Date of Approval: June 16, 2011

FREEDOM OF INFORMATION SUMMARY -

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-328

ZACTRAN

Gamithromycin -
Injectable Solution -
Beef and Non-Lactating Dairy Cattle -

For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in beef and non-lactating dairy cattle. ZACTRAN is also indicated for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD associated with *M. haemolytica* and *P. multocida*.

Sponsored by:

Merial Ltd.

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I. GENERAL INFORMATION:

A. File Number:	NADA 141-328
B. Sponsor:	Merial Ltd. - 3239 Satellite Blvd., Bldg. 500 - Duluth, GA 30096-4640 - Drug Labeler Code: 050604
C. Proprietary Name:	ZACTRAN
D. Established Name:	Gamithromycin
E. Pharmacological Category:	Antimicrobial
F. Dosage Form:	Injectable Solution
G. Amount of Active Ingredient:	150 mg gamithromycin/mL
H. How Supplied:	100, 250, and 500 mL bottles
I. How Dispensed:	Rx
J. Dosage:	6 mg/kg body weight (2 mL per 110 lb BW), administered one time
K. Route of Administration:	Subcutaneous injection in the neck
L. Species/Class:	Cattle/Beef and non-lactating dairy
M. Indications:	ZACTRAN is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in beef and non-lactating dairy cattle. ZACTRAN is also indicated for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD associated with *Mannheimia haemolytica* and *Pasteurella multocida*.

II. EFFECTIVENESS:

A. Dosage Characterization:

Four dose selection studies conducted in the U.S. were used to evaluate the relative effectiveness of various doses of gamithromycin (also referred to as ML-1,709,460) in the treatment of cattle naturally infected with bovine respiratory disease (BRD). The studies were conducted according to a randomized, controlled, masked design with 15 to 20 beef cattle per treatment group with body weights ranging from 150 to 270 kg. Doses of 1, 3, 4.5, 6, or 9 mg gamithromycin/kg body weight (BW) were compared to saline-treated controls. Animals were enrolled that exhibited BRD as determined by depression and/or respiratory character scores and a rectal temperature of $\geq$ 104ºF. Clinical (depression and respiratory) scores and rectal temperature were assessed daily for 14 days following treatment. On each observation day, any animal that died, did not improve, or showed elevation of clinical scores or temperature compared to enrollment was classified as a treatment failure. Treatment success was defined as any animal not classified as a treatment failure by the end of the study.

All gamithromycin doses except 1 mg/kg BW resulted in a significant ($p < 0.05$) increase in the numbers of treatment successes, compared to controls. The differences between gamithromycin doses were not significant ($p > 0.05$). However, the 6 mg/kg BW dose showed a numerical improvement over lower doses, and the 9 mg/kg BW dose showed no apparent advantage over 6 mg/kg BW. Therefore, 6 mg/kg BW was selected for use in the field effectiveness studies.

B. Substantial Evidence:

1. Dose Confirmation Study (Treatment of BRD)

a. - Title: "Field Efficacy Studies of ML-1,709,460 for the Treatment of Bovine Respiratory Disease in Cattle," Study Numbers PR&D 0115401, 0115402, 0115403, and 0115404. September to November 2004.

b. - Investigators and Study Locations:

Larry L. Smith, D.V.M., B.S., M.S.; Larry Smith Research and Development, Lodi, WI
Roger L. Sifferman, D.V.M.; Bradford Park Veterinary Hospital, Springfield, MO; Study Location: Rockdale, TX
William A. Wolff, D.V.M.; Columbia, MO
Marlene D. Drag, D.V.M., M.S., DACLAM; Merial Missouri Research Center, Fulton, MO

c. - Study Design:

1) - *Objective*: To evaluate the effectiveness of gamithromycin for the treatment of BRD when administered to cattle subcutaneously (SC) one time at 6 mg/kg BW.

2) - *Study Animals*: Multi-origin, commingled, purebred and crossbred, male (intact and castrated) and female beef cattle weighing 130 to 262 kg were obtained from sale barns and trucked 4 to 19.5 hours to the study sites. At each study site, all animals were from a single supplier of commingled animals from multiple origins. There were 497 animals used in the study (229 females, 268 males) across all sites.

3) - *Experimental Design*: The study was conducted at four sites in Wisconsin, Texas, and Missouri. Animals were evaluated for up to seven days after arrival and were enrolled if they exhibited clinical signs of BRD (depression score ≥ 1, AND respiratory character score ≥ 1, AND rectal temperature ≥ 104.0°F).

Depression score was evaluated using the following criteria:

0 = Normal (nothing unusual in animal's attitude);
1 = Mild Depression (somewhat slow coming to feed bunk, but did eat);
2 = Moderate Depression (slight head/ears drooping, reluctant to move about, reluctant to come to the feed bunk);
3 = Severe Depression (pronounced head/ear droop; very reluctant to move);
4 = Moribund (recumbent)

Respiratory character was evaluated using the following criteria:

0 = Normal (No abnormal respiratory symptoms present. Respiratory rate and effort were appropriate for the environment);
1 = Mild Respiratory Distress (serous nasal or ocular discharge and/or cough);
2 = Moderate Respiratory Distress (mucous or mucopurulent nasal or ocular discharge and/or increase in respiratory rate or effort);
3 = Severe Respiratory Distress (marked increase in respiratory rate or effort including one or more of the following: open mouth breathing, abdominal breathing, or extended head)

At each site, enrolled cattle were randomly allocated to replicate and to treatment group. Each replicate included three animals (two treated, one control). Day 0 was the day of enrollment and was the same for each animal in a replicate. The experimental unit was the animal.

4) - *Drug Administration*: Gamithromycin was administered to animals assigned to the treated group as a SC injection of 6 mg/kg BW in the neck on their day of enrollment (Day 0). An equivalent volume of sterile saline was administered subcutaneously to animals assigned to the control group on their day of enrollment (Day 0). A maximum of 10 mL was administered per injection site.

5) - *Measurements and Observations*: The primary variable was the number of treatment successes on Day 10. Animals were clinically scored and their rectal temperatures were recorded daily from Days 1 to 10. From Days 4 to 9 and on Day 10, animals that met treatment failure criteria were classified as treatment failures and were removed from the study. On Day 10, remaining animals that met treatment success criteria were classified as treatment successes.

Treatment failure was defined as any mortality or animal euthanized due to BRD as confirmed by necropsy, or any animal that met the following clinical criteria:

From Days 4 to 9: -
Depression Score > 1, OR -
Respiratory Score > 1, AND -
Temperature ≥ 104.0°F -

On Day 10: -
Depression Score > 1, OR -
Respiratory Score > 1, OR -
Temperature ≥ 104.0°F -

Treatment success on Day 10 was defined as any remaining animal that met the following clinical criteria:

Depression Score = 0, OR -
Respiratory Score = 0, AND -
Temperature < 104.0°F -

Nasal swab samples were collected from each animal prior to treatment for identification of BRD pathogens. Animals were monitored for adverse experiences beginning on Day 0, and injection sites were monitored beginning on Day 1. Lung lesions were sampled and tracheal swabs were collected for identification of BRD pathogens from any animal that died or was euthanized. The persons performing post-treatment evaluations were masked to the treatment assignment.

d. - Statistical Analysis: Treatment success rates were compared between treated and untreated animals using a generalized linear model with a logit link function and a binomial error distribution. Treatment was included as a fixed

effect, and site and site-by-treatment were included as random effects. Depression and respiratory scores were compared between treatment groups using the Cochran-Mantel-Haenszel (CMH) chi-squared test. Scores were collapsed into "normal" or "abnormal" for each day with study site included as a stratum. Body temperatures were analyzed using a mixed model for repeated measures analysis of variance (ANOVA). Treatment, day, and treatment-by-day were included as fixed effects. Site, site-by-treatment, site-by-day, and site-by-day-by-treatment were included as random effects. The pre-treatment value was included as a covariate in the model. Compound symmetry was used as the variance-covariance matrix. Significance for all comparisons was evaluated using $\alpha = 0.05$.

e. - Results: Treatment success rates were statistically significantly different ($p = 0.03$) between the gamithromycin-treated group (58%) and the saline control group (19%).

f. - Adverse Events: Injection site swelling was noted in 44/327 (13.5%) of gamithromycin-treated animals. Swelling resolved by Day 6 in 89% of treated animals, and was resolved in all except one animal by Day 10. There were no other adverse events in the study that were attributed to gamithromycin administration.

g. - Conclusion: Gamithromycin is effective for the treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* when administered to beef cattle as a SC injection of 6 mg/kg BW given one time.

2. Dose Confirmation Studies (Control of BRD)

The effectiveness of gamithromycin for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD associated with *M. haemolytica* and *P. multocida* was demonstrated in two separate field studies that used similar study designs and were analyzed independently. Sufficient numbers of isolates of *M. haemolytica* and *P. multocida* were recovered across both studies for inclusion in the BRD control indication. *H. somni* and *M. bovis* were also recovered during the study, but not in sufficient numbers to justify inclusion in the BRD control indication at this time.

a. - Field Study

1) - Title: "Field Efficacy Study of ML-1,709,460 [gamithromycin] for the Control of Bovine Respiratory Disease in Cattle." Study No. PR&D 0101405. November 2006.

2) - Investigator and Study Location: Kelly Lechtenberg, D.V.M., Ph.D.; Midwest Veterinary Services, Oakland, NE

3) - Study Design:

a) - *Objective*: To evaluate the effectiveness of gamithromycin for the control of respiratory disease in cattle at high risk of developing BRD.

b) - *Study Animals*: A total of 201 multi-origin, commingled, crossbred male (intact and castrated) beef cattle, approximately 6 to 8 months old, weighing 170 to 256 kg were obtained from auction markets in Kentucky and Tennessee, and were transported by truck to the study site.

Cattle were received at the site on Day -1. On Day 0, animals were processed according to standard cattle arrival procedures and evaluated for signs of BRD. A total of 159 animals with depression scores = 0 (on a scale from 0 [normal] to 4 [moribund]), respiratory character scores ≤ 1 (on a scale from 0 [normal] to 3 [severe respiratory distress]), and rectal temperatures < 104.0 °F were enrolled, randomly assigned to treatment group, and treated.

c) - *Treatment Groups*: Animals were randomly assigned to one of two treatment groups – gamithromycin (106 animals) or saline (53 animals).

d) - *Drug Administration*: The test article was gamithromycin injectable solution (150 mg gamithromycin/mL), administered at 6 mg/kg (2 mL per 110 lb) BW. The control article was 0.9% sterile saline for injection, administered at 2 mL per 110 lb BW. The test and control articles were administered by SC injection one time on Day 0. Treatments were administered in the left side of the neck, with a maximum volume of 10 mL per injection site.

e) - *Measurements and Observations*: General health observations were performed daily from Days 0 to 10. Animals were observed for mortality and clinical signs of BRD once daily from Days 1 to 10 at approximately the same time each day by personnel who were masked to the treatment assignments. Injection site irritation was monitored daily from Days 1 to 10.

From Days 1 to 10, animals with a depression score ≥ 1 or respiratory score ≥ 2 had rectal temperatures taken. Animals that met treatment failure criteria on Days 1 to 10 were declared treatment failures and

removed from the study pens. Treatment failure was defined as any one of the following:

- Mortality/euthanasia due to BRD (confirmed by necropsy);
- Any animal with a depression score ≥ 1 OR respiratory score ≥ 2, AND rectal temperature ≥ 104.0 °F;
- Any animal with a respiratory score = 3 (regardless of rectal temperature); or
- Any animal with a depression score ≥ 3 (regardless of rectal temperature).

Nasopharyngeal swabs were collected for identification of BRD pathogens from animals that were removed from the study due to BRD (treatment failures).

4) - Statistical Analysis: The primary variable was treatment success on Day 10. Treatment success was defined as any animal that remained alive and in the study on Day 10 and was not classified as a treatment failure.

The proportion of treatment successes in the gamithromycin-treated and saline-treated groups was analyzed by a generalized linear mixed model with treatment as the fixed effect and pen and pen-by-treatment interaction as random effects. The model had a logit link function and a binomial error distribution. A two-sided test was used at the significance level of 0.05.

5) - Results: There was a statistically significant difference ($p = 0.0019$) for Day 10 treatment success between the gamithromycin-treated group (91/106, 86%) and the saline-treated control group (19/53, 37%).

Isolates of *M. haemolytica* (from 12 animals) and *P. multocida* (from 23 animals) were recovered from post-treatment nasopharyngeal swabs.

6) - Adverse Events: Palpable injection site swellings were observed at least once in 52 of 106 gamithromycin-treated animals. All visible injection site swellings resolved prior to Day 4. No other test-article related adverse events were reported in this study.

7) - Conclusions: This study demonstrates that gamithromycin injectable solution is effective for the control of respiratory disease in cattle at high risk of developing BRD when administered one time as a SC injection of 6 mg/kg BW (2 mL per 110 lb BW).

b. - Field Study

1) - Title: "Field Efficacy Study of ML-1,709,460 [gamithromycin] for the Control of Bovine Respiratory Disease in Cattle." Study No. PR&D 0194401. December 2008.

2) - Investigator and Study Location: C. Scanlon Daniels, DVM; Agri-Research, Inc., Canyon, TX -

3) - Study Design:

a) - *Objective*: To evaluate the effectiveness of gamithromycin for the control of respiratory disease in cattle at high risk of developing BRD.

b) - *Study Animals*: A total of 339 multi-origin, commingled, crossbred female beef cattle, approximately 6 to 10 months old, weighing 130 to 293 kg were obtained from auction markets in Arkansas, and were transported by truck to the study site.

Cattle were received at the site on Day -1. On Day 0, animals were processed according to standard cattle arrival procedures and evaluated for signs of BRD. A total of 308 animals with depression scores = 0 (on a scale from 0 [normal] to 4 [moribund]), respiratory character scores ≤ 1 (on a scale from 0 [normal] to 3 [severe respiratory distress]), and rectal temperatures < 104.0 °F were enrolled, randomly assigned to treatment group, and treated.

c) - *Treatment Groups*: Animals were randomly assigned to one of two treatment groups – gamithromycin (154 animals) or saline (154 animals).

d) - *Drug Administration*: The test article was gamithromycin injectable solution (150 mg gamithromycin/mL), administered at 6 mg/kg (2 mL per 110 lb) BW. The control article was 0.9% sterile saline for injection, administered at 2 mL per 110 lb BW. The test and control articles were administered by SC injection one time on Day 0. Treatments were administered in the left side of the neck, with a maximum volume of 10 mL per injection site.

e) - *Measurements and Observations*: General health observations were performed daily from Days 0 to 10. Animals were observed for mortality and clinical signs of BRD once daily from Days 1 to 10 at approximately the same time each day by personnel who were masked to treatment assignments. Injection sites were monitored for visible signs of inflammation daily from Days 1 to 10.

From Days 1 to 10, animals with a depression score ≥ 1 or respiratory score ≥ 2 had rectal temperatures taken. Animals that met treatment failure criteria on Days 1 to 10 were declared treatment failures and removed from the study pens. Treatment failure was defined as any one of the following:

- Mortality/euthanasia due to BRD (confirmed by necropsy);
- Any animal with a depression score ≥ 1 OR respiratory score ≥ 2, AND rectal temperature ≥ 104.0 °F;
- Any animal with a respiratory score = 3 (regardless of rectal temperature); or
- Any animal with a depression score ≥ 3 (regardless of rectal temperature).

Nasopharyngeal swabs were collected for identification of BRD pathogens from each animal at enrollment and from animals that were removed from the study due to BRD (treatment failures).

4) - Statistical Analysis: The primary variable was treatment success on Day 10. Treatment success was defined as any animal that remained alive and in the study on Day 10 and was not classified as a treatment failure.

The proportion of treatment successes in the gamithromycin-treated and saline-treated groups was analyzed by a generalized linear mixed model with treatment as the fixed effect and pen and pen-by-treatment interaction as random effects. The model had a logit link function and a binomial error distribution. A two-sided test was used at the significance level of 0.05.

5) - Results: There was a statistically significant difference ($p = 0.0016$) for Day 10 treatment success between the gamithromycin-treated group (120/154, 78%) and the saline-treated control group (90/154, 58%).

M. haemolytica (88 isolates) and *P. multocida* (110 isolates) were isolated from pre-treatment and post-treatment nasopharyngeal swabs.

6) - Adverse Events: No test-article related adverse events were reported in this study.

7) - Conclusions: This study demonstrates that gamithromycin injectable solution is effective for the control of respiratory disease in cattle at high risk of developing BRD when administered one time as a SC injection of 6 mg/kg BW (2 mL per 110 lb BW).

III. TARGET ANIMAL SAFETY:

The target animal safety of gamithromycin was demonstrated in a toxicity and injection site irritation study using the final formulation of the drug.

A. Toxicity/Injection Site Irritation Study

1. - Title: "Evaluation of the Safety of ML-1,709,460 Injection When Administered as a Single Subcutaneous (SC) Dose to Cattle at 1X, 3X, and 5X the Recommended Use Level for 3X the Projected Duration of Use". Study No. PR&D 0097101. September to November 2003.

2. - Study Director: Dr. John Holste; Missouri Research Center, Fulton, MO/Holste Biological and Pharmaceutical Services, Inc., Columbia, MO

3. - Study Design:

   a. - *Objectives:* To evaluate the safety of gamithromycin injection in the proposed market formulation when administered SC to cattle at 1X, 3X, and 5X the labeled dose of 6 mg/kg BW, for a duration of 15 days (three times the labeled duration of use); and to determine the acceptability of gamithromycin injectable formulation after SC administration in cattle at 6 mg/kg BW. The study was conducted in compliance with FDA Good Laboratory Practice Regulations (21 CFR Part 58).

   b. - *Study Animals:* A total of 32 healthy, ruminating, intact male and female, approximately six-month old, Angus cattle, weighing an average of 196 kg, were enrolled in the study. Animals were housed individually in pens.

   c. - *Treatment Groups:* Cattle were randomly assigned to one of four treatment groups – saline, or one of three gamithromycin dose levels. There were 8 animals (4 males and 4 females) per treatment group.

   d. - *Test Article Administration:* The test article was gamithromycin, supplied as an injectable solution containing 150 mg/mL. Cattle were treated with gamithromycin by SC injection at 6.0 (1X), 18.0 (3X), or 30.0 mg/kg BW (5X) on Days 0, 5, and 10 (three times the maximum labeled duration). The Day 0 injection was given in the left middle neck of all animals and was used to evaluate injection site irritation. A maximum volume of 10 mL was administered per injection site.

   The control article was 0.9% saline solution for injection, administered at 10 mL per 50 kg BW (the volume equivalent to the 5X dose of gamithromycin) by SC injection. Control animals were treated on the same schedule as treated animals.

e. - *Measurements and Observations:* Blood samples for hematology and serum chemistry analyses and urine samples for routine urinalysis were collected from all animals pre-treatment and on Days 5, 10, and 15 post-treatment. General health observations were conducted hourly for the first four hours after dosing and then twice daily. Clinical examinations (including hydration status) were performed pre-treatment and on Days 0, 5, and 10 (four to eight hours post-injection), and on Day 15 prior to necropsy. Injection sites were evaluated on Days 1, 4, 5, 10, and 15. Body weights were recorded during acclimation and on Days 5, 10, and 15. Feed consumption was recorded daily. Animals were euthanized on Day 15. At necropsy, a gross examination was performed, and organs and tissues (including Day 0 injection sites in the 1X group) were collected for histological examination.

f. *Statistical Methods:* All analyses were performed using the PROC MIXED procedure of SAS Version 8.2. Analyses of plasma chemistry, hematology, quantitative urinalysis variables of specific gravity and pH, body weight, heart rate, and temperature were performed using analysis of covariance for a repeated measures design. Factors included "replicate (nested within sex)" and "replicate by day (nested within sex)" as random effects; and sex, treatment, sampling day, and all interactions among these three factors as fixed effects. An average pre-test value was used as a covariate if there were more than one pre-test value for a variable.

Average feed consumption was analyzed for every two- to three- day period (Days 1 through 3, 4 through 6, 7 through 9, 10 through 12, and 13 through 14) per subject, with the average daily feed consumption from Days -7 through -1 as the baseline covariate. Replicate, replicate-by-treatment, and replicate-by-days were included as random effects. A heterogeneous first-order autoregressive covariate structure was used to account for the repeated measures.

4. - Results:

a. - *Clinical Observations:* There were no unscheduled deaths or post-treatment animal removals during this study. Some animals in the 3X and 5X groups exhibited signs of injection site discomfort at 10 minutes post-treatment after each injection. Injection site observations included transient swelling, heat, edema, pain, and firmness at the injection site. There were no other treatment-related abnormalities observed.

b. - *Feed Consumption:* Mean feed consumption was statistically significantly decreased ($p < 0.10$) in the 5X group, and numerically decreased in the 3X group, compared to the control group from Days 1 to 6. However, mean feed consumption in the 3X and 5X groups was numerically higher than the control group for the remainder of the study. In addition, mean feed consumption in the 3X and 5X groups was numerically decreased compared to the 1X group

for all study day periods, with the exception of the 3X group for Days 13 to 14.

c. - *Hematology/Clinical Chemistry:* Dose-related increases in platelet counts and decreases in red cell variables (hematocrit, hemoglobin, and red blood cell count) were noted in all gamithromycin-treated groups, and were considered to be related to injection site inflammation. Red cell variables remained within normal limits in the 1X group. Hematocrit and hemoglobin decreased slightly below the reference range in some animals in the 3X and 5X groups. No adverse effects on bone marrow cytology were seen. Mean activated partial thromboplastin time (APTT) exhibited a statistically significant ($p < 0.10$) dose-related increase. A similar trend was seen with prothrombin time (PT). Thrombin clotting time (TT) was observed to decrease in the 5X group and no dose-related trend was observed. None of the observed changes were considered clinically significant.

d. - *Necropsy Evaluation/Histopathology:* Other than injection site reactions, no clinically significant, treatment-related gross lesions were observed. Thickened injection site regions and fascial plane edema in the right shoulder were observed in some treated animals in all dose groups. Minimal perivascular inflammation was seen in the brains of five animals in the 5X group, and one animal in the 3X group. This was not considered clinically significant.

e. - *Injection Sites (Day 0 Injection Site, Control and 1X Group):* On Day 15, gross lesions in the 1X group were consistent with inflammation and included: thickened skin and subcutis (6/8 animals), muscle discoloration (1/8 animals), and red discoloration of the injection site (1/8 animals). One animal showed no gross lesions. No gross lesions were observed in the control group. Histopathological examination showed chronic and/or granulomatous inflammation accompanied by fibrin deposition and hemorrhage in all 1X group animals. Inflammation ranged from mild to severe, with most animals exhibiting mild to moderate inflammation. One control animal showed mild hemorrhage at the injection site microscopically.

5. - Conclusions: This study demonstrates that gamithromycin injection is safe when administered to cattle one time subcutaneously at 6 mg/kg BW.

IV. HUMAN FOOD SAFETY:

A. Toxicology

1. Summary of Toxicology Studies

a. 13-Week Oral (Diet Admixture) Toxicity Study in Mice. Study No. PR&D 0085201. June 2004.

1) *Study Director and Location:* John Findlay, B.S.; Experimur, Chicago, IL

2) -*Experimental Design:* CD-1 mice (10 per sex per dose) were administered gamithromycin at the constant diet concentrations of 0, 210, 420, and 2100 ppm (corresponding to the target doses of 0, 50, 100, or 50 mg/kg BW/day, respectively) for 13 consecutive weeks. Body weights and food consumption were recorded. Daily drug intake was calculated based on food consumption. At the end of the study, all mice were euthanized and subjected to gross necropsy. Blood was collected prior to necropsy for clinical chemistry and hematology. Histopathology was performed on all tissues in control and high-dose animals, and on livers of all animals.

3) -*Results and Conclusion:* No treatment-related clinical signs of toxicity were observed. There were no treatment-related effects noted in food consumption, body weight or body weight gain, and clinical chemistry or hematology parameters. Treatment with 500 mg/kg BW/day resulted in microscopic liver lesions, characterized by centrilobular vacuolation, pale hepatocytes with coarse, granular-staining cytoplasm, and microvesicular vacuoles. The incidence of these lesions observed was 40% in males and 70% in females. Based on the liver effect and with consideration of the actual test article intakes for the 100 mg/kg BW/day group, a no-observed-effect-level (NOEL) for gamithromycin was established at 65.2 mg/kg BW/day for males and 135.6 mg/kg BW/day for females.

b. 13-Week Oral (Diet Admixture) Toxicity Study in Rats. Study No. PR&D 0085101. June 2003.

1) *Study Director and Location:* John Findlay, B.S.; Experimur, Chicago, IL

2) -*Experimental Design:* Sprague-Dawley rats (10 per sex per dose) were administered gamithromycin at the constant diet concentrations of 0, 300, 600, and 1000 ppm (corresponding to the target doses of 0, 30, 60, or 100 mg/kg BW/day, respectively) for 13 consecutive weeks. Body weights and food consumption were recorded. Daily drug intake was calculated based on food consumption. Ophthalmic examinations were performed. Blood was collected periodically for clinical chemistry and hematology

analysis. Urine samples were collected and analyzed. At the end of the study, all rats were euthanized and subjected to gross necropsy. Histopathology was performed on all tissues collected from control and high-dose animals, and on livers from the low- and mid-dose animals.

3) -*Results and Conclusion:* No treatment-related clinical signs of toxicity were observed. There were no treatment-related effects noted in food consumption, body weight or body weight gain, and clinical chemistry or hematology parameters. The high-dose animals showed high incidences (80% for males and 100% for females) of multifocal vacuolation in the bile duct epithelium, in contrast to the absence of such lesions in control animals. Based on the effect on bile ducts and with consideration of the actual test article intakes for the 60 mg/kg BW/day group, a NOEL for gamithromycin was established at 26.7 mg/kg BW/day for males and 35.6 mg/kg BW/day for females.

c. 52-Week Oral (Capsule) Toxicity Study in Beagle Dogs. Study No. PR&D 0090601. October 2004.

1) *Study Director and Location:* John Findlay, B.S.; Experimur, Chicago, IL

2) -*Experimental Design:* Beagle dogs (6 per sex per dose) were orally dosed with gamithromycin via gelatin capsule at dose levels of 0, 0.3, 1, and 3 mg/kg BW/day for 52 consecutive weeks. Body weights were recorded. Clinical pathology parameters in blood and urine were measured periodically. Ophthalmic and electrocardiogram examinations were performed. At the end of the study, all dogs were euthanized and subjected to gross necropsy. Histopathology was performed on all tissues collected from control and high-dose animals and on liver, gall bladder, eyes, heart, pituitary gland, aorta, epididymides, and lymph nodes (mandibular and mesenteric) from the low- and mid-dose animals.

3) -*Results and Conclusion:* No treatment-related clinical signs of toxicity were observed. There were no treatment-related effects noted in food consumption, body weight or body weight gain, ophthalmic and electrocardiogram observations, urinary or hematology parameters, and organ weights. The high-dose group had elevated triglyceride levels at all time points examined. Treatment-related microscopic changes occurred in the eyes and epididymis in the high-dose animals. Minimal to moderate vacuolation of the non-pigmented epithelial cells of the retina was noted in one male and one female at the high dose, while an increase in the incidence and severity of epithelial vacuoles in the epididymis was noted in four high-dosed males. A NOEL for gamithromycin was established at 1 mg/kg BW/day based on microscopic changes in the eyes of both

genders and epididymis and elevated triglyceride levels in males at the high dose (3 mg/kg BW/day).

d. Oral (Gavage) Development Toxicity Study in Rats. Study No. PR&D 0070301. March 2005.

1) -*Study Director and Location:* Raymond G. York, Ph.D.; Charles River Discovery and Development Services, Argus Division, Horsham, PA

2) -*Experimental Design:* Pregnant Sprague Dawley rats (25 per dose) were administered gamithromycin (150, 300, and 450 mg/kg BW/day) or vehicle (aqueous 0.5% methylcellulose) orally by gavage once daily on gestation days (GD) 6 through 18. Dams were euthanized on GD 21, and subjected to Caesarian section and gross necropsy. Clinical observation, feed consumption, and body weights were recorded. Maternal endpoints measured included weight of gravid uterus with ovaries, number and distribution of corpora lutea, number and distribution of implantation sites, and size, color and shape of placentae. Fetal endpoints measured included number of live and dead fetuses, early and late resorptions (defined as gross presence or absence of organogenesis, respectively), fetal weight and sex, and gross external alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations, and the remaining half were examined for skeletal alterations.

3) -*Results and Conclusion:* The test article was clearly toxic to dams at the highest dose as indicated by a high incidence (68%) of premature death. At the two lower doses, non-fatal toxic effects were prevalent. A maternal NOEL for gamithromycin could not be determined due to adverse clinical observations, reduced feed consumption, and decreased body weights at the lowest dose (150 mg/kg BW/day). With regard to fetal effects, the highest dose (450 mg/kg BW/day) reduced fetal weight by 41% and was clearly teratogenic with 71.4% of the litters examined showing fetal variations. At the 300 mg/kg BW/day dose, soft tissue and skeletal variations were also noted but the reduction in fetal body weight was less severe (8%). The fetal NOEL for gamithromycin was 150 mg/kg BW/day. Because treatment was initiated on GD 6, this study did not evaluate potential effects on development during the pre-implantation period. Therefore, this study was considered together with additional studies in which gamithromycin was administered to pregnant animals during the peri-implantation period (e.g., reproductive toxicity testing under item f below) to make the final conclusions regarding the human food safety of gamithromycin.

e. Oral (Gavage) Developmental Toxicity Study in Mice. Study No. PR&D 0070401. December 2004.

1) -*Study Director and Location:* Raymond G. York, Ph.D.; Charles River Discovery and Development Services, Argus Division, Horsham, PA

2) -*Experimental Design:* Pregnant CD-1 mice (25 per dose) were administered gamithromycin (100, 300, and 1000 mg/kg BW/day) or vehicle (aqueous 0.5% methylcellulose) orally by gavage once daily on GD 6 to 17. Dams were euthanized on GD 18, and subjected to Caesarian section and gross necropsy. Clinical observations and body weights were recorded. Maternal endpoints measured included weight of gravid uterus with ovaries, number and distribution of corpora lutea, number and distribution of implantation sites, and size, color and shape of placentae. Fetal endpoints measured included number of live and dead fetuses, early and late resorptions (defined as gross presence or absence of organogenesis, respectively), fetal weight and sex, and gross external alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations and the remaining half were examined for skeletal alterations.

3) -*Results and Conclusion:* The high dose (1000 mg/kg BW/day) was toxic to dams, as evident by increased mortality, increased incidence of adverse clinical observations, and decreased body weight gain (35%). A maternal NOEL could not be accurately determined due to the lack of food consumption data. The high dose caused clear fetotoxicity (increased numbers of early resorptions), inhibited fetal growth (reduced gravid uterine and fetal body weights without a change in litter size), and exhibited teratogenic effects (cleft palate, malpositioned testes, large frontal sutures, incompletely ossified ribs, delayed sternal ossification, and decreased number of ossification sites at multiple locations). At the mid-dose (300 mg/kg BW/day), gravid uterine weight was reduced by 10%, but this decrease was not accompanied by smaller litter size or reduced fetal weight. Other significant fetal effects at this dose included increased incidence of medially rotated hind limbs in 4 (of 254) fetuses from 3 (of 22) litters. These findings were considered equivocal because rotated hind limbs were not observed in the high-dose group. The developmental NOEL for gamithromycin was 300 mg/kg BW/day. Because treatment was initiated on GD 6 (implantation in the mouse occurs on day 5 post-mating), this study did not allow the evaluation of potential effects on development during the pre-implantation period. Therefore, this study was considered together with additional studies in which gamithromycin is administered to pregnant animals during the peri-implantation period (e.g., reproductive toxicity testing under item f below)

to make the final conclusions regarding the human food safety of gamithromycin.

f. Two-Generation Oral (Diet Admixture) Reproductive Toxicity Study in Rats. Study No. PR&D 0100501. August 2005.

1) - *Study Director and Location:* Bernadette Ryan, Ph.D., D.A.B.T.; Experimur, Chicago, IL

2) - *Experimental Design:* Parental male and female Sprague-Dawley rats (P1) (26 per sex per dose) were fed diet admixture containing 0, 100, 300, or 1000 ppm (equivalent to 0, 10, 30, or 100 mg/kg BW/day, respectively) gamithromycin for 10 weeks prior to mating, 2 weeks during mating, and 22 days following parturition (15 weeks total). The offspring (F1) were fed gamithromycin for 11 weeks, 2 weeks during mating, and 22 days following parturition (16 weeks total). Body weight and food consumption were recorded. P1 and F1 rats were analyzed for signs of general toxicity and effects on reproductive performance. All P1 and F1 rats were subject to necropsy. Histopathology was performed on a variety of reproductive and non-reproductive tissues from P1 and F1 parents, and F1 and F2 pups. F2 pups were analyzed at weaning.

3) - *Results and Conclusion:* Treatment with the high dose resulted in reduced weight gain during gestation in P1 dams, increased pituitary to body weight ration in P1 dams, decreased size of F1 litters (and an insignificant decrease in the size of F2 litters), increased pup weight at birth and weaning (F1), decreased pup survival on postnatal day (PND) 21 (F2), increased thymus to body weight ratio (F1 male weanlings), decreased brain to body weight ratio in F1 weanlings and adults of both sexes, increased feed consumption and body weight gain in F1 males, decreased numbers of primordial ovarian follicles in F1, and vacuolization of the hepatic bile duct in both the parental and F1 generations. Treatment with the mid-dose resulted in increased thymus to body weight ratio (F1 male weanlings), and decreased pup survival on PND 21 (F2). Treatment with the low dose resulted in increased thymus to body weight ratio (F1 male weanlings). The NOEL for systemic toxicity was 30 mg/kg BW/day based on histological changes in the bile duct of the liver at the highest dose. The reproductive NOEL was 10 mg/kg BW/day based on decreased pup survival in the F2 generation and equivocal effects on thymus to body weight ratio at the mid dose. The developmental NOEL was determined from the developmental studies listed above.

g. Bacterial Reverse Mutation Assay using *Salmonella typhimurium/Escherichia coli.* Plate Incorporation Test with and without metabolic activation (Ames Test). Study No. PR&D 0061501. November 2001.

1) -*Study Director and Location:* Valentine O. Wagner, III, M.S.; BioReliance, Rockville, MD

2) -*Experimental Design:* Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and one strain of *Escherichia coli* WP2*uvr*A were used. Acetone was used as the solvent for the test article, gamithromycin. To determine the toxicity, ten concentrations of the test article ranging from 6.7 µg per plate to 5000 µg per plate, or 0.00033 µg per plate to 10.0 µg per plate were tested in the presence and absence of S9 mix. The reverse mutation was assessed with test concentrations from 0.033 to 3.3 µg per plate (for *Salmonella* strains) or 0.033 to 10 µg per plate (for the *E. coli* strain) in the presence and absence of S9.

3) -*Results and Conclusion:* No positive increases in the number of revertants were observed with any of the test strains either in the presence or absence of S9. In the confirmatory assay, no positive increases in the number of revertants per plate were observed with any of the test strains either in the presence or absence of S9. Gamithromycin was not mutagenic under the conditions of this assay.

h. *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$) Mouse Lymphoma Assay. Study No. PR&D 0055901. February 2002.

1) -*Study Director and Location:* Richard H.C. San, Ph.D.; BioReliance, Rockville, MD

2) -*Experimental Design:* Mouse lymphoma L5178Y cells, clone 3.7.2C were used in the assay. Dimethyl sulfoxide (DMSO) was used as the solvent for the test article, gamithromycin. A preliminary toxicity test was performed to establish the optimal dose levels for the mutagenesis assessment. In the non-activation phase of the mutation assay using 4 or 24 hours exposure, concentrations ranging from 15 to 300 µg/mL were cloned; in the S9-activation system, concentrations of 300 to 600 µg/mL were cloned. The induction of forward mutation at the thymidine kinase (TK) locus in the presence and absence of S9 was assayed by colony growth of L5178Y/TK mouse lymphoma cells in the presence of 5-trifluorothymidien (TFT).

3) -*Results and Conclusions:* No increases in the mutant frequency (≥ 100 mutants) were observed at any of the doses tested. Though the

study does not meet the current assay standards, the results of the L5178Y/TK$^{+/-}$ mouse lymphoma mutagenesis assay indicate that, under the conditions of this study, gamithromycin was concluded to be negative.

i. Mammalian Erythrocyte Micronucleus Test Rat Micronucleus Assay, Oral Route. Study No. PR&D 0061301. February 2002.

1) -*Study Director and Location:* Ramadevi Gudi, Ph.D.; BioReliance, Rockville, MD

2) -*Experimental Design:* ICR mice were given a single dose of gamithromycin (up to 2000 mg/kg BW) or the vehicle alone (1% methylcellulose in water) by oral gavage, or a single dose of cyclophosphamide (50 mg/kg BW as a positive control) by intraperitoneal injection, at a constant volume of 20 mL/kg BW. Mice were observed for clinical signs of toxicity after dose administration. Bone marrow cells -polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs), collected 24 and 48 hours after the treatment, were examined for the presence of micronuclei (MPCEs and MNCEs).

3) -*Results and Conclusion:* The ratio of PCEs to total erythrocytes was reduced in the male and female 24 hour test article-treated groups relative to the vehicle controls. The number of MPCEs per 10,000 PCEs in test article-treated groups was not statistically increased relative to the respective vehicle control in either male or female mice, regardless of dose level or bone marrow collection time. Under the conditions of the assay, gamithromycin did not induce significant increases in the micronucleated PCEs in the bone marrow of ICR mice and is concluded to be negative.

j. *In Vitro* Mammalian Chromosome Aberrations Test of Gamithromycin using Chinese Hamster Ovary (CHO) Cells. Study No. PR&D 0055801. November 2001.

1) -*Study Director and Location:* Ramadevi Gudi, Ph.D.; BioReliance, Rockville, MD

2) -*Experimental Design:* The clone CHO-K1 of the Chinese hamster ovary (CHO) cell line was used in the assay. Acetone was used as the solvent for the test article, gamithromycin. Metabolic activation system consisted of Aroclor 1254-induced rat liver (Sprague-Dawley) homogenate (S9) and co-factor pool. Positive controls consisted of cyclophosphamide (+S9) and mitomycin (-S9). The cells were exposed to test article continuously for 4 hours (+/- S9) followed by a 16-hour recovery time, or for 24 hours (-S9) with no recovery time. Doses ranged from 50 µg/mL to 1200 µg/mL (-S9) or from 50 µg/mL to 1800 µg/mL (+S9).

3) -*Results and Conclusion:* In the 4-hour treatment time (- S9) group, the mitotic index was reduced by 60% at 800 µg/mL, the highest test concentration evaluated for chromosome aberrations. The percentage of cells with structural aberrations in the test article-related groups was significantly increased above that of the solvent control at 400 µg/mL and 800 µg/mL dose levels ($p \leq 0.01$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p \leq 0.05$). In the 4-hour treatment time (+S9) group, the mitotic index was reduced by 49% at 1200 µg/mL, the highest test concentration evaluated for chromosome aberrations. The percentage of cells with structural aberrations in the test article-related groups was significantly increased above that of the solvent control at 800 µg/mL and 1200 µg/mL dose levels ($p \leq 0.01$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p \leq 0.05$). In the confirmatory assay that included a 20-hour exposure (-S9), the test article induced statistically significant structural chromosome aberrations at all exposure times except in the 4-hour exposure time in the absence of S9. It is concluded that gamithromycin was positive for the induction of structural chromosome aberrations in the absence and presence of a metabolic activation system using CHO cells.

k. *In Vitro* Mammalian Chromosome Aberrations Test using Chinese Hamster Ovary (CHO) Cells. Study No. PR&D 0076701. December 2001.

1) -*Study Director and Location:* Ramadevi Gudi, Ph.D.; BioReliance, Rockville, MD

2) -*Experimental Design:* The clone CHO-K1 of the CHO cell line was used in the assay. DMSO was used as the solvent for the test article, gamithromycin. Positive controls consisted of cyclophosphamide (+S9) and mitomycin (-S9). Metabolic activation system consisted of Aroclor 1254-induced rat liver (Sprague-Dawley) homogenate (S9) and co-factor pool. The cells were exposed to test article continuously for 4 hours (+/- S9) and for 20 hours (-S9).

3) -*Results and Conclusion:* In the 4-hour treatment time group (-S9 and +S9), doses employed ranged from 62.5 µg/mL to 2000 µg/mL. In the -S9 experiment, the mitotic index was reduced by 48% at 1000 µg/mL, the highest test concentration evaluated for chromosome aberrations. The percentage of cells with structural aberrations in the test article-related groups was not significantly increased above that of the solvent control, regardless of the dose level. In the +S9 experiment, the mitotic index was reduced by 53% at 1000 µg/mL, the highest test concentration evaluated for chromosome aberrations. The percentage of cells with structural aberrations in the test article-related groups was not significantly increased

above that of the solvent control. In the 20-hour treatment time group (-S9) the doses employed ranged from 62.5 µg/mL to 2000 µg/mL. The mitotic index was reduced by 53% at 500 µg/mL, the highest test concentration evaluated for chromosome aberrations. The percentage of cells with structural aberrations in the test article-related groups was significantly increased above that of the solvent control at dose levels 125 µg/mL and 500 µg/mL ($p \leq 0.05$ and 0.01 respectively, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$). It is concluded that gamithromycin was positive for the induction of structural chromosome aberrations in the absence and presence of a metabolic activation system using CHO cells.

l. *In Vitro* Mammalian Chromosome Aberrations Test of (Z)-Oxime using Chinese Hamster Ovary (CHO) Cells. Study No. PR&D 0055401. April 2001.

1) -*Study Director and Location:* Ramadevi Gudi, Ph.D.; BioReliance, Rockville, MD

2) -*Experimental Design:* The clone CHO-K_1 of the CHO cell line was used in the assay. Ethanol was used as the solvent for the test article, (Z)-Oxime (a metabolite of gamithromycin). Positive controls consisted of cyclophosphamide (+S9) and mitomycin (-S9). Metabolic activation system consisted of Aroclor 1254-induced rat liver (Sprague-Dawley) homogenate (S9) and cofactor pool. Based on a preliminary toxicity test, the high dose selected for the definitive assays (+/- S9, 4-hour treatment and -S9, 20-hour treatment) was 1500 µg/mL (dose range 65 µg/mL to 1500 µg/mL for all the assays). Visible precipitate was observed in the treatment medium at dose levels of ≥ 250 µg/mL. Dose levels of ≥ 125 µg/mL were soluble in the treatment medium.

3) -*Results and Conclusion:* In the -S9, 4-hour treatment time group, the mitotic index was reduced by 31% at 1000 µg/mL, the highest test concentration evaluated for chromosome aberrations. The percentage of cells with structural aberrations in the test article-treated groups was not significantly increased above that of the solvent control. In the +S9, 4-hour treatment time group, the mitotic index at the highest dose level evaluated for chromosome aberrations, 1500 µg/mL, was 23% reduced relative to the solvent control. The percentage of cells with structural aberrations in the test article-related groups was significantly increased above that of the solvent control at a dose level of 1000 µg/mL. The Cochran-Armitage test was negative for a dose response ($p > 0.05$). In the -S9, 20-hour treatment group, the mitotic index was 74% at 750 µg/mL, the highest test concentration evaluated for chromosome aberrations. The

percentage of cells with structural aberrations in the test article-treated groups was not significantly increased above that of the solvent control at all dose levels ($p > 0.05$, Fisher's exact test). On the basis of comparison of the significantly increased structural aberrations observed at dose level of 1000 µg/mL ($p \leq 0.05$, Fisher's exact test) in the 4-hour +S9 group to that of the concurrent solvent control value, it is concluded that (Z)-Oxime induced a biologically significant positive response in the chromosome aberration assay using CHO cells *in vitro*.

m. Bacterial Reverse Mutation Assay of (Z)-Oxime using *Salmonella typhimurium*/*Escherichia coli* Plate Incorporation Test with and without metabolic activation (Ames Test). Study No. PR&D 0057101. February 2001.

1) -*Study Director and Location:* Valentine O. Wagner, III, M.S.; BioReliance, Rockville, MD

2) -*Experimental Design*: Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and one strain of *Escherichia coli* (WP2*uvr*A) were used. The test article, (Z)-Oxime (metabolite of gamithromycin), was not completely soluble in the vehicle solvent, ethanol. To determine the toxicity, ten concentrations of the test article ranging from 0.25 µg per plate to 5000 µg per plate were tested in the presence and absence of S9 mix. The reverse mutation was assessed with the top dose of 90 µg per plate (for *Salmonella* strains) or 270 µg per plate (for the *E. coli strain*) in the presence and absence of S9.

3) -*Results and Conclusion:* No positive responses were observed with any of the test strains in the presence and absence of S9. It was concluded that under the conditions of this study, (Z)-Oxime did not cause a positive response in the presence and absence of Aroclor-induced rat liver S9.

n. 18-Month Oral (Diet Admixture) Carcinogenicity Study in Mice. Study No. PR&D 0100401. September 2008.

1) -*Study Director and Location:* John Findlay, B.S.; Experimur, Chicago, IL

2) -*Experimental Design:* Gamithromycin was administered as an admixture in the diet to three treatment groups of CD-1 mice (70 per sex per dose) for 78 weeks at target doses of 30, 110, and 325 mg/kg BW/day (males) and 40, 150, and 440 mg/kg BW/day (females). The concurrent control group (120 per sex) was given the basal diet for at least 78 weeks. Mortality, morbidity, and clinical observations were performed throughout the study. Body weights and food consumption were measured. Blood samples for differential blood counts were taken from moribund/humanely

euthanized mice and all surviving mice at week 78. At terminal sacrifice, surviving animals were euthanized and necropsied. Preserved tissues from the control and high-dose groups or animals that died before treatment termination were processed and examined microscopically. In addition, the liver and Harderian gland from the remaining (*i.e.*, low- and mid-dose groups) terminal necropsy animals were evaluated microscopically.

3) -*Results and Conclusion:* There was an increase in mortality in the males of mid- and high-dose groups and females of the high-dose group. Biologically relevant decreased body weights and a decreased mean total weight gain (approximately 11%) with a concurrent decrease in mean food consumption were noted in high-dose females. The low dose (30 mg/kg BW/day) was the NOEL for gamithromycin based on the increased mortality seen in the mid-dose male group. Due to lack of treatment-related neoplastic findings, gamithromycin was not considered carcinogenic to mice under the conditions of the study.

o. 2-Year Oral (Diet Admixture) Carcinogenicity Study in Rats. Study No. PR&D 0090501. September 2008.

1) -*Study Director and Location:* John Findlay, B.S.; Experimur, Chicago, IL

2) -*Experimental Design:* Gamithromycin was administered as an admixture in the diet to three treatment groups of Sprague-Dawley rats (70 per sex per group) for a minimum of 100 weeks at target doses of 10, 30, and 100 mg/kg BW/day. The concurrent control group (120 per sex) was given the basal diet only. Mortality, morbidity, and clinical observations were performed throughout the study. Body weights and food consumption were measured. Blood samples for differential blood counts were taken from moribund animals, control groups and high-dose groups during months 12, 18, 23 (males) and 24 (females). At the interim and terminal sacrifice point, rats were euthanized and subject to a complete necropsy. Preserved tissues from all the control and high-dose animals or animals that died before treatment termination were processed and examined microscopically. In addition, the thyroid glands from the remaining (*i.e.*, low- and mid-dose groups) terminal necropsy animals were evaluated microscopically.

3) -*Results and Conclusion:* Treatment-related effects included increased mortality and shortened survival time in males of the high-dose group, decreased body weight in the high-dose groups of both sexes, decreased food consumption in males in the high-dose group, and decreased body weight in females at the high dose. A NOEL for gamithromycin was 10 mg/kg BW/day based on the increased mortality seen in the mid-dose male group. Due to lack of treatment-related neoplastic findings,

gamithromycin was not considered carcinogenic to rats under the - conditions of the study. -

An assessment was prepared to determine if a microbiological Acceptable Daily Intake (ADI) was needed for gamithromycin residues present in edible tissues of cattle. The following studies listed under items p, q and r were performed to provide data needed to answer the questions asked in a stepwise approach to establish a microbiological ADI.

p. Determination of the Minimum Inhibitory Concentration (MIC) against Bacterial Strains Representing the Normal Human Intestinal Microbiota. Study No. PR&D 0122601. June 2006.

1) -*Study Director and Location:* Andrew Pridmore, BSc; PhD.; Don Whitley Scientific Ltd. (DWS), Shipley, West Yorkshire, United Kingdom

2) -*Experimental Design:* The minimum inhibitory concentration (MIC) of gamithromycin was determined against 100 bacterial strains representing the normal human intestinal microbiota. These comprised 10 isolates from each of the following groups listed in the table below. All bacterial strains were isolated from the feces of healthy unmedicated human volunteers. The test system was standardized agar dilution MIC methodology, as described in Clinical and Laboratory Standards Institute guidelines M7-A6 and M11-A6.

3) Results and Conclusion: It is concluded from the study that gamithromycin is active against a majority of bacterial genera, most prominent for *Bifidobacterium* species and least for *Lactobacillus* species.

Table 4.1. Gamithromycin activity against selected human fecal bacterial isolates.

Bacterial Group	***In vitro* Activity (µg/mL)**		
	MIC_{50}	**MIC_{90}**	**MIC range**
B. fragilis	4	32	1 to 32
Other *Bacteroides*	4	32	1 to 32
Bifidobacterium	0.125	0.5	0.125 to 1
Clostridium	0.25	16	0.062 to >128
Enterococcus	2	4	0.062 to 4
E. coli	2	8	2 to 8
Eubacterium	0.5	1	0.5 to 8
Fusobacterium	0.5	32	0.125 to >128
Lactobacillus	32	32	0.25 to 128
Peptostreptococcus	4	16	0.25 to 64

Note: MIC_{50} and MIC_{90} indicate the concentration that inhibited 50% and 90% of the isolates tested, respectively.

q. Effect of Fecal Binding on Antibacterial Activity. Study No. PR&D 0122801. June 2006.

1) - *Study Director and Location:* Andrew Pridmore, BSc; PhD.; Don Whitley Scientific Ltd. (DWS), Shipley, West Yorkshire, United Kingdom

2) - *Experimental Design:* A quantitative assessment of fecal binding was made by incubating selected concentrations of test article with increasing concentrations of sterile pooled human feces prepared in Mueller Hinton Broth. After incubation of each test article/feces combination for various time periods, fecal solids were removed by centrifugation. The supernatant liquid was inoculated with an *Enterococcus* strain and incubated for approximately 24 hours, and antibacterial activity in each supernatant was assessed by the presence or absence of bacterial growth after incubation. Differences in antibacterial activity before and after interaction with feces were used to calculate the percentage of test article bound to feces.

3) - *Results and Conclusion:* It is concluded from the study that gamithromycin bound readily to human fecal material at a high level (> 83.3%) and that the unbound portion of gamithromycin (up to 17%) has antibacterial activity.

r. Prevalence and Gamithromycin Susceptibility of *Enterococcus*, *Bifidobacterium* and *Clostridium* Species within the Feces of Healthy Humans. Study No. PR&D 0122901. January 2007.

1) -*Study Director and Location:* Andrew Pridmore, BSc; PhD.; Don Whitley Scientific Ltd. (DWS), Shipley, West Yorkshire, United Kingdom

2) -*Experimental Design:* The viable count and gamithromycin susceptibility of *Enterococcus*, *Bifidobacterium,* and *Clostridium* species within fecal samples were determined using spiral plate and surface spread plate techniques. For each agar formulation, fecal dilutions were deposited on antibiotic-free agar and agar containing increasing gamithromycin concentrations. Plates were counted for bacterial colonies using an automated counter. The viable counts obtained for a given bacterial group in the presence of each gamithromycin concentration were compared with those obtained in the absence of gamithromycin.

3) -*Results and Conclusion:* MICs against isolates of *Enterococcus* spp. collected from this study were between < 0.016 µg/mL and > 4 µg/mL, and any strain with an MIC of > 4 µg/mL may be regarded as resistant to gamithromycin (in reference to interpretive criteria for erythromycin). Thus, a non-susceptible population of *Enterococcus* spp. is pre-existing among healthy subjects. The susceptibility of human fecal *Enterococcus* spp. to gamithromycin also varied considerably amongst individual donors. Similarly, a detectable proportion of *Bifidobacterium* and *Clostridium* spp. was inhibited by gamithromycin at 0.125 µg/mL, but a large proportion of *Bifidobacterium* and *Clostridium* spp. was not inhibited by gamithromycin at 8 µg/mL. The data from this study with fecal samples of healthy human volunteers show that there are significant levels of non-susceptible populations that pre-exist in the intestinal tract, supporting the conclusion that determination of a microbiological ADI for resistance development is not appropriate.

2. Determination of No Observed Effect Level (NOEL) for chronic exposure.

The toxicological Acceptable Daily Intake (ADI) of total gamithromycin-related residues was determined from the lowest NOEL in the most sensitive species tested in the various toxicology studies conducted. Studies considered in establishing the toxicological ADI are summarized in Table 4.2.

Table 4.2. No-Observed-Effect Levels (NOEL) in toxicology studies for gamithromycin.

Study	Study No.	Lowest NOEL (mg/kg BW/day)
Subchronic Oral Toxicity Study in Mice	PR&D 0085201	65
Subchronic Oral Toxicity Study in Rats	PR&D 0085101	27
Chronic Oral Toxicity Study in Dogs	PR&D 0090601	1
Developmental Toxicity Study in Mice	PR&D 0070401	300
Developmental Toxicity Study in Rats	PR&D 0070301	150
Two-Generation Rat Reproduction Study	PR&D 0100501	10

Based on these toxicology studies, the chronic oral toxicity study in dogs was determined to be the most appropriate study (based upon the lowest NOEL) to determine the toxicological ADI. The NOEL from the dog study was 1 mg/kg BW/day.

3. Acceptable Daily Intake (ADI)

Toxicological ADI

$$Toxciological\ \ Acceptable\ \ Daily\ \ Intake\ \ (ADI) = \frac{Lowest\ \ NOEL}{Safety\ \ Factor}$$

A safety factor of 100 was used because the NOEL was from a chronic study. Because the lowest NOEL is 1 mg/kg BW/day, the toxicological ADI is calculated as follows:

$$ADI = \frac{1\ mg/kg\ BW/day}{100\ -}$$
$$= 0.01\ mg/kg\ BW/day\ \ or\ 10\ \ \mu g/kg\ BW/day$$

Microbiological ADI

Based on data obtained from *in vitro* studies, and the assessment presented by the sponsor, it is concluded that there is a need to determine a microbiological ADI for gamithromycin residues. Of the two endpoints of concern (*i.e.*, disruption of colonization barrier and increase of the population(s) of resistant bacteria), a microbiological ADI is determined for disruption of the colonization barrier.

The microbiological ADI for gamithromycin residues is determined from *in vitro* susceptibility data and following an accepted step-by-step approach.

Steps to determine a microbiological ADI

Step 1*:* Are residues of gamithromycin and (or) its metabolites microbiologically active against representatives of the human colonic flora?

Yes, the compound is active against relevant human intestinal flora. This conclusion is substantiated by data derived from Study PR&D 0122601, *Determination of the Minimum Inhibitory Concentration (MIC) against Bacterial Strains Representing the Normal Human Intestinal Microbiota,* listed under the section "Summary of toxicological studies."

Step 2: Do gamithromycin residues enter the human colon?

Yes. Based on gamithromycin comparable metabolism data in dogs, in conjunction with available human absorption, distribution, metabolism and elimination data on azithromycin, about 30% of ingested gamithromycin residues enter the human colon.

Step 3: Do the residues entering the human colon remain microbiologically active?

Yes. From Study PR&D 0122801, *Effect of Fecal Binding on Antibacterial Activity*, it is concluded that up to 17% of gamithromycin residues remain biological active in the human colon.

The combination of information and data derived from the assessments for Steps 2 and 3 is used to determine the "fraction of oral dose available for microorganisms," which is the denominator of the formula used for calculating a microbiological ADI. Thus, the "fraction" in this case is 5% (30% x 17%).

Step 4: Is there any scientific justification to eliminate testing for either one or both endpoints of concern?

Yes. A determination of a microbiological ADI for the endpoint of "change in resistant population" is not practical, as non-susceptible populations of *Enterococcus*, *Bifidobacterium,* and *Clostridium* are already present in the intestinal flora of the donors tested. In addition, the proportion of the non-susceptible populations varies noticeably among donors, making it difficult to accurately define a no-observable adverse effect level (NOAEL) or no-observable adverse effect concentration (NOAEC) for this endpoint of concern.

Step 5: Determine the NOAECs/NOAELs for the endpoint(s) of concern as established in Step 4. The most appropriate NOAEC/NOAEL is used to determine the microbiological ADI.

The microbiological ADI for disruption of the colonization barrier of the human colon is determined based on *in vitro* MIC data and by applying the following formula:

$$Microbiological\ ADI = \frac{MIC_{calc} \times Mass\ of\ colonic\ content}{Fraction\ of\ oral\ dose\ available\ to\ microorganisms\ x\ 60\ kg}$$

Where:

1) MIC_{calc} was calculated based on the MIC_{50} data from Study No. PR&D 0122601 (see item 1.p. above). The MIC_{calc} was determined to be 0.477 µg/mL (derived from calculation of eight of the 10 bacterial groups tested);
2) The "fraction" is 5% (see Step 3 above);
3) Mass of colonic content = 220 grams (as stated in CVM GFI No. 159).

Thus, the microbiological ADI is 35 µg/kg BW/day or 2.1 mg/person/day.

Because the toxicological ADI of 10 µg/kg BW/day is lower than the calculated microbiological ADI of 35 µg/kg BW/day, we assign the toxicological ADI (10 µg/kg BW/day) as the final ADI for total gamithromycin residues.

4. Safe Concentrations for Total Residues (edible tissues and injection sites, if applicable)

The calculation of the tissue safe concentrations is based on the *General Principles for Evaluating the Safety of Compounds used in Food-Producing Animals* (FDA/CVM, revised July 2006). The safe concentration for total gamithromycin residues (ppm) in each edible tissue of cattle is calculated using the following formulation:

$$Safe\ Concentration\ (SC) = \frac{Acceptable\ Daily\ Intake\ (ADI) \times Human\ Weight}{Consumption\ Value}$$

The average human weight is approximated at 60 kg. The daily consumption values of edible tissues of cattle are approximated as 300 g for muscle, 100 g for liver, 50 g for kidney, and 50 g for fat.

Therefore, the safe concentrations for the edible tissues are calculated as - (summarized in Table 4.3): -

$$SC(muscle) = \frac{ADI \times 60\ kg}{300\ g/day} = \frac{10\ \mu g/kg\ BW/day \times 60\ kg}{300\ g/day} = 2\ \mu g/g = 2\,ppm$$

$$SC(liver) = \frac{ADI \times 60\ kg}{100\ g/day} = \frac{10\ \mu g/kg\ BW/day \times 60\ kg}{100\ g/day} = 6\ \mu g/g = 6\,ppm$$

$$SC(kidney) = \frac{ADI \times 60\ kg}{50\ g/day} = \frac{10\ \mu g/kg\ BW/day \times 60\ kg}{50\ g/day} = 12\ \mu g/g = 12\,ppm$$

$$SC(fat) = \frac{ADI \times 60\ kg}{50\ g/day} = \frac{10\ \mu g/kg\ BW/day \times 60\ kg}{50\ g/day} = 12\ \mu g/g = 12\,ppm$$

Table 4.3. Safe Concentrations (SCs) for total gamithromycin-related residues in edible tissues of cattle using the food consumption factors.

Edible Tissue	Amount Consumed/Day	Safe Concentration (SC)
Muscle	300 g	2 ppm
Liver	100 g	6 ppm
Kidney	50 g	12 ppm
Fat	50 g	12 ppm

Ten times (10X) the muscle safe concentration was considered as the injection site safe concentration. Therefore,

SC(injection site) = 10 x SC(muscle) = 10 x 2 ppm = 20 ppm

B. Residue Chemistry:

1. Summary of Residue Chemistry Studies

a. Total Residue and Metabolism Studies

1) Distribution and Excretion of Total Residues after Subcutaneous Dosing in Cattle with [^{3}H] Gamithromycin. Study No. PR&D 0078101. December 2004.

a) *Study Director and Location:* Niranjan Banav, Ph.D.

In-Life Phase: Merck & Co., Inc., Branchburg Farm, Somerville, NJ
Analytical Phase: Merial Limited, North Brunswick, NJ

b) - *Test Animals:* 14 (7 male castrated and 7 female) healthy Angus-cross beef cattle, 6 to 7 months old, 191.0 to 235.5 kg BW

c) - *Route of Drug Administration and Time and Duration of Dosing:* Test animals were administered a single dose of gamithromycin of ^{3}H-gamithromycin by subcutaneous injection at 6 mg/kg BW. The animals were sacrificed by groups at 21, 49, and 70 days after dose administration.

d) - *Total Residue Concentration:* The following tissues were collected in this study: liver, kidney, fat, lung, muscle, injection site, and plasma. Total radioactive residues were determined for the tissues, plasma, and excreta. Selected samples were extracted and the extracts were analyzed by radio-HPLC (high performance liquid chromatography) to determine the nature of the radioactive residues of ^{3}H-gamithromycin. Mean concentrations of total residues and marker residues for liver and injection site are shown in the table below. The concentration of total residues was highest in the injection site and liver samples. The concentration of parent gamithromycin in edible tissues followed the same order as for total residue levels: injection site > liver > kidney > fat ≈ muscle. Parent drug is the major residue that depletes at a similar rate to total residues in edible tissues at 21 days post-dosing.

Table 4.4. Mean (± standard deviations) concentrations of total residues (µg/g) in tissues of cattle administered a single dose of ^{3}H-gamithromycin by subcutaneous injection at 6 mg/kg BW.

Days after Treatment	Liver	Kidney	Muscle	Fat	Injection Site
21	2.35 ± 0.39	0.74 ± 0.13	0.038 ± 0.009	1 @ 0.061 3 @ <LOQ	16.05 ± 10.78
49	0.307 ± 0.15	0.051 ± 0.02	4 @ <LOQ	1 @ 0.014 3 @ <LOQ	0.649 ± 0.27
70	0.057 ± 0.01	0.01 ± 0.003	0.004 ± 0.001 1 @ <LOQ	4 @ <LOQ	0.080 ± 0.09

LOQ (limit of quantitation) for total residues ranged from 0.0025 µg/g to 0.0397 µg/g. -

Table 4.5. Mean concentrations of marker residue (μg/g) in tissues of cattle administered a single dose of ^{3}H-gamithromycin by subcutaneous injection at 6 mg/kg BW.

Days after Treatment	Liver	Kidney	Muscle	Fat	Injection Site
21	0.499 ± 0.123	0.350 ± 0.111	1 @ 0.011 3 @ <LOQ	<LOQ	10.36 ± 7.3
49	0.30 ± 0.02 1 @ <LOQ	0.0247 3 @ <LOQ	<LOQ	<LOQ	0.187 ± 0.10
70	<LOQ	<LOQ	<LOQ	<LOQ	1 @ 0.056 3@<LOQ

LOQ for marker residue was 0.01 ppb.

2) Metabolite Profiles of ^{3}H-Gamithromycin in Selected Cattle Tissue Samples. Study No. PR&D 0078501. September 2004.

a) - *Study Director and Location:* Rose A. Huang, Ph.D.; Merial Limited, Pharmacokinetics and Drug Metabolism, North Brunswick, NJ

b) - *Metabolite Profiles:* Selected cattle tissues from study PR&D 0078101 (reported above) were used to determine the metabolite profiles of ^{3}H-gamithromycin and to identify the major metabolites. The following tissue samples were analyzed using reversed-phase HPLC: liver, kidney, lung, injection site, urine, and feces. The major metabolite identified was parent gamithromycin. The declad metabolite is formed when gamithromycin loses the cladinose moiety.

Table 4.6. Summary of metabolites identified in tissues of cattle administered a single dose of ^{3}H-gamithromycin by subcutaneous injection at 6 mg/kg BW.

Tissue	M1	M2	M3	Declad -	M4	TDO[1] -	M5	M6	M7	Gamithromycin	M8	M9
Liver	+	+	+	+	+	+	+	+	+	+	+	+
Kidney	+	+	**x**	+	+	+	**x**	+	+	+	+	+
Lung	+	+	+	+	+	+	**x**	+	+	+	**x**	+
Injection Site	+	+	**x**	+	+	+	**x**	+	+	+	+	+
Urine	+	+	**x**	+	+	+	+	+	+	+	+	+
Feces	+	+	+	+	+	+	+	+	+	+	+	+

+: observed
x: not observed
[1]the translactone of parent drug

b. Comparative Metabolism Study

Determination of Total Radioactive Residues and Metabolite Profiles of ^{3}H-Gamithromycin in Selected Dog Tissues, Fluids and Excreta from the In-Life Phase of Study PR&D 0093201. Study No. PR&D 0098601. November 2004.

1) - *Study Director and Location:* Rose A. Huang, Ph.D.; Merial Limited, Pharmacokinetics and Drug Metabolism, North Brunswick, NJ

2) - *Test Animals:* 5 male and 5 female healthy beagle dogs

3) - *Route of Drug Administration and Time and Duration of Dosing:* Test animals were administered two oral doses of approximately 10 mg/kg BW of ^{3}H-gamithromycin.

4) - *Total Residue Concentration:* Total residues and metabolic profiles were determined in liver, kidney, fat, lung, muscle, plasma, bile, colonic lumen contents, urine, and feces.

Total radioactive residue levels in tissues followed the order: liver > lung > kidney > fat ≈ muscle, which is the same order as found in cattle. Total radioactive residue concentrations in both bile and colonic lumen contents were high, which corresponds to a high total radioactive residue concentration in feces. Biliary excretion is the predominant route of elimination of gamithromycin and its metabolites.

Table 4.7. Summary of metabolites identified in tissues of dogs administered two oral doses of nominal 10 mg/kg BW of ^{3}H-gamithromycin.

Tissue	**M1**	**M2**	**M3**	**Declad**	**M4**	**TDO**	**M5**	**M6**	**M7**	**Gamithromycin**	**M8**	**M9**
Liver	+	+	x	+	+	+	x	+	+	+	+	+
Kidney	+	+	x	+	+	+	+	+	+	+	+	+
Fat	+	x	x	+	+	+	x	+	+	+	+	+
Muscle	+	+	x	+	+	+	x	+	+	+	+	+
Lung	+	+	x	+	+	+	+	+	+	+	+	+
Bile	+	+	x	+	+	+	x	+	+	+	+	+
Plasma	+	+	x	+	+	+	+	+	+	+	+	+
Urine	+	x	x	+	+	+	+	+	+	+	+	+
Colonic Lumen	+	+	x	+	+	+	+	+	+	+	+	+
Feces	+	+	+	+	+	+	x	+	+	+	+	+

+: observed
x: not observed

The metabolite profiles in cattle and dog tissues are qualitatively similar. In general, gamithromycin, declad, and/or metabolite M2 (*N*-dealkylated declads, M2a and M2b) were the major residues observed in cattle tissues. Gamithromycin and declad were the major residues in dog tissues.

Table 4.8. Metabolites in Cattle and Dogs.

Species	Major Metabolites (>10%)	Minor Metabolites (<10%)
Cattle	Declad *N*-despropyl *N*-desmethyl declads (M2a + M2b)	TDO
Dog	Declad *N*-methylhydroxylate of Gamithromycin (M8)	M2 (M2a + M2b) TDO *N*-despropyl Gamithromycin (M6)

c. Residue Depletion Study

A Definitive Study to Determine the Marker Residue in cattle after a Single Subcutaneous Dosing of Gamithromycin at 6 mg/kg body weight. Study No. PR&D 0110801. April 2005.

1) -*Study Directors and Location:*

In-Life Phase: Gregory C. Royer, D.V.M.; Merial Limited, Missouri Research Center, Fulton, MO

Analytical Phase: Niranjan Banav, Ph.D.; Merial Limited, Pharmacokinetics and Drug Metabolism, North Brunswick, NJ

2) -*Test Animals:* 17 male castrated and 17 female healthy Angus beef cattle, less than 6.5 to 8.5 months old, 185 to 281 kg BW.

3) -*Route of Drug Administration and Time and Duration of Dosing:* Test animals were administered a single dose of gamithromycin by subcutaneous injection at 6 mg/kg BW. The animals were sacrificed by groups at 10, 21, 35, 49, and 70 days after dose administration.

4) -*Marker Residue Concentration:* The following tissues were collected: liver, kidney, fat, muscle, and injection site. The processed tissues were analyzed for marker residue using validated liquid chromatography tandem mass spectrometry (LC-MS/MS) methods. The concentration of marker residue, gamithromycin, in edible tissues followed the same order as reported in the radiolabel study (PR&D 0078101): injection site > liver > kidney > fat ≈ muscle. The level of marker residue in muscle and fat

was very low at Day 10 sacrifice and was below the quantitation limit at the remaining sacrifice time points.

Table 4.9. Mean Marker Residues of Gamithromycin (ng/g) in Tissues of Cattle after a Single Dose of Gamithromycin at 6 mg/kg BW.

Sacrifice Day	Liver	Kidney	Fat	Muscle	Injection Site
10	2790 ± 545	2470 ± 792	30.2 ± 16.5	BLOD*	25563 ± 12430
21	642 ± 263	303 ± 77	BLOQ	BLOD	1@BLOQ 7669 ± 6783
35	175 ± 57	93.4 ± 22	BLOQ	BLOD	2@BLOD 3998 ± 2983
49	6@BLOQ**	1@BLOQ 18.9 ± 3.9	BLOQ	BLOD	1@BLOD 444 ± 295
70	1@BLOQ 4@BLOD	BLOQ	BLOQ	BLOD	3@BLOQ 3@BLOD 81.80

*BLOD = below limit of detection (liver, injection site, muscle 10 ppb; kidney, fat, 1 ppb)
**BLOQ = below limit of quantitation (liver, injection site, 100 ppb; muscle, 75 ppb; kidney, fat, 10 ppb)

2. Target Tissue and Marker Residue Assignment

Based on the results of the total residue study (PR&D 0078101), liver is assigned as the target tissue in cattle. Based on the results of the tissue metabolism study (PR&D 00785101), the marker residue for cattle liver is gamithromycin.

3. Tolerance Assignments

A statistical analysis of the 99% tolerance limit with 95% confidence for the 35-day data for liver from Study PR&D 0110801 was used to establish a liver tolerance of 500 ppb. The muscle tolerance is 150 ppb.

4. Withdrawal Period

Based on the liver tolerance of 0.5 ppm, the statistical analysis of the depletion phase of the liver marker residue level (target tissue), and the established safe concentration of marker residue at the injection site (20 ppm), a withdrawal period of 35 days is assigned.

C. Microbial Food Safety:

1. Antimicrobial resistance

Microbial food safety information for gamithromycin was evaluated using a qualitative risk assessment procedure. The dosage regimen evaluated was 6 mg of gamithromycin per kg of body weight. The indications associated with the dosage regimen evaluated are 1) for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* in beef and non-lactating dairy cattle, and 2) for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD associated with *Mannheimia haemolytica* and *Pasteurella multocida*.

The qualitative risk assessment procedure involved conducting 1) a *release assessment* to describe the probability that gamithromycin and its use in beef cattle will result in the emergence of macrolide-resistant bacteria or macrolide resistance determinants in or on treated beef cattle under proposed conditions of use; 2) an *exposure assessment* to describe the likelihood of human exposure to macrolide-resistant bacteria or macrolide resistance determinants through consumption of edible products from treated cattle; and 3) a *consequence assessment* to describe potential human health consequences arising from exposure to the defined resistant bacteria or resistance determinants by considering the human medical importance of azalides (comparable to macrolides) used in the treatment of human infectious disease.

It was determined that the risk of development of resistant bacteria of human health concern (i.e., *Campylobacter* spp) from the proposed use of gamithromycin in beef cattle is medium. In conjunction with the classification of exposure risk as low, and azalides ranking of high due to its critical importance in human medicine, an overall risk estimation of **high** was assigned. Thus, the proposed conditions of use are compatible with the Agency's risk management strategies for Category 1 drug, which include prescription (Rx) marketing status, use in individual animals, and monitoring for resistance through FDA/CDC/USDA's National Antimicrobial Resistance Monitoring System.

2. Impact of residues on human intestinal flora

See above under Section IV.A. Toxicology. 1.p., A.1.q., and A.1.r.

D. Analytical Method for Residues:

1. Determinative Procedure

Internal standard is added to ground bovine tissues which are homogenized in the presence of phosphate buffer, with a back-wash with hexane for muscle tissue. The supernatant is placed on a solid phase extraction cartridge; gamithromycin is

eluted, evaporated to dryness, and reconstituted in methanol. After dilution, quantitation is by positive electrospray LC-MS/MS. A sponsor monitored multi-laboratory transfer trial for the method was successfully completed.

2. Confirmatory Procedure

The sample extraction and preparation for the confirmatory procedures are identical to the ones for the determinative procedures. Three additional ion transitions from gamithromycin are monitored to obtain ion ratios, signal-to-noise ratios, and retention times that meet the required acceptability criteria.

3. Availability of the Method

The method is on file with the Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to ZACTRAN:

For use in cattle only. Not for use in humans. Keep this and all drugs out of reach of children. The material safety data sheet (MSDS) contains more detailed occupational safety information. To report adverse effects, obtain an MSDS or for assistance, contact Merial at 1-888-637-4251.

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that ZACTRAN, when used according to the label, is safe and effective for the treatment of BRD associated with *M. haemolytica*, *P. multocida*, and *H. somn*i in beef and non-lactating dairy cattle; and for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD associated with *M. haemolytica* and *P. multocida*. Additionally, data demonstrate that residues in food products derived from cattle treated with ZACTRAN will not represent a public health concern when the product is used according to the label.

A. Marketing Status:

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). This decision was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product to treat and control BRD, and

(b) restricting this drug to use by or on order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues.

B. Exclusivity:

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of the approval because no active ingredient of the new animal drug has previously been approved.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.