicity
Seven studies were conducted according to relevant requirements regarding repeat dose toxicity. Three of those studies were in rats, two in mice and two in dogs.

Rats: Three oral administrations, repeat dose studies were conducted in rats.
In a 13 week study in rats doses of 0, 1, 3, 10 and 100 mg/kg bw were used. The no-effect level (NOAEL) was considered to be 10mg/kg/day.

A second 13 week study in rats was performed with doses of 0, 3, 60 and 100 mg/kg bw. No clinical signs were observed and clinical pathology observations did not reveal any consistent pattern of alterations. Bile duct epithelial alterations were observed in the high dose group (mild cytoplasmic vacuolation). The NOAEL was considered to be 60mg/kg/day.

The third rat study was a 21 day study conducted to elucidate the mechanism underlying the toxic effects and lesions seen in the 13 week studies. Animals were dosed with 0 or 300mg/kg of drug. In the treated group, hunched posture and clear oral discharge was noted in both genders. Body weight gain decreased in both genders, with a more severe depression in females. Histopathological findings included minimal to moderate hepatocellular vacuolation, hepatocellular hypertrophy, karyomegaly and diffuse bile duct vacuolation in treated animals. Phospholipidosis was observed and is considered to be a class effect of macrolides.

Mice: Two 13 week studies were performed in mice with oral dose levels of 0, 50, 100, 500mg/kg/day and 0, 350, 450mg/kg/day. No treatment-related deaths or clinical signs of toxicity were observed. The NOAELs for the mouse studies were 100mg/kg/day and 350mg/kg/day respectively.

Dogs: Two studies were performed in dogs; a 13 week study with doses of 0, 1, 3, 10, 30 mg/kg/day and a 52 week study with doses of 0, 0.3, 1, 3 mg/kg/day. No treatment related deaths were reported. In both studies the NOAEL was 1 mg/kg/day.

Conclusions: In the rat studies a minor effect was detected on liver cells at high doses, indicative of drug-induced phospholipidosis. All macrolides have the potential to cause phospholipidosis which is considered a class effect. The studies in mice were consistent with the studies in rats (liver lesions).

Tolerance in the target species of animal
See Part IV.

Reproductive toxicity, including teratogenicity
Studies on the effects on reproduction
A two generation study was performed in rats with oral doses of 0, 10, 30 and 100 mg/kg/day.
It was concluded that drug related effects on reproduction were only seen in the high dose group (reduced litter size). The NOAEL was set at 30 mg/kg bw/day.

Embryotoxicity/foetotoxicity, including teratogenicity
Two studies were conducted in mice and two in rats, one preliminary and one definitive in each species. The outcome of the preliminary (dose finding) study in rats appears to have led to excessively high doses (0, 150, 300 and 450mg/kg/day) in the definitive study as maternal toxicity was seen at the lowest dose of 150 mg/kg/day. The NOAEL for foetal toxicity was set at 150 mg/kg/day.
Oral doses of 0, 100, 300 and 1000mg/kg/day were administered in the definitive mouse study. A reduction in gravid uterine weight in the dams at a dose rate of 300 mg/kg/day was ascribed to reduced foetal body weight and was not considered as maternal toxicity. The NOAEL for maternal toxicity was set at 300 mg/kg/day. Foetal effects included cleft palate, malpositioned testes, enlarged frontal sutures and incomplete ossification. The NOAEL for foetal effects was considered to be 300 mg/kg bw/day.

Mutagenicity
The following tests were conducted in relation to mutagenicity:
a) Ames test,
b) *in vitro* gene mutation in mouse lymphoma cells,
c) *in vitro* chromosomal aberration test in Chinese hamster ovary cells,
d) *in vivo* micronucleus test in mice, and
e) unscheduled DNA synthesis assay in rat liver.

All tests were conducted according to OECD guidelines and in compliance with current GLP procedures.
Negative results were obtained in the Ames test, mouse lymphoma cell assay, *in vivo*-micronucleus assay and the unscheduled DNA synthesis assay. Positive results were obtained at the highest doses in the *in vitro* chromosomal aberration test in Chinese hamster ovary cells, both when gamithromycin was dissolved in DMSO and when it was dissolved in acetone.
Based on this mutagenicity package, gamithromycin is considered not to be genotoxic *in vivo*.

Carcinogenicity
Two oral dosing carcinogenicity studies were conducted: a two year study in rats and an 18 month study in mice. In the rat study dose levels were 0*,* 10, 30, 100 mg/kg/day and in the mouse study dose levels were 30, 110, 325 mg/kg/day for males and 40, 150 and 440 mg/kg/day for females. Incidence of all gross and microscopic lesions remained within normal ranges for each species/strain and without any dose-response relationship.

There was no evidence of carcinogenic effects of gamithromycin.

Studies of other effects

Special studies
Neurotoxicity
Not performed

Skin sensitation
One study in 20 guinea pigs was performed and showed that gamihromycin was not considered a skin sensitiser in guinea pigs.

Skin irritation
One study was performed in three rabbits and showed that gamithromycin is slightly irritant.

Eye irritation
One study in three New Zealand white rabbits was conducted. Gamithromycin was administered at a dose of 0.1 g to the right eye of each of three rabbits. It was concluded that gamithromycin is an irritant of the eyes of the rabbit.

The skin and eye irritation are mentioned in the relevant sections of the SPC and a user safety recommendation has been made.

Observations in humans
No data are available in humans as gamithromycin has been developed for use in animals only.

Microbiological studies (studies on human gut flora and organisms used in food processing)

Minimum Inhibitory Concentration (MIC) of bacteria from the human intestinal flora
A GLP-compliant study against a range of human intestinal flora that met the requirements of the CVMP/VICH guidance "Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI" (CVMP/VICH/467/03-FINAL) was carried out. MIC data were determined against 100 bacterial strains, comprising 10 isolates from each of 10 genera representing the normal human intestinal microbiota. All strains were sourced from the faecal microbiota of healthy non-medicated humans.

The descriptive MIC data from the study against normal human gut flora is summarised below:

Bacterial Group	Summary MIC parameters (µg/ml)			
	MIC_{50}	MIC_{90}	Geometric mean MIC	MIC range
Bacteroides fragilis	4	32	5.7	1 to 32
Other *Bacteroides* spp.	4	32	7.5	1 to 32
Bifidobacterium	0.125	0.5	0.25	0.125 to 1
Clostridium	0.25	16	0.6	0.062 to >128
Enterococcus	2	4	1.3	0.062 to 4
Escherichia coli	2	8	3.3	2 to 8
Eubacterium	0.5	1	0.7	0.5 to 8
Fusobacterium	0.5	32	1.4	0.125 to >128
Lactobacillus	32	32	8.6	0.25 to 128
Peptostreptococcus	4	16	3.5	0.25 to 64

Based on the MIC_{50} data the MIC_{calc} was calculated to be 0.74 µg/ml for gamithromycin (MIC_{cal} is defined as the lower 90% confidence limit for the mean MIC_{50} of the most relevant genera).

Using the input data of MIC_{calc} (0.74 µg/ml) and the value obtained from an assay on the fraction of oral dose available (0.22) the microbiological ADI with respect to the colonisation barrier was calculated as follows:

$$ADI = \frac{0.74 \times 220}{(0.44 \times 0.5) \times 60} = 12.33 \text{ µg/kg bw (i.e. 740 µg/person)}$$

MICs of Food-Borne Pathogens and Commensal Organisms
Whilst conventionally macrolides are not active against *Enterobacteriaceae*, the azalide subclass represented here by gamithromycin does show activity against *Enterobacteriaceae*. In a GLP compliant study the activity of gamithromycin against commensal bacteria from the bovine intestinal tract and food borne pathogens of human clinical significance was reported. MICs were determined using internationally recognised standardised agar dilution MIC methodology, as described by the Clinical and Laboratory Standards Institute (CLSI). The results showed that gamithromycin has good activity against *Campylobacter* spp. and reduced activity against *Enterococcus* spp., *E. coli* and *Salmonella* spp.
The numbers of strains tested allows further breakdown of activity to species level, from which it was seen that gamithromycin was equally active against *Campylobacter jejuni* and *Campylobacter coli* and against the principal *Salmonella* isolates from cattle, yet was less active against *Enterococcus faecalis* than against *Enterococcus faecium.*

Assessment of Microbial Safety
The effect of the prolonged exposure of the intestinal flora on selection for resistance in intestinal bacteria with a special focus on *Campylobacter jejuni* was also assessed. The data with regard to the concentration of gamithromycin in the colon after administration at 6 mg/kg body weight showed that after 5 days, concentrations would fall below the MIC for *Campylobacter* spp. and thus there will be a period of at least 5 days when *Campylobacte*r spp. will be exposed to sub-MIC concentrations of gamithromycin. Within the same study *Bacteroides fragilis* and *Enterococcus* spp. counts were also monitored. *Bacteroides fragilis* was clearly inhibited whereas there was no change in the total anaerobe or *Enterococcus* spp. counts over the experimental period suggesting no gross changes to the overall gut flora ecosystem. The Applicant acknowledged that while there is no data relating to resistance development in *Campylobacter* spp. there is much that can be learned from other resistance development studies concerning azalide antimicrobials and in particular studies that have addressed resistance development at sub-MIC concentrations. *In vitro* and *in vivo* resistance emergence studies indicate that sub-MIC concentrations of the related (surrogate) antimicrobial, azithromycin do not induce significant bacterial resistance.

User safety

The user safety assessment addressed several key points relating to the use of gamithromycin such as exposure, hazard identification and user risk and safety assessment.

Exposure assessment
Only acute exposure was anticipated and evaluated because gamithromycin is a single one-off injection product. Exposure may occur via accidental self-injection of small volumes (estimated at 0.25 ml), with an assumed rapid and complete (100%) absorption from this site. This can be considered as the major route of exposure as compared to other routes of exposure. This worst-case scenario would result in an exposure to 37.5 mg gamithromycin (0.625 mg/kg). Other routes of exposure include accidental dermal and ocular exposure.

Hazard identification
Based on the toxicity studies conducted in rodents and dogs, and using data from acute and sub-chronic tests, a NOEL of 10 mg/kg was established in a 28-day toxicity test. There is no evidence of dermal toxicity. Gamithromycin is not mutagenic and is not considered to be toxic to reproduction except at maternotoxic doses. Gamithromycin is moderately irritant to the skin and irritant to the eye. It is not sensitising, under the test conditions used. A number of the excipients are known irritants and/or potential sensitisers and this is reflected in the user warnings in the SPC.

User risk and safety assessment
It is unlikely that significant user exposure will occur due to mitigating factors, such as the product's presentation in filled, ready-to-use bottles, prescription only use, limited single-dose indications in beef cattle, and route and method of administration with appropriate animal handling procedures. In rare cases of user exposure, professional users may come into contact with small volumes of product as the result of accidental topical, needle stick or incidental oral exposures during the product administration phase. Accidental needle sticks followed by product injection represent the worst-case exposure scenario as compared to other types of exposures since this product is readily absorbed from an injection site as demonstrated in gamithromycin marker residue studies in cattle. This presumption of 100% bioavailability after accidental injection is a conservative presumption since most needle stick injuries reported among veterinarians are classified as mild and localised at the site of injection and are rarely associated with severe systemic effects or time lost from work.

From the above assessment and the other presented data it was concluded that that there is a low health risk to professional users and minimal risk management measures are necessary.

Environmental safety

Phase I Assessment
The environmental impact of gamithromycin was evaluated on the basis of its intended use in cattle

and in line with the relevant VICH guideline. Two scenarios were investigated. One was the use of manure as fertilizer and the other one was the treatment of cattle in a pasture. In both cases the calculated maximum values for the PEC in soil were below the threshold level of 100 μg/kg and therefore there was no need for a Phase II evaluation, in accordance with the guideline. Further the excipients of gamithromycin are naturally occurring organic acids and one common anti-oxidant. These compounds should be extensively metabolised after sub-cutaneous injection and should not reach the soil as such.

Depletion of residues

Depletion of residues studies
Five studies were conducted regarding the depletion of residues. One was performed in rats, one in dogs and three in cattle. The dosing formulations used for experimental purposes in the metabolic studies of gamithromycin were prepared by spiking tritium-labeled gamithromycin into the appropriate formulation (3 batches).

The study performed in rats aimed to determine the stability of tritiated gamithromycin during metabolic transformations, and ensure that this radiolabelled compound could be used for metabolic profiling in cattle, the target species.

In the dog study eight dogs that received approximately 10 mg/kg of the tritiated compound were sacrificed at 6 or 24h after dosing. Total radioactive residues were determined and selected tissues, fluids and excreta were analysed to monitor gamithromycin and its metabolites. Total radioactivity levels were highest in the liver, followed by lung, kidney, fat and muscle, as observed in cattle.

Three studies were conducted in cattle: the first study was designed to determine the distribution and excretion of total residues of gamithromycin in cattle after SC administration. From the results, the liver is considered the target organ, since residue levels were highest in this organ at all sampling times.

The second study in cattle was designed to determine the metabolic profile of gamithromycin in cattle. The radioactive tissues collected from the first study were used. Total radioactivity levels were highest in the liver, followed by lung, kidney, fat and muscle. By 2 weeks post administration, most of the excreted radioactivity was primarily eliminated in the faeces and secondarily in the urine. The parent compound and the metabolites Declad (loss of a cladinose, sugar moiety) and M2 (N-dealkylated-declad) were the major residues in the selected tissues, fluids and excreta.

The third study in cattle was designed to determine the marker residue for gamithromycin in cattle after SC administration at the recommended therapeutic dose on days 10, 21, 35, 49 and 70. As described in previous studies, concentrations of the marker residue followed the order, injection site > liver > kidney > fat = muscle. In fat and muscle, the marker residue levels were very low after day 10 and remained below the limit of detection for the rest of the study. In other tissues, depletion followed a first order kinetics, with elimination half-lives of 7.0, 6.2 and 5.8 days in injection site, liver and kidney, respectively.

Based on the above studies in cattle, the liver appears as the target organ for gamithromycin, followed by kidney. Non-injection site muscle and fat may contain some detectable traces of residues. In rats and dogs, the major residue in urine and faeces consists of the parent compound, while declad and the translactone derivative are major metabolites.

Gamithromycin is considered as the marker residue, since it is the major residue, its distribution is comparable to the total residue, and its decline is similar to the decline of total residue level.

MRL

The Commission Regulation (EC) No.203/2008 inserted gamithromycin in Annex III of Council Regulation (EEC) No 2377/90 for cattle and pigs in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Gamithromycin	Gamithromycin	Bovine	20 µg/kg 200 µg/kg 100 µg/kg	Fat Liver Kidney	Provisional MRLs expire on 1.7.2009 Not for use in animals producing milk for human consumption

The excipients are included in Annex II of Council Regulation (EEC) No 2377/90.

An overall ADI of 10 µg/kg bw (i.e. 600 µg/person) was established for gamithromycin, based on the lowest NOEL identified in the toxicological evaluation of 1 mg/kg bw/day observed in the 52-week repeated dose toxicity study in dogs and using an uncertainty factor of 100 to account for inter and intra-species variability.

Withdrawal period

The withdrawal period was calculated in accordance with the CVMP guideline on injection site residues (EMEA/CVMP/542/03-FINAL) and the CVMP Note for guidance: approach towards harmonisation of withdrawal periods (EMEA/CVMP/036/95 FINAL). The statistical approach described in the second of these two guidance documents was used to demonstrate that at the selected withdrawal period of 64 days, marker residue concentrations in the target tissues liver, kidney and fat were below their respective MRLs and the total residues exposure that would result from ingestion of a standard food basket (including 300g of injection site muscle) would be below the ADI of 600µg per person.

Since no MRL has been established for milk, Zactran should not be used in animals producing milk for human consumption. An appropriate warning has been included in sections 4.3 and 4.11 (Contraindications and Withdrawal Period) of the SPC / product literature. This restriction on use should be extended to pregnant cows and heifers as well because of the long withdrawal period. The following text is, therefore, included in section 4.11 of the SPC: “Not permitted for use in lactating animals producing milk for human consumption. Do not use in pregnant cows or heifers, which are expected to produce milk for human consumption, within 2 months of expected parturition.”

Analytical method(s)

Full details of the analytical method (based on HPLC/MS/MS) for quantification of gamithromycin, which is both the parent compound and the marker residue in liver, kidney, muscle and fat, were provided in the MRL application and are briefly summarised in the summary report.

The Applicant committed to provide samples, standards, internal standard and other information as needed to the EMEA designated laboratory upon request to verify that the proposed analytical detection method allows the determination of the residue levels as decided by the Community in accordance with the provisions of Regulation (EEC) No 2377/90. The Applicant also committed to follow Volume 8 to address the deficiencies as suggested by CVMP to complete further validation of the method for residue monitoring.

OVERALL CONCLUSIONS ON SAFETY AND RESIDUES

Gamithromycin exerted no reproductive or developmental toxicity except at maternotoxic doses in the two rodent species tested. It was shown not to be mutagenic, according to 4 of the 6 studies reported, including *in vivo* tests and only in the *in vitro* chromosomal aberration assay was reported to have some effects at the highest doses tested. Gamithromycin was also shown not to be carcinogenic in rodents (rats and mice).

The pharmacokinetic properties of gamithromycin were examined in cattle and calves and found to be similar. Protein binding was determined in 4 species and found to be consistent across species. A specific method was developed by LC-MS and was validated for specificity, accuracy and precision.
The metabolic profile of gamithromycin was studied in three species (dog, cattle, rat). In rats and dogs, the major residue in urine and faeces consists of the parent drug, while Declad (loss of the cladinose sugar moiety) and the translactone derivative were the major metabolites. Metabolite M2 was not found in rats, but was found at very low levels in dogs. Although the metabolites found in cattle are also found in rats/dogs, the quantitative metabolite profile is different between rats/dogs and cattle. Gamithromycin was found to be concentrated in the liver, secondarily in the kidneys and in the fat. Muscle was shown to have low concentrations of residues, except at the site of injection.

The appropriate ratios of marker to total residues were determined, following a tissue residue depletion study. These ratios varied between 24% (liver) and 65% (injection site, high concentration) or 36% (injection site, median range). Rates of disappearance were similar for marker and total residues. The withdrawal period was set at 64 days.

4. EFFICACY ASSESSMENT

Pharmacodynamics
Gamithromycin is an azalide, 15-membered semisynthetic macrolide class antibiotic with uniquely positioned alkylated nitrogen at 7a-position of the lactone ring. This special chemistry facilitates rapid absorption at physiological pH and a long duration of action at the target tissue, lung.

Macrolides in general have both bacteriostatic and bactericidal action mediated through disruption of bacterial protein synthesis. Macrolides inhibit bacterial protein biosynthesis by binding to the 50S ribosomal subunit and by preventing peptide chain elongation. The *in vitro* data show that gamithromycin acts in a bactericidal manner. The broad spectrum antimicrobial activity of gamithromycin includes *Mannheimia haemolytica, Pasteurella multocida and Histophilus somni*, the bacterial pathogens most commonly associated with BRD. The MIC and MBC data are reported from a representative sample of isolates from field materials within different geographic EU areas.

Species	MIC_{90}	MBC_{90}
	µg/ml	
Mannheimia haemolytica	0.5	1
Pasteurella multocida	1	2
Histophilus somni	1	2

Three mechanisms are generally considered responsible for resistance to the macrolide class of compounds. This is often referred to as MLS_B resistance as it affects macrolides, lincosamides and streptogramins. The mechanisms involve the alteration of the ribosomal target site, the utilisation of active efflux mechanism and the production of inactivating enzymes.

In the target species, the main reaction observed were local signs at injection sites. There were no severe detrimental effects in overdosed animals. Non-published studies on the *in vitro* effect of the product on main chemical mediators, enzyme systems and transporters involved in physiological functions of the organism (including, e.g., alpha-adrenergic, beta-adrenergic) did not reveal any effect at the doses tested.

Impact of resistance development to efficacy

Gamithromycin is not used in human medicine, however, the macrolide class is widely used and so gamithromycin use in veterinary medicine might be expected to select for cross-resistance to those macrolides used in man. Further cross-resistance to other antimicrobials such as lincosamides and streptomycin B, also used in humans and animals is expected. Potential transmission of resistance to commencal and zoonotic bacteria has been described.

As there is no documented evidence of resistance development to gamithromycin in the field, it was not possible to make any substantial comment on the likely speed of resistance development.

Target animal safety

Tolerance in the target species of animal
Target species tolerance of gamithromycin 150 mg/ml injectable solution was assessed in a GLP compliant study in which the market formulation was administered subcutaneously to cattle at 1X, 3X, and 5X the recommended use level, (6mg/kg body weight), for a duration of 15 days (three times the recommended duration of use). Systemic and local injection site tolerances were evaluated. Different locations were used for administering each dose.

Clinical investigations revealed that animals from the 3X or 5X group exhibited some head twisting, attempting to lick and scratch the site of injection (3X) and/or pawing the ground (5X) at 10 minutes after treatment. Local reactions were detected (site-swelling, with heat). At the end of the study, only some skin thickening and/or discolouration could be observed at injection sites.

Mild, dose dependent, alterations in several red blood cell parameters were detected. These alterations were considered secondary to the local inflammation process at the site of injection. Since all mean values remained within normal ranges and since abnormal values were rare and not consistent, these changes are not considered clinically relevant.

Injection site reactions
The CVMP concluded that gamithromycin 150 mg/ml injectable solution in the proposed commercial formulation administered subcutaneously to cattle at 6.0 mg/kg body weight and at not more than the recommended 10 ml per injection site caused visible, oedematous or firm injection site swellings in 46% of treated animals. The reactions were largely transient, mainly resolving after 3 days without significant pain or visible skin changes and with only 14% still evident and largely resolving at 14/15-days after treatment. This transient injection site reaction pattern was considered typical of macrolides. Further there was no evidence of systemic treatment related adverse effects in the various pre-clinical and clinical efficacy studies conducted with the final formulation when administered at the recommended treatment dose in the target species. The principal adverse effect arising from the use of gamithromycin is local injection site reaction, which is considered typical of macrolides.

Laboratory studies:

Laboratory trials were not conducted

Dose titration study(ies)

Dose determination

Treatment of BRD
A GLP compliant dose titration study was carried out using an experimental *Mannheimia haemolytica* challenge model in ruminating cattle in order to demonstrate the efficacy of gamithromycin for the treatment of Bovine Respiratory Pneumonia (BRD). The proposed dose of gamithromycin given once at 6.0 mg/kg was tested by comparison with 3.0 and 9.0 mg gamithromycin/kg doses and a saline placebo treatment.

The results demonstrated that treatment with gamithromycin at 3, 6 or 9 mg/kg administered once, subcutaneously after the onset of clinical signs of respiratory disease, reduced the mortality and significantly ($p<0.05$) reduced the clinical signs and the lung lesions compared to the saline placebo treatment. A trend for the 6 mg/kg and 9 mg/kg doses to more consistently reduce the lung lesions in cattle was observed. Clinical parameter and lung lesion score improvements after treatment at 9 mg/kg gamithromycin were not generally significantly ($p>0.05$) greater than after treatment at 6 mg/kg.

Further four dose-ranging/dose determination GCP compliant studies were provided in support of the recommended treatment dose of 6 mg/kg for the treatment of bovine respiratory disease. The studies had the same basic design (controlled, blinded and randomised). Three test product (gamithromycin) treatment groups were included in each of the studies, using doses in the range 1 mg to 9 mg gamithromycin/kg. The results of these studies, when taken as a whole, suggested that 6 mg/kg was the appropriate dose to take use for the dose confirmation/field studies relating to the treatment claim.

Overall it was concluded that the results of the dose determination study together with those from the dose-ranging studies provided adequate clinical evidence that gamithromycin administered once subcutaneously at 6 mg/kg body weight is the optimum dose for the therapeutic treatment of BRD.

Prevention of BRD
The Applicant in order to support the 6.0mg gamithromycin/kg bodyweight prevention dose performed two controlled GCP compliant, challenge model dose determination studies comparing three doses of gamithromycin at 3.0, 6.0 and 9.0 mg/kg bodyweight and a saline (0.9%)-treated control. All doses were administered subcutaneously.

Although it was noted that the studies did not demonstrate clear statistical difference between doses because of the small number of animals within groups and the short observation periods, the results of those studies, supported the conclusion that the 6 mg/kg dose was superior over the 3 mg/kg and that the 9 mg/kg dose did not provide improved efficacy over the 6 mg/kg . Therefore the CVMP considers that there was sufficient data in support of the efficacy of the 6.0 mg/kg dose for the prevention claim.

FIELD STUDIES

The efficacy of gamithromycin 150 mg/ml injectable solution administered subcutaneously at 6 mg/kg bodyweight in the therapeutic and preventive treatment of BRD was evaluated in two field trials in the EU. The studies were conducted according to a controlled, blinded, randomized block design.

Treatment of BRD
One multi-centred field efficacy trial for the treatment of BRD was conducted across a wide range of cattle rearing units in different EU countries utilising 18 groups of animals. The trial was conducted according to Good Clinical Practice (GCP) standards. A total of 455 cattle including non-ruminating and ruminating animals of various breeds (beef and dairy) were enrolled. None of the animals had received bacterial vaccines for BRD within 6 months, or antimicrobials within 30 days prior to the trial. Prior to inclusion all cattle were confirmed as showing signs of naturally acquired BRD based on a standardised system of clinical scoring for Depression and Respiratory Character. By culturing nasal swabs collected pre-treatment from the 18 included groups of animals it was confirmed that 16 groups were infected with *Pasteurella multocida*, 15 *Mannheimia haemolytica*, 3 *Histophilus somni* and 12 *Mycoplasma bovis*. At one trial location no BRD target pathogens were isolated pre-treatment, and this group of animals was excluded from the assessment of treatment success.

The cattle were randomly divided into two approximately equal groups; one treated with gamithromycin 150 mg/ml injectable solution at 6 mg/kg bodyweight and the second positive control group treated with tulathromycin 10% injectable solution (Draxxin, Pfizer) at 2.5 mg/kg (the recommended dose).

Efficacy of therapeutic treatment of BRD was assessed by determining the number of treatment successes at 14 days after treatment, defined as cattle that were not declared treatment failures during the study. In addition, microbiological cure was assessed by monitoring target BRD pathogen isolations from nasal swabs collected at the end of each study from treatment successes and from nasal swabs or lungs of treatment failures including mortalities on the days these animals were declared treatment failures. The determination of efficacy was based on a non-inferiority comparison. A one-sided lower 95% confidence interval for the difference in the proportions of treatment success between the two groups was created. This was evaluated against the margin of difference of 20%.

For all of the variables (treatment success, depression scoring, respiratory character scoring and rectal temperature) gamithromycin was shown to be non-inferior to tulathromycin. The non-inferiority comparison of the results showed that the efficacy of Zactran was non-inferior to that of Draxxin.

Prevention of BRD
One multi-centred field efficacy trial for the prevention of BRD was conducted across a range of cattle rearing units in different EU countries, aiming to evaluate the preventive treatment efficacy of gamithromycin. The trial was conducted according to Good Clinical Practice (GCP) standards. A total of 575 cattle ranging in age from 11 days to 97 weeks and weighing 54 to 576 kg body weight were used.

At the start of the study included animals were at high BRD-risk but showing no clinical signs of disease. The high BRD-risk status of cattle was confirmed pre-enrolment by a greater than 5% of non-study animals in the same air-space as study animals at each location having presented with clinical signs of BRD within 48 to 72 h after the first BRD case, and the isolation of BRD target pathogens, *P. multocida* (2 locations), *M. haemolytica* (4 locations) and/or *M. bovis* (4 locations), from nasal swabs collected pre-enrolment from the BRD cases. None of the animals had received bacterial vaccines for BRD within 6 months, or antimicrobials within 30 days prior to enrolment in the trial.

The cattle were randomly divided into two equal groups; one treated with gamithromycin 150 mg/ml injectable solution at 6 mg/kg bodyweight and the second placebo, control group treated with 0.9% saline at 2.0 ml/ 50kg bodyweight.

Efficacy of preventive treatment against BRD was assessed by determining the number of treatment successes at 14 days after treatment defined as cattle that were not declared treatment failures during the study.

There were no BRD-related mortalities recorded during this trial. Data for all animals, ruminating and non-ruminating, were combined for the assessment of treatment success. The preventive treatment successes was 82% in the gamithromycin-treated group and 56% in the saline-treated controls ($p<0.01$).

The CVMP accepted that the results of the field trial for the preventative claim supported such a claim.

OVERALL CONCLUSION ON EFFICACY

Based on the dose-ranging studies and the challenge model using *Mannheimia haemolytica* the CVMP accepted that there was adequate clinical evidence to select a dose of gamithromycin of 6mg/kg b.w. administered once subcutaneously as the optimum dose for the therapeutic and preventive treatment of BRD.

It was also concluded that the results of the efficacy, clinical and field trials can support the treatment and prevention claims of the product against BRD.

5. BENEFIT RISK ASSESSMENT

In general the quality part of the dossier takes into account current rules and guidelines. Some outstanding points have been identified; however, the Applicant has committed to provide these data as post-authorisation commitments.

Gamithromycin inhibits the essential protein biosynthesis by selective binding to bacterial 50S ribosomal subunits in susceptible bacteria. The drug has a low oral toxicity. Reproduction studies were conducted with rats and developmental studies were performed in rats and rabbits. No teratogenic effects were observed. Adverse effects on maternal reproductive parameters or foetal development could only be induced with high oral doses. It was concluded that gamithromycin can be used in pregnant heifers/cows. The genotoxic potential of gamithromycin was evaluated in a number of *in vitro* and *in vivo* toxicological tests. The results of the generic toxicology assays indicate that gamithromycin is not genotoxic. Studies in rats and mice confirmed that gamithromycin has no carcinogenetic potential.

Gamithromycin was found to be a severe ocular irritant in rabbits and was considered "Irritating to eyes". Furthermore, gamithromycin was also considered to be a potential skin sensitiser. Therefore, appropriate user warnings have been included in the product literature for Zactran.

The withdrawal period was set by the CVMP at 64 days.

Since no MRL has been established for milk, Zactran should not be used in animals producing milk for human consumption. An appropriate warning has been included in sections 4.3 and 4.11 (Contraindications and Withdrawal period) of the SPC / product literature:
"Not permitted for use in lactating cattle producing milk for human consumption. Do not use in pregnant cows or heifers, which are intended to produce milk for human consumption, within 2 months of expected parturition."

Many calves exhibited injection site reactions, which persisted for about 14 days. This has been taken into account by including an appropriate warning in the SPC and product literature.

Shortcomings of the clinical field trial data provided were noted, especially with regard to the methodology of microbiological sampling in cattle, the short duration of post treatment observation periods, low completion rates at the end of the observation period and lack of post-treatment microbiological samples. However, the CVMP agreed that the efficacy of a single dose of 6 mg gamithromycin / kg b.w. had been sufficiently demonstrated for the treatment and prevention of Bovine Respiratory Disease (BRD) associated with *Mannheimia (Pasteurella) haemolytica*, *Pasteurella multocida* and *Histophilus somni.* Also, the efficacy of gamithromycin was demonstrated comparable to that of an already approved veterinary medicinal product containing tulathromycin.

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

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