Date of Approval: November 19, 2014

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-349

DRAXXIN 25 Injectable Solution

Tulathromycin Injection

Cattle (suckling calves, dairy calves, and veal calves)

For treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* in suckling calves, dairy calves, and veal calves.

Sponsored by:

Zoetis Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-349

B. Sponsor

Zoetis Inc.
333 Portage St.
Kalamazoo, MI 49007

Drug Labeler Code: 054771

C. Proprietary Name

DRAXXIN 25 Injectable Solution

D. Established Name

Tulathromycin injection

E. Pharmacological Category

Antimicrobial

F. Dosage Form

Sterile injectable solution

G. Amount of Active Ingredient

25 mg/mL

H. How Supplied

50 mL, 100 mL, and 250 mL bottles

I. Dispensing Status

Rx

J. Dosage Regimen

2.5 mg/kg body weight (BW), administered once

K. Route of Administration

Subcutaneous injection

L. Species/Class

Cattle (suckling calves, dairy calves, and veal calves)

M. Indication

Suckling Calves, Dairy Calves, and Veal Calves

BRD – DRAXXIN 25 Injectable Solution is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*.

N. Effect of Supplement

This supplement provides for use in suckling calves, dairy calves, and veal calves for the treatment of BRD associated with *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis*.

II. EFFECTIVENESS

A. Dosage Characterization

The dosage regimen for DRAXXIN 25 Injectable Solution in cattle is identical to the dosage regimen for DRAXXIN Injectable Solution (100 mg tulathromycin/mL, NADA 141-244). The Freedom of Information (FOI) Summary for the approval of DRAXXIN Injectable Solution dated May 24, 2005, contains dosage characterization information for cattle.

B. Substantial Evidence

The following pharmacokinetic study provides evidence that DRAXXIN 25 Injectable Solution is therapeutically equivalent to DRAXXIN Injectable Solution when used in cattle (suckling calves, dairy calves, and veal calves).

1. Pharmacokinetic Study

a. Title: Pharmacokinetic Comparison of DRAXXIN Injectable Solution and a Lower Concentration Formulation of DRAXXIN Injectable Solution Administered to Cattle by Subcutaneous Injection at 2.5 mg Tulathromycin/kg Body Weight. Study Number 1532N-60-11-886; January 2012 to September 2012.

b. Study Director: Terry N. TerHune, DVM, PhD, HMS Veterinary Development, Tulare, CA

c. Study Design:

1) *Objective*: To assess the relative bioavailability of the currently-approved DRAXXIN Injectable Solution (100 mg/mL, NADA 141-244) to DRAXXIN 25 Injectable Solution [lower concentration formulation (25 mg/mL)] in cattle when administered by subcutaneous (SC) injection at 2.5 mg tulathromycin/kg body weight (BW).

2) *Animals*: 80 healthy Holstein calves (40 heifers and 40 steers), approximately 11 weeks old, weighing 72 to 113 kg at the beginning of the study.

3) *Experimental Design*: This two-treatment parallel study compared pharmacokinetic (PK) characteristics of DRAXXIN 25 Injectable Solution (25 mg/mL) to the currently-approved DRAXXIN Injectable Solution (100 mg/mL).

4) *Test Article Administration*: Animals were dosed with their assigned treatment once subcutaneously in the neck at 2.5 mg tulathromycin/kg BW.

5) *Measurements and Observations*: Blood samples were collected from each animal at the following time points: 0 (prior to the first dose), and at 20 and 40 minutes, and 1, 1.5, 2, 3, 4, 7, 10, 24, 48, 96, 144, 192, 240, 288, and 336 hours after dosing. General health observations were made once daily throughout the study. Injection sites were visually evaluated at 2, 24, and 48 hours after injection. Abnormal injection sites continued to be evaluated approximately every 24 hours until the site no longer had visible or palpable swelling.

d. Analysis: The concentrations of tulathromycin in plasma were measured using a validated liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) assay validated for bovine plasma. Pharmacokinetic parameters were determined for each individual animal.

Plasma concentrations and PK variables were analyzed using a general linear mixed effect model in SAS. Values for C_{max}, AUC_{0-4}, and AUC_{0-LOQ} were transformed using a natural logarithm prior to analysis. The back-transformed least squares (LS) means and 90% confidence intervals (CI) were reported as appropriate. Bioequivalence was assessed for C_{max}, AUC_{0-4}, and AUC_{0-LOQ}. The criteria used to demonstrate bioequivalence between DRAXXIN and DRAXXIN 25 were as follows:

- For AUC_{0-LOQ} the 90% confidence limits for the ratio of LS means were within the limits of 80 to 125%; and either of the following:

- For AUC_{0-4} the 90% confidence limits on the LS means ratio were within the limits of 80 to 125%; or

- For C_{max} the 90% confidence limits on the LS means ratio were within the limits of 70 to 143%.

e. Results: The PK profiles of DRAXXIN Injectable Solution and DRAXXIN 25 Injectable Solution in cattle showed comparable extents of absorption, as demonstrated statistically by the 90% confidence intervals about the ratio of the 25 mg/mL to 100 mg/mL tulathromycin AUC_{0-LOQ} values being contained within the limits of 0.80 – 1.25 (Table 2). Given tulathromycin's rapid distribution phase relative to its terminal elimination half-life ($T_{1/2}$), the relationship between

antimicrobial activity and extent of exposure rather than peak concentrations, and its margin of safety, it was determined that tulathromycin AUC_{0-4} values rather than C_{max} provided an appropriate metric for evaluating therapeutic comparability with respect to product rate of absorption. The 90% confidence interval for AUC_{0-4} values was contained within the bounds of 0.80 - 1.25. The largest differences in plasma profiles were observed in the first 24-hour period after dosing.

Summary statistics for PK parameters for each treatment group are shown in Table 1.

Table 1. A summary of PK results (average ± standard deviation [SD]) by treatment (n =40 animals per treatment group) following the administration of DRAXXIN Injectable Solution (reference) and DRAXXIN 25 Injectable Solution (test) in cattle as a single SC injection of 2.5 mg tulathromycin/kg BW.

PK Parameter	DRAXXIN Injectable Solution (reference)	DRAXXIN 25 Injectable Solution (test)
C_{max} (ng/mL)	643 ± 302	474 ± 194
AUC_{0-LOQ} (h*ng/mL)	9806 ± 1440	11408 ± 2542
AUC_{0-4} (h*ng/mL)	1358 ± 293	1229 ± 332
T_{max} (h)	0.509 ± 0.276	1.18 ± 1.88
$T_{½}$ (h)	103 ± 25.6	111 ± 86.6

C_{max} - maximum plasma concentration
AUC_{0-LOQ} - the area under the plasma concentration vs. time curve from time of injection to the limit of quantification of the assay
AUC_{0-4} - the area under the plasma concentration vs. time curve from time of injection extrapolated to four hours after injection
T_{max} - the time after initial injection to when C_{max} occurs
$T_{½}$ - the plasma elimination half-life of tulathromycin

Table 2. Back-transformed least squares (LS) means and 90% confidence intervals (CI) for AUC_{0-4} and AUC_{0-LOQ} following a single SC injection of 2.5 mg tulathromycin/kg BW in swine administered as DRAXXIN Injectable Solution (reference) and DRAXXIN 25 Injectable Solution (test).

PK Parameter	LS Mean DRAXXIN Injectable Solution (Reference)	LS Mean DRAXXIN 25 Injectable Solution (Test)	Ratio of Test to Reference	90% CI
AUC_{0-LOQ} (h*ng/mL)	9701	11121	1.15	1.07 - 1.23
AUC_{0-4} (h*ng/mL)	1326	1180	0.89	0.806 – 0.982

f. Adverse Events: Two animals receiving DRAXXIN 25 Injectable Solution had measurable injection site swelling at 2 hours post-

injection. By 24 hours post-injection, only one animal had measurable injection site swelling, which resolved by five days post-injection. No animals receiving DRAXXIN Injectable Solution had measurable injection site swelling. Except for injection site swelling, no other treatment-related adverse reactions were reported.

g. Conclusion: Based on the statistical comparison of AUC_{0-4} and AUC_{0-LOQ} between the groups, DRAXXIN 25 Injectable Solution is therapeutically equivalent to DRAXXIN Injectable Solution (NADA 141-244) when administered to cattle once by SC injection at a dose of 2.5 mg tulathromycin/kg BW. Therefore, DRAXXIN 25 Injectable Solution is effective for treatment of BRD associated with *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis* in suckling calves, dairy calves, and veal calves when administered once by SC injection at a dose of 2.5 mg tulathromycin/kg BW.

III. TARGET ANIMAL SAFETY

A. Systemic Safety

Evaluation of target animal safety in cattle (suckling calves, dairy calves, and veal calves) was based on a PK comparison between DRAXXIN 25 Injectable Solution and DRAXXIN Injectable Solution (100 mg/mL). Tulathromycin administered to calves as DRAXXIN 25 Injectable Solution at a dose of 2.5 mg tulathromycin/kg BW once by SC injection was demonstrated to be therapeutically equivalent to a corresponding SC injection of DRAXXIN Injectable Solution based upon comparability of their respective AUC_{0-LOQ} and AUC_{0-4} values (see EFFECTIVENESS section above). Therefore, this study confirms the systemic safety of DRAXXIN 25 Injectable Solution in cattle (suckling calves, dairy calves, and veal calves) when administered by SC injection at a dose of 2.5 mg tulathromycin/kg BW once.

The FOI Summary for the approval of DRAXXIN Injectable Solution, NADA 141-244, dated May 24, 2005, contains the results of the systemic target animal safety study confirming the safety of tulathromycin when administered to cattle by SC injection at a dose of 2.5 mg tulathromycin/kg BW once.

B. Injection Site Safety

1. Title: Injection Site Tolerance of DRAXXIN Lower Concentration (DRAXXIN 25) in Cattle. Study Number 1433N-60-11-934; December 2011 to June 2012.

2. Study Director: Gregory A. Inskeep, DVM, Pfizer Animal Health, Kalamazoo, Michigan.

3. Study Design:

   a. *Objective*: To characterize the injection site tolerance of DRAXXIN 25 Injectable Solution when injected subcutaneously to growing cattle once at the maximum proposed dose volume of 11.5 mL per injection site or at a dose of 2.5 mg/kg body weight, whichever was higher.

b. *Animals*: Sixteen healthy, Angus and Angus cross, castrated male calves, weighing about 100 kg at arrival, were enrolled in the study. Animals were housed in pens in groups of four, and were randomly assigned to pen, treatment group, and necropsy day.

c. *Test Article Administration*: The test article was tulathromycin as DRAXXIN 25 Injectable Solution (25 mg tulathromycin/mL). The control article was sterile saline injectable solution. Calves were injected subcutaneously with 11.5 mL or 2.5 mg tulathromycin/kg BW (whichever resulted in a higher volume per injection site) in the neck on Day 0.

d. *Measurements and Observations*: General health observations were conducted by trained personnel on all animals at least once daily from Day -14 until Day 42, the last day of the study. Clinical observations were conducted by the study veterinarian at least once on Days -14, -1, 0, 7, 14, 28, and 42. Two saline-treated calves and two DRAXXIN 25-treated calves were euthanized on Days 7, 14, 28, and 42.

Injection site observations (visual observation and palpation) were conducted by trained personnel on Day -14, and once daily from Day -1 until Day 28, on Days 32, 35, 39, and 42. Erythema, heat, sensitivity, firmness, necrosis, and drainage were documented as present or absent. Swelling was evaluated by measuring the shortest and longest superficial dimensions and the elevation of the swelling.

4. Statistical Analysis: None.

5. Results:

a. *General Health and Clinical Observations*: There were no abnormal clinical or general health observations related to test article administration. All treated animals completed the study.

b. *Injection Site Observations*: There were no findings of erythema, heat, sensitivity, necrosis, or drainage at any of the injection sites in either treatment group. Firmness was observed in one DRAXXIN 25-treated calf on a single day (Day 5) but was not observed in saline-treated calves. Swelling was noted in two DRAXXIN 25-treated calves but was not observed in any of the saline-treated calves. One calf had swelling noted on Days 1 and 2, with maximal swelling of 18.33 cm^3 on Day 1. The other calf had swelling noted on Days 4 to 7 (the calf was necropsied on Day 7, so no further observations were possible), with maximal swelling of 2.09 cm^3 on Day 5.

c. *Gross Necropsy and Histopathology*: Gross necropsy observations revealed discoloration and changes in consistency of connective tissue and skeletal muscle. The discoloration was described as either red, dark red, or brown. The number of calves with abnormal gross necropsy observations is provided in Table 3.

Table 3. The number of calves with abnormal injection site findings during gross necropsy.

Necropsy Day	DRAXXIN 25 group	Saline group
Day 7	Both calves	Neither calf
Day 14	Both calves	One of two calves
Day 28	One of two calves	Both calves
Day 42	Neither calf	Neither calf

Microscopic changes consistent with inflammation were seen in three saline-treated calves and in five DRAXXIN 25-treated calves. The changes in DRAXXIN 25-treated calves were more commonly graded mild to marked than the changes in saline-treated calves, which were mostly graded minimal to moderate. No microscopic findings were observed in the DRAXXIN 25-treated calves necropsied on Day 42 of the study.

6. Conclusion: This study demonstrated that DRAXXIN 25 Injectable Solution was well tolerated when injected subcutaneously in growing cattle once at the maximum proposed dose volume of 11.5 mL per injection site or at a dose of 2.5 mg/kg body weight, whichever was higher. Injection site irritation (as evidenced by grossly visible lesions at necropsy) extended beyond the assigned pre-slaughter withdrawal period.

IV. HUMAN FOOD SAFETY

A. Antimicrobial Resistance:

The impact of the addition of a lower concentration formulation of tulathromycin (DRAXXIN 25 Injectable Solution), as well as the impact of the proposed inclusion of suckling calves, dairy calves, and veal calves on the tulathromycin label as it pertains to microbial food safety (antimicrobial resistance) were carefully considered by the Agency. The Agency determined that this supplemental action should not significantly impact public health with respect to antimicrobial resistance, because the two formulations were determined to have comparable bioavailability, and there were no changes in dosage, route of administration, or duration of use.

Low contamination rates of retail beef, combined with low prevalence of *Campylobacter* in cattle and low consumption of veal indicate that the potential for human infection with erythromycin-resistant *Campylobacter* from consumption of veal is low. Inclusion of suckling calves, dairy calves, and veal calves to the product label represents a minor increase in extent of use of the product, and therefore a significant increase in antimicrobial resistance selection pressure is not anticipated with this approval. Further evaluation of microbial food safety was not necessary for this new formulation.

B. Impact of Residues on Human Intestinal Flora:

1. Determination of the need for establishing a microbiological acceptable daily intake (mADI)

A step-by-step approach, supported with study data, was followed to determine whether there is a concern for effects of tulathromycin residues on human intestinal flora.

a. Step 1: Are residues of the drug and (or) its metabolites microbiologically active against representatives of the human intestinal flora?

Yes, tulathromycin is active against representative human intestinal bacteria. This conclusion was confirmed by an *in vitro* susceptibility study performed by the firm, which is described below.

Activity of tulathromycin against 100 bacterial strains of human gut origin: determination of Minimum Inhibitory Concentration (MIC).

Study No.	Pfizer Study No. 1671-N-03-00-217
Study Period	November 2000 to February 2001
Study Director	Dr. Andrew Pridmore
Study Location	Don Whitley Scientific Limited, Shipley, West Yorkshire, United Kingdom

Study Design: The objective of this study was to examine the activity of tulathromycin against 10 representative *genera* of dominant human fecal bacteria. Ten isolates from each *genus* or group were tested *via* agar dilution methodology for aerobes and anaerobes as described by the Clinical and Laboratory Standard Institute (CLSI). Two different bacterial densities differing by a factor of 100 were tested. All bacterial strains were obtained from feces of healthy volunteers in the UK (no antibiotic treatment for three months before isolation) between 1998 and 2000, with the exception of *Eubacterium* (1994) and two isolates of *Fusobacterium* (1994 and 1995).

Results and conclusions: MIC_{50}, MIC_{90}, and the geometric MICs were determined for each *genus*/group. Among the bacterial groups tested, *Bifidobacterium* spp. were the most susceptible to tulathromycin at both inoculum sizes; i.e., MIC_{50} at 0.5 with inoculum of 10^4 to 10^6 cfu/ml, and MIC_{50} at 1 µg/ml with inoculum of 10^6 to 10^8 cfu/ml. *Enterococcus* spp., *Escherichia coli,* and *Fusobacterium* spp. were the next most susceptible bacteria. In general, MIC_{50} values increased by 2-fold as the inoculum size increased. A decision was made to use the most sensitive group for assessing the *in vitro* activity of the compound.

b. Step 2: Do tulathromycin residues and/or its metabolites enter the human colon?

Yes, tulathromycin and its metabolites enter the human colon. The firm states that data obtained with tulathromycin demonstrated that no more than 50% of ingested tulathromycin residues will reach the colon and be excreted in feces. No human studies have been conducted with the drug. However, in a study performed in swine, as summarized below, the firm demonstrated that tulathromycin is excreted in feces, indicating that tulathromycin residues enter the human colon.

Excretion and Pharmacokinetics of tulathromycin in swine urine/feces and plasma/lung, respectively, following an oral gavage or intramuscular dose at 2.5 mg/kg body weight.

Study No.	Pfizer Study No. 1521E-60-01-194
Study Period	August 6, 2001 through December 21, 2001
Study Director	Philip Inskeep, Ph.D.
Study Location	Pfizer R&D, Veterinary Medicine Safety and Metabolism, Groton, CT

Study Design: The study evaluated concentrations of tulathromycin in plasma, lung, urine, and feces following a single oral or intramuscular dose of 2.5 mg/kg bw to growing swine. For the excretion phase, urine, and feces were collected 9 times from animals dosed orally every 24 hours. Drug concentration in feces was determined by high pressure liquid chromatography (HPLC) and mass spectrometry analyses.

Results and conclusions: Average urinary concentration was < 0.456 µg/ml, compared with fecal concentrations of the parent drug ranging from 3 to 99 µg/g. Maximum fecal concentrations occurred at 24 to 48 hours after dosing. After oral administration, 30 to 53% of the administered dose was recovered in feces as unchanged drug over a 14-day period. Upon comparison, the study concluded that fecal excretion of tulathromycin in pigs is similar to the excretion in rats (46%), dogs (36%), and cattle (42%). In addition, excretion of tulathromycin is similar to excretion of other macrolides used in human medicine, such as erythromycin, clarithromycin, and azithromycin (40 to 55% of an oral dose is absorbed in humans). The firm concluded from the study that ingested tulathromycin residues will be eliminated in feces and urine, with no more than 50% being eliminated in feces as parent drug.

c. Step 3: Do residues and/or metabolites of tulathromycin entering the human colon remain microbiologically active?

The answer is No. Through a series of studies, the firm demonstrated that tulathromycin at a range of concentrations is inactivated in the digestive system and there is virtually no biological activity in the colon. Those studies supporting the conclusion are summarized below.

Study #1. Effect of tulathromycin against *Bifidobacterium* and *Fusobacterium* strains of human gut origin following passage through a simple *in vitro* gut model.

Study No.	Pfizer Study No. 1671-N-03-01-231
Study Period	July through September, 2001
Study Director	Dr. Andrew Pridmore
Study Location	Don Whitley Scientific Limited, Shipley, West Yorkshire, United Kingdom

Study Design: Tulathromycin was added to Cooked Meat Medium, and the mixture was incubated in the presence of pepsin at pH 2.0 for 1 hour. After adjusting the pH to 7.0, bile salts and pancreatin were added to the mixture and incubated for 4 hours. *Bifidobacterium* (two strains) and *Fusobacterium* (two strains) were tested. An inoculum at a density of 10^6 cfu/ml of each strain was introduced to the mixture, and their viability assessed after 18 hours of incubation. Different concentrations of tulathromycin (0, 2, and 8 µg/ml) were tested against each bacterial strain. The highest concentration tested represents at least 4 X the MIC for each bacterial strain.

Results and conclusions: No effects of tulathromycin (at 2 or 8 µg/ml) were observed with any of the 4 bacterial strains tested in the study. All four strains were able to multiply under the conditions of the test systems (changes in pH, addition of pancreatin and bile acids) in the absence of drug and in the presence of 2 and 8 µg/ml of the drug. The conclusion of the study was that tulathromycin at concentrations up to 4X the MIC of the tested strains did not inhibit growth of the strains in the model system.

Study #2. Effect of tulathromycin against *Bifidobacterium* and *Fusobacterium* strains of human gut origin following passage through a simple *in vitro* gut model.

Study No.	Pfizer Study No. 1671-N-03-01-240
Study Period	January through February, 2002
Study Director	Dr. Andrew Pridmore
Study Location	Don Whitley Scientific Limited, Shipley, West Yorkshire, United Kingdom

Study Design: This study is an extension of study #1 (summarized above). Tulathromycin was added to Cooked Meat Medium at 10, 15, and 20 µg/ml. The concentrations of tulathromycin used represent at least 10X the MIC determined for each bacterial strain. Bacterial strains tested in this study were the same ones used in the previous study.

Results and conclusions: Tulathromycin did not inhibit growth of the tested strains after 18 hours of incubation in the test system. The findings further show a lack of effect of tulathromycin on cell viability

of two groups of the most sensitive anaerobic bacteria of the GI tract at concentrations from 2 to 20 µg/ml. Therefore, it is expected that the activity of ingested residues will be significantly reduced during passage through the GI tract alone, and remaining biological activity is negligible.

Study #3. Adsorption/desorption of ^{14}C-tulathromycin in soils, cattle, and human feces.

Study No.	Pfizer Study No. 1A72N-60-00-203
Study Period	October, 2000 to December, 2001
Study Director	Riyadh N. Fathulla, PhD
Study Location	Covance Laboratories Inc., Madison, WI

Study Design: Adsorption and desorption characteristics of tulathromycin were studied in soil, cattle feces, and human feces following Organization of Economic Cooperation and Development (OECD) Guideline 106. A kinetic test was performed at a sorbent:solution ratio of 1:10 for human feces, allowing 24 hours for both adsorption and desorption to reach equilibrium. The adsorption/desorption isotherm tests were conducted at 0.1, 1, 5, and 25 ppm of ^{14}C-tulathromycin. Blank (feces and $CaCl_2$ with no drug) and control (drug and $CaCl_2$ with no sorbent) samples were run in triplicate. Coefficient (Koc and Kd) values were calculated for sorption and desorption. Liquid scintillation and HPLC analyses were performed for one sample of each sorbent:solution ratio to determine radioactivity and amount of unchanged radioactive drug extracted from feces. Adsorption supernatants and pellets were analyzed for radioactivity and unchanged drug concentration.

Results and conclusions: At a sorbent:solution ratio of 1:5, the range of mean percent of absorption to feces was 40 to 55%, with maximum adsorption occurring within the first 4 hours. The range of mean percent desorbed of amount adsorbed was 18.7 to 22.8%, with a plateau reached at 11.8 hours. The adsorption Kd was 8.5, and Koc 61. The desorption Kd was 115, and adsorption Koc 821. The parent drug was stable in the sorbent both during adsorption and desorption periods for 48 hours. The firm stated that the Kd value is a conservative estimate of the binding properties of tulathromycin to feces in the colon because the study was conducted at an incubation temperature of 20° C. Binding to feces would be greater at body temperature (37° C). In order to confirm this, another study on the effects of temperature on binding was conducted as summarized below (Study #4).

Study #4. Binding of [14C] tulathromycin to human feces – effect of temperature on the sorption coefficient (Kd).

Study No.	Pfizer Study No. 53056/54866
Study Period	April of 2002
Study Director	Mark Moen
Study Location	Environmental Sciences, Chemical Research and Development, Pfizer Global Research and Development

Study Design: A sorption study with ^{14}C-tulathromycin was performed to compare binding to human feces at 20 and 37° C. Kd values were calculated at each temperature based on a single point determination. Experimental procedures are similar to the description in Study #3. Each assay was done in triplicate for each temperature.

Results and conclusions: The results showed that percentages of binding at 37 and 20° C were 76.4% and 63.3%, respectively. Kd values were 32 and 17 at 37° C and 20° C, respectively. Mass balance determination showed that over 90% of radiolabeled tulathromycin was accounted for under both conditions. Thus, it was concluded from the study that tulathromycin is more likely to bind to feces at body temperature, limiting the amount of biologically active tulathromycin residues in the colon.

Study #5. Effect of fecal binding and pH on antibacterial activity of tulathromycin: comparative MIC determinations.

Study No.	Pfizer Study No. 1671N-03-01-226.
Study Period	February-March, 2001
Study Director	Dr. Andrew Pridmore
Study Location	Don Whitley Scientific Limited, Shipley, West Yorkshire, United Kingdom

Study Design: MIC values of tulathromycin against 4 strains each of *E. coli*, *Enterococcus,* and *Bifidobacterium* were determined under 4 growth conditions: 1) culture media at pH 7.2, 2) culture media at pH 6.5, 3) culture media and sterilized fecal material at pH 7.2, and 4) culture media and sterilized fecal material at pH 6.5. MIC testing was performed according to CLSI guidelines for aerobic bacterial strains. Tulathromycin was tested at various concentrations ranging between 128 and 0.031 µg/mL under each condition. Activity in the presence of feces was measured as the Concentration Preventing Growth (CPG) following sub-culture from the fecal mixture. The lowest concentration of drug that prevented visible growth following sub-culture was recorded as the CPG. Calculation of the binding to feces was performed under the premises that the drug binds to proteins and other fecal material, so that tulathromycin residues are, therefore, unavailable to interact with bacteria.

Results and conclusions: Compared with pH 7.2, growth medium with a pH of 6.5 caused a marked reduction in tulathromycin activity against *E. coli* and *Enterococcus* stains, and a moderate reduction against *Bifidobacterium* strains. Comparison between CPGs in medium alone and in the presence of feces showed that >75% of the test compound was bound to feces the strains tested. It was concluded that tulathromycin activity can be markedly reduced by low pH, and the compound is extensively bound to feces.

Summary of Step 3: From the description of five studies above, it is clear that feces has a detrimental effect on the activity of tulathromycin. As much as 20 µg/ml of tulathromycin (> 10X the MIC) did not inhibit the growth of bacteria tested, which represented the most susceptible groups studied. Fecal binding studies using radiolabeled tulathromycin demonstrated that as much as 65% of the compound is in bound form. In addition, pH changes in the testing system had a noticeable effect on the loss of activity for tulathromycin. Therefore, as demonstrated by the studies, the amount of tulathromycin entering the human colon has no detectable biological activity.

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

- Colonization barrier disruption
- Increase in populations of resistant intestinal bacteria

Yes, based on results from the studies described above in the Steps 2 and 3, the amount of tulathromycin residues able to enter the human colon has insignificant and negligible biological activity. Therefore, testing of either endpoint of concerns - colonization barrier disruption or resistance development - is not needed.

2. Determination of the final mADI

There is no need to determine a mADI for the proposed application.

Final conclusion: Under the proposed conditions of use, the amount of microbiologically active residues of tulathromycin reaching the human colon and remaining biologically active is negligible, and is not expected to have any adverse effect on human intestinal flora. It is concluded that the toxicological ADI of tulathromycin (15 µg/kg BW/day) is well protective of consumers from impact on human intestinal flora.

C. Toxicology:

Reassessment of the toxicological Acceptable Daily Intake (ADI) was not needed for this supplemental approval. The FOI Summary for the original approval of NADA 141-244, dated May 24, 2005, contains a summary of all toxicology studies and information.

D. Assignment of the Final ADI:

The final ADI is the toxicological ADI of 15 µg/kg BW/day for total tulathromycin residues derived from the no-observed-effect level (NOEL) of 15 mg/kg BW/day of the developmental toxicity study in rats, and a safety factor of 1000.

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

The safe concentration of total tulathromycin residues in edible tissues of cattle and swine is 3 ppm for muscle, 9 ppm for liver, 18 ppm for kidney, and 18 ppm for fat.

F. Residue Chemistry:

1. Summary of Residue Chemistry Studies

a. Total Residue and Metabolism Studies

CVM did not require a total residue and metabolism study for this approval. The FOI Summary for the original approval of NADA 141-244 dated May 24, 2005, contains a summary of the total residue and metabolism studies for tulathromycin in cattle.

b. Comparative Metabolism Study

CVM did not require comparative metabolism studies for this approval. The FOI Summary for the approval of NADA 141-244 dated May 24, 2005, contains a summary of comparative metabolism studies for tulathromycin in cattle.

c. Study to Establish Withdrawal Period

Study No. 1531N-60-11-882 – Determination of the Concentration of Tulathromycin Residues (CP-60,300) in Injection Site and Edible Tissues of Veal Calves Receiving One Subcutaneous Injection of DRAXXIN (25 mg/mL) at 2.5 mg/kg

The purpose of this GLP-study was to measure the concentration of CP-472,295(e) marker residue (CP-60,300) in the tissues of preruminating (veal) calves following a single subcutaneous injection of DRAXXIN (25 mg/mL) Injectable Solution at 2.5 mg/kg.

Study Dates: September 15, 2011 – April 30, 2012

Study Director: Tracie Wolthuis

In-Life Testing Site: Halbert Dairy Farm, LLC, Battle Creek, MI and Pfizer Animal Health – VMRD, Richland, MI

Analytical Laboratory: Pfizer Animal Health VMRD Metabolism and Safety, Kalamazoo, MI

Test Animals: Thirty-six healthy preruminating male Holstein calves weighing 44 to 62 kg at study initiation

Test Article Administration: Animals were randomly assigned to one of nine treatment groups (n=4 animals/group) and received a single subcutaneous injection of 2.5 mg tulathromycin/kg BW in the neck.

Sampling and Analysis: Tissue samples from both kidneys, primary (500 ± 20% g) and secondary (300 ± 20% g) injection sites, whole liver, perirenal fat and muscle from the hindquarter with skin and fat removed were collected at 4, 8, 12, 16, 20, 28, 35, 42 and 49 days post-dose. The concentration of CP-472,295(e) (tulathromycin) residue as the CP-60,300 "common fragment" in the liver, kidney, muscle, and fat samples was measured using a validated LC-MS/MS method for tulathromycin marker residue CP-60,300 in tissues using a matrix standard curve.

Tissue Results: The mean tulathromycin (CP-472,295(e)) concentrations in liver, injection site muscle, muscle, kidney and fat are presented in Table 4. The mean CP-60,300 (common fragment) concentrations in liver are presented in Table 5.

Table 4. Mean Tulathromycin (CP-472,295(e)) Residue Concentrations (ppm) in Preruminating (Veal) Calf Liver, Injection Site Muscle, Muscle, Kidney and Fat Tissue Samples.

Withdrawal Time (days)	Tulathromycin Residues in Liver (ppm ± SD)	Tulathromycin Residues in Injection Site Muscle (ppm ± SD)	Tulathromycin Residues in Muscle (ppm ± SD)	Tulathromycin Residues in Kidney (ppm ± SD)	Tulathromycin Residues in Fat (ppm ± SD)
4	7.935 ± 1.56	6.683 ± 1.56	0.902 ± 0.13	5.033 ± 0.62	1.463 ± 0.64
8	5.623 ± 0.20	3.397 ± 0.20	0.498 ± 0.05	3.160 ± 0.55	0.836 ± 0.28
12	4.980 ± 0.67	2.853 ± 0.67	0.380 ± 0.06	2.850 ± 1.79	0.519 ± 0.21
16	3.630 ± 0.22	1.793 ± 0.22	0.233 ± 0.13	1.990 ± 0.21	0.444 ± 0.13
20	3.083 ± 0.32	1.391 ± 0.32	0.124 ± 0.06	0.912 ± 0.27	0.287 ± 0.08
28	0.922 ± 0.28	1.427 ± 0.28	BLQ	0.504 ± 0.15	0.438 ± 0.57
35	0.744 ± 0.32	0.622 ± 0.32	BLQ	0.338 ± 0.06	0.097 ± 0.10
42	0.335 ± 0.09	0.482 ± 0.09	BLQ	BLQ	BLQ
49	0.344 ± 0.22	0.309 ± 0.22	BLQ	BLQ	BLQ

BLQ – predicted concentration is less than validated limit of quantitation (liver<300 ppb, kidney<200 ppb, muscle and fat<50 ppb)

Table 5. Mean Residue CP-60,300 (common fragment) concentrations (ppm) in Preruminating (Veal) Calf Liver.

Withdrawal Time (days)	Mean Tulathromycin Residues in Liver (ppm ± SD)
4	5.668 ± 1.24
8	4.252 ± 0.20
12	3.557 ± 0.77
16	2.593 ± 0.31
20	2.202 ± 0.42
28	0.659 ± 0.30
35	0.532 ± 0.07
42	0.239 ± 0.17
49	0.246 ± 0.12

Using the residue depletion data from Study Number 1531N-60-11-882, the tolerance of 5.5 ppm for the marker residue (common fragment, CP-60,300) in the target tissue (liver) and a statistical algorithm that determines the upper 95% confidence limits on the 99th percentile for liver residues, a 22-day withdrawal period is calculated.

2. Target Tissue and Marker Residue

No reassessment of target tissue and marker residue was needed for this approval. The FOI Summary for the approval of NADA 141-244 dated May 24, 2005, contains a summary of information used to determine liver as the target tissue and CP-60,300 as the marker residue for cattle.

3. Tolerance(s)

The tolerance for CP-60,300 (the marker residue) is 5.5 ppm in cattle liver as described in the approval of NADA 141-244 dated May 24, 2005.

4. Withdrawal Period

A 22-day withdrawal period is assigned for the tulathromycin 25 mg/mL product in calves (suckling calves, dairy calves, and veal calves). A withdrawal period of 22 days is consistent with depletion of residues at the injection site.

G. Analytical Method for Residues:

1. Description of Analytical Method

The regulatory method for determination of tulathromycin in bovine liver is an LC-MS/MS assay involving a solution standard curve, which successfully completed a sponsor monitored multilaboratory method trial. For this

approval, the standard curve was matrix-based and validated. For the confirmatory procedures, the sample extraction and preparation are identical to the sample extraction and preparation for the determinative procedures with the monitoring of an additional two ions resulting in two ions ratios that meet the ±10% relative abundance matching criteria.

2. Availability of the Method

The validated regulatory method for detection and confirmation of residues of tulathromycin is available from the Center for Veterinary Medicine, 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 DRAXXIN 25 Injectable Solution:

For use in animals only. Not for human use. Keep out of reach of children.

To report a suspected adverse reaction or to request a safety data sheet call 1-888-963-8471. For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS or online at http://www.fda.gov/AnimalVeterinary/SafetyHealth.

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 DRAXXIN 25 Injectable Solution, when used according to the label, is safe and effective for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* in suckling calves, dairy calves, and veal calves. Additionally, data demonstrate that residues in food products derived from species treated with DRAXXIN 25 Injectable Solution will not represent a public health concern when the product is used according to the label.

A. Marketing Status

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

B. Exclusivity

This supplemental approval for DRAXXIN 25 Injectable Solution qualifies for THREE years of marketing exclusivity under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act because the supplemental approval included safety studies. This exclusivity begins as of the date of our approval letter and only applies to the addition of the BRD treatment claim in suckling calves, dairy calves, and veal calves.

C. Supplemental Applications

This supplemental NADA did not require a reevaluation of the safety or effectiveness data in the original NADA (21 CFR 514.106(b)(2)).

D. Patent Information:

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