Date of Approval: October 16, 2014

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-244

DRAXXIN

Tulathromycin

Injectable Solution

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

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

For removal of the veal calf restriction (residue warning statements).

Sponsored by:

Zoetis Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-244

B. Sponsor

Zoetis Inc.
333 Portage St.
Kalamazoo, MI 49007

Drug Labeler Code: 054771

C. Proprietary Name

DRAXXIN

D. Established Name

Tulathromycin

E. Pharmacological Category

Antimicrobial

F. Dosage Form

Sterile injectable solution

G. Amount of Active Ingredient

100 mg/mL

H. How Supplied

50 mL, 100 mL, 250 mL, and 500 mL glass vials

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 Injectable Solution is indicated for the treatment of BRD associated with *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis*.

N. Effect of Supplement

This supplement provides for 1) the treatment of bovine respiratory disease associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* in suckling calves, dairy calves, and veal calves; and 2) removal of the veal calf restriction (residue warning statements).

II. EFFECTIVENESS

A. Dosage Characterization

This supplemental approval does not change the previously approved dosage. The Freedom of Information (FOI) Summary for the original approval of NADA 141-244 dated May 24, 2005, contains dosage characterization information for cattle.

B. Substantial Evidence

DRAXXIN was previously approved for the treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovi*s in beef and non-lactating dairy cattle.

Zoetis Inc. conducted a Bayesian meta-analysis to determine the effectiveness of DRAXXIN for the treatment of BRD associated with *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovi*s in suckling calves, dairy calves, and veal calves.

1. Bayesian Analysis

a. Title: "Bayesian Analysis in Support of the Use of DRAXXIN for the Treatment of Bovine Respiratory Disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovi*s in Pre-ruminating Dairy Calves and those Processed for Veal". Zoetis Inc query number V7010Q070. June 2014.

b. Study Methods:

1) Objective: To evaluate the effectiveness of DRAXXIN for the treatment of BRD associated with *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovi*s in suckling calves, dairy calves, and veal calves.

2) Data Source and Study Selection: The analysis included data from BRD treatment effectiveness studies conducted for the approval of DRAXXIN in the U.S. and contemporaneous studies conducted in Europe. To establish an appropriate database for comparison, young calves in the studies were defined as calves weighing 250 lbs or less and fed

primarily a milk-based diet, and older calves were defined as calves weighing more than 250 lbs, and fed primarily a roughage and grain-based diet. Based both on the available body of evidence associated with DRAXXIN effectiveness in young animals and a cross-study evaluation of available pharmacokinetic data, there are no differences in the tulathromycin exposure achieved in preruminating calves and ruminating calves weighing less than 250 lbs. Therefore, the weight cutoff of 250 lbs was used for pooling effectiveness data from multiple dose determination and dose confirmation studies.

Table 1 includes a list of studies and animals included in the analysis.

Table 1. List of studies and animal information.

Study Number	Region	Type of Calves	Number of Treated Calves Included in Analysis[1]	Number of Treatment Successes
5133C-03-98-200	Europe	young	14	14
5133E-03-98-201	Europe	young	10	10
5133E-10-99-205	Europe	young	41	27
5133C-10-99-208	Europe	young	42	41
5133C-03-99-221	Europe	young	36	31
5133C-10-99-223	Europe	young	63	59
5133C-10-02-238	Europe	young	54	46
5133C-12-99-209	Europe	older	43	37
5133C-12-99-222	Europe	older	58	54
1133C-60-99-305	U.S.	older	80	61
1133C-60-99-306	U.S.	older	74	56
1133C-60-99-307	U.S.	older	80	71
1133C-60-99-308	U.S.	older	80	57

[1]Some animals enrolled in the studies were subsequently excluded from the analysis for reasons such as protocol deviations, development of non-BRD related disease, or missing data.

3) Summary Measure: The BRD treatment success rate in young calves treated with tulathromycin at 2.5 mg/kg BW as a single subcutaneous injection at enrollment was compared to the treatment success rate in older calves treated with tulathromycin at the same dosage regimen.

c. Analysis:

1) Null Hypothesis: The null hypothesis was that the treatment success rate in young calves (less than or equal to 250 lb or 114 kg BW) was worse than in older calves (greater than 250 lb or 114 kg BW). The 95% highest posterior density (HPD) credible interval for the older calves was calculated. The decision rule was to reject the null

hypothesis if the lower bound of the 95% HPD credible interval for the treatment success rate in young calves was greater than or equal to the lower bound of the 95% HPD credible interval for the older calves.

2) Prior Elicitation: Under the alternative hypothesis, the treatment success rate in young calves would be at least as good as in older calves. The data from the U.S. studies in older calves was considered "historical data" and was used to construct the prior for the treatment success rate in the young calves. In addition, a scale precision parameter a_0 ($0 \leq a_0 \leq 1$) was used to quantify the uncertainty in the historical data and control the "degree of borrowing" from the historical data. This type of prior is called power prior. This elicitation scheme is minimally subjective because the prior itself is just a weighted likelihood. Moreover, the precision parameter (a_0) allows more flexible control of the influence of the historical data in posteriors.

   Before conducting the analysis, it was established that the data from Europe should not dominate the posterior distribution for the treatment success rate in the young calves in the U.S., so the scale precision parameter was set at 1, allowing a full borrowing from the U.S. studies. An assumption was made that the prior distributions for the success rates in the young calves and older calves were uniform because, in general, a uniform prior on the success rates is noninformative and expected to have minimal impact on the posterior distribution of the success rates.

3) Sensitivity Analysis: A sensitivity analysis was used to investigate how the change in scale precision parameter (a_0) impacted the posterior distribution of the treatment success rate for the young calves under two scenarios ($a_0 = 0$ and $a_0 = 0.5$).

d. Results:

1) Primary Scenario ($a_0 = 1$): Proc MCMC in SAS 9.2 was used for the analysis. Table 2 summarizes the posterior distributions for the two variables, treatment success in young calves (p1) and treatment success in older calves (p2) when $a_0 = 1$.

Table 2. Posterior Distribution ($a_0 = 1$)

Type of Calves	Mean Proportion of Success	Standard Deviation	95% HPD Lower Limit	95% HPD Upper Limit
young (p1)	0.8229	0.0159	0.7920	0.8544
older (p2)	0.8082	0.0193	0.7705	0.8457

The mean treatment success rate (0.8229) for the young calves was slightly higher than the older calves (0.8082). The 95% HPD credible intervals for the two groups (p1 and p2) had considerable overlap. In addition, p1 had less variation than p2.

Based on the decision rule, the null hypothesis was rejected using this analysis. The treatment success rate for the young calves was at least as good as the treatment success rate for the older calves.

2) Sensitivity Analysis: Tables 3 and 4 show the posterior distributions for treatment success when $a_0 = 0$ and $a_0 = 0.5$, respectively.

Table 3. Posterior Distribution for Sensitivity Analysis ($a_0 = 0$).

Type of Calves	Mean Proportion of Success	Standard Deviation	95% HPD Lower Limit	95% HPD Upper Limit
young (p1)	0.8740	0.0204	0.8336	0.9132
older (p2)	0.8930	0.0303	0.8322	0.9480

Table 4. Posterior Distribution for Sensitivity Analysis ($a_0 = 0.5$).

Type of Calves	Mean Proportion of Success	Standard Deviation	95% HPD Lower Limit	95% HPD Upper Limit
young (p1)	0.8388	0.0179	0.8030	0.8732
older (p2)	0.8247	0.0236	0.7777	0.8700

When $a_0 = 0$, the mean treatment success rate for the young calves (0.8740) was slightly lower than the older calves (0.8930). When $a_0 = 0.5$, the mean treatment success rate for the young calves (0.8388) was slightly higher than the older calves (0.8247). Under both scenarios, the 95% HPD credible intervals for the two groups (p1 and p2) had considerable overlap. In addition, p1 had less variation than p2, and the 95% HPD credible intervals for p2 covered p1 when a_0 was set to 0. Therefore, under either scenario, the treatment success rate for the young calves was still "equivalent" to the treatment success rate for the older calves per the decision rule.

e. Conclusion: Based on the analysis, the treatment success rate for the young calves in these studies was at least as good as the treatment success rate for the older calves. Therefore, DRAXXIN is considered effective for the treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* in suckling calves, dairy calves, and veal calves.

2. Microbiology

Across the studies described above, sufficient isolates of *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis* were recovered from pre-treatment nasopharyngeal swabs to demonstrate that these pathogens contributed to the observed BRD outbreaks. Among the calves that were *M. bovis*-positive at pre-treatment and subsequently treated with tulathromycin, a majority were classified as treatment successes.

III. TARGET ANIMAL SAFETY

The FOI Summary for the original approval of NADA 141-244 dated May 24, 2005, contains a summary of target animal safety studies conducted in beef and non-lactating dairy cattle. The findings from these studies were used in conjunction with the margin of safety study conducted in preruminating calves summarized below to demonstrate safety of DRAXXIN injectable solution (100 mg tulathromycin/mL) in suckling calves, dairy calves, and veal calves.

A. Margin of Safety Study:

1. Title: "Margin of safety of CP-472,295(e) 10% injectable solution in pre-ruminant cattle." Study Number 5432N-03-00-236. October 2000.

2. Study Director: Susan C. Hill, BSc., PhD. Moredun Scientific Limited (MSL), Penicuik, Midlothian, Scotland

3. Study Design:

   a. Objective: To assess the safety of DRAXXIN injectable solution (known during the study as CP-472,295(e) 10% injectable solution) when administered to preruminating cattle by subcutaneous (SC) injection at 2.5 mg/kg BW (the labeled dose) or 7.5 mg/kg BW (three times the labeled dose) given once.

   b. Study Animals: The test animals were 12 male and 12 female healthy calves, which, on the day of treatment, were between 13 and 27 days of age and weighed between 38.5 to 59.5 kg. The calves were Holstein Friesian or Aberdeen Angus crossbred.

   c. Treatment Groups: Four male and four female calves were randomly assigned to each of three treatment groups as indicated in Table 5.

Table 5. Summary of treatment groups.

Treatment Group	Treatment Regimen	Number of Animals
Control (0X)	saline 0.075 mL/kg BW* SC	8 (4 males and 4 females)
1X	tulathromycin 2.5 mg/kg BW SC	8 (4 males and 4 females)
3X	tulathromycin 7.5 mg/kg BW SC	8 (4 males and 4 females)

*volume equivalent to tulathromycin at 7.5 mg/kg BW

   d. Drug Administration: The test article was the final formulation of DRAXXIN injectable solution containing 100 mg tulathromycin /mL. The control article was a commercial 0.9% sterile saline injectable solution. The calves were randomly assigned to treatment and given either the control article at 0.075 mL/kg BW or the test article at either 2.5 mg/kg BW or 7.5 mg/kg BW.

Test and control articles were administered on Day 0 by subcutaneous injection in the right lateral neck, using a sterile, 18 gauge, 1 inch, disposable needle.

e. Measurements and Observations: The following parameters were measured and/or observed during the study: clinical observations (including physical examination, injection site observations, and body weight), clinical pathology, and post-mortem examination. The individuals performing clinical observations were masked to treatment assignment. The treatment administrator and Study Director were not masked to treatment assignment and did not perform clinical observations.

General health observations were made once on the day of arrival (Day -10) and twice daily up to and including the day before treatment (Day -1). The study veterinarian performed physical examinations on the calves the day before treatment (Day -1). On the day of treatment (Day 0), the study veterinarian performed clinical observations just before treatment, and then 1 to 2, 4 to 5, and 8 to 10 hours after treatment. On Days 2 to 6, the study veterinarian made clinical observations twice daily. On Day 7, clinical observations were made once prior to euthanasia.

Clinical pathology, consisting of hematology and clinical chemistry, was assessed on Day -7 and Day -1, at the midpoint of the study (Day 2), and at the end of the study (Day 7).

Body weights were determined on Days -7, -1, and 7.

All study animals were euthanized on Day 7. Each animal was subjected to necropsy and gross pathological evaluation by a masked veterinary pathologist. Heart, liver, kidney, and representative tissues from gross lesions, including injection site tissue, were collected for histopathology.

4. Statistical Analysis: Repeated measures analysis of covariance models were used to test for treatment effects on clinical pathology variables. Gross necropsy and histopathology abnormalities were summarized. Body weights were analyzed using a general linear mixed model for repeated measurements. *A priori* contrasts were used to compare treatments in terms of average daily live weight gain from Day -7 to Day -1 and from Day -1 to Day 7.

5. Results:

a. No adverse test article-related effects were observed during clinical observations. No adverse test article-related effects were observed for body weights or clinical pathology parameters. Injection site observations consisted of small, soft swellings or diffuse thickening of the tissue. Injection site swellings in the 3X animals (8 of 8) were larger in surface area than in the 1X animals (7 of 8): on average, these swellings were approximately 13.6 cm^2 compared with 4.7 cm^2, respectively, on Day 1. Swellings gradually diminished in surface area over the duration of the study, but were still apparent on Day 7 (average approximately 5.8 cm^2 in

the 3X group (8 of 8) and 2.2 cm^2 in the 1X group (7 of 8)). No injection site swellings occurred in the control group.

b. No abnormal findings on gross and histopathologic examination, other than injection site-related findings, were attributed to administration of tulathromycin. Injection site histopathologic changes included edema (4 of 4 in the 1X group; 4 of 4 in the 3X group), mild suppurative inflammation (4 of 4 in the 1X group; 4 of 4 in the 3X group), and damage to the dermis (3 of 4 in the 1X group; 2 of 4 in the 3X group). These findings were considered to be minimal or mild to moderate, and were considered to be resolving.

6. Conclusions: This study demonstrated that DRAXXIN injectable solution (100 mg tulathromycin/mL) is safe in preruminating calves when administered once as a subcutaneous injection at a dosage of 2.5 mg tulathromycin/kg BW.

IV. HUMAN FOOD SAFETY

A. Antimicrobial Resistance:

The impact of the proposed removal of the veal calf restriction from the tulathromycin label on microbial food safety (antimicrobial resistance) was carefully considered by the Agency. The Agency determined that this supplemental action should not significantly impact public health with respect to antimicrobial resistance. 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. Removal of the veal calf restriction from 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.

B. Impact of Residues on Human Intestinal Flora:

1. Determination of the need for establishing a microbiological ADI

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 with 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 3 months before isolation) between 1998 and 2000, with the exception of *Eubacterium* (1994) and 2 isolates of *Fusobacterium* (1994 and 1995).

Results and conclusions: MIC_{50}, MIC_{90}, and the geometric MIC were determined for each genus/group. Among the bacterial groups tested, *Bifidobacterium* spp. were the most susceptible bacteria 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 at the increased inoculum size. It was decided to use the most sensitive group for assessing the *in vitro* activity of the compound.

b. Step 2: Do residues 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 pigs, 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 pigs. 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 the excretion of other macrolides used inhuman 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 the residues 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 4X 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 4 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 the ingested residues will be significantly reduced by the 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 samples (drug and $CaCl_2$ with no sorbent) were run in triplicate.

Coefficients (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 effect of temperature on binding was conducted as summarized below (Study #4).

Study #4. Binding of [^{14}C] 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 (> 10 x 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, the pH changes in the testing system have a noticeable effect on the loss of activity for tulathromycin. Therefore, as demonstrated by the studies, the amount of tulathromycin that enters the human colon has no detectable biological activities.

d. Step 4: Determination if there is any scientific justification to eliminate testing for either one 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 that is able to enter the human colon has an 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 Microbiological ADI

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

Decision Statement: Under the proposed 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 effects on human intestinal flora. It is concluded that the tADI of tulathromycin (0.9 mg/person/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 or 0.9 mg/person/day for total tulathromycin residues derived from the 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 in Edible Tissues:

The safe concentrations of total tulathromycin residues in edible tissues are 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 supplemental 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

b. Comparative Metabolism Study

Comparative metabolism of tulathromycin in rats and dogs (the animals used in the toxicity tests) was satisfactorily demonstrated by data in the original approval of NADA 141-244 (FOI Summary dated May 24, 2005).

c. Study to Establish Withdrawal Period and/or Milk Discard Time

(1) Tissue Residue Depletion Study

A tissue residue depletion study in preruminating calves was conducted to evaluate tulathromycin residues (as the marker residue CP-60,300) in tissues from liver, kidney, muscle, fat and injection site after a single administration of DRAXXIN injectable solution (2.5 mg tulathromycin/kg body weight). Tulathromycin residue data determined the target tissue and allowed a withdrawal period to be established for all preruminating calves.

Title: "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 Injectable Solution at 2.5 mg/kg"

In-Life Facility: Halbert Dairy Farm, LLC, Battle Creek, MI

Study Director: Tracie Wolthuis
Pfizer Animal Health, Kalamazoo, MI

Analytical Facility: Pfizer Animal Health, Kalamazoo, MI

The study was conducted according to Good Laboratory Practices (21 CFR 58). Thirty-six male preruminating calves weighing between 38 to 69 kg were individually housed. Four animals were randomly assigned to each of the nine treatment groups. Calves received a single subcutaneous injection of DRAXXIN (100 mg/mL) injectable solution in the neck at 2.5 mg tulathromycin/kg body weight. Tissue samples were collected after the dosing period and were analyzed with the determinative method for tulathromycin. Injection site tissue samples were analyzed in triplicate, while all other tissue samples were analyzed in duplicate.

Table 6. CP-60,300 Concentrations (ppb) in Liver

Sampling timepoint (days)	Mean ± Std Deviation (n=4)
4	4948 ± 849
8	3666 ± 914
12	2790 ± 511
16	2394 ± 809
20	1573 ± 379
28	1192 ± 235
35	755 ± 84
42	429 ± 172
49	348 ± 56

2. Target Tissue and Marker Residue

The target tissue for tulathromycin residues in cattle is liver, as described in the original approval of NADA 141-244 dated May 24, 2005. The marker residue is the common fragment, CP-60,300.

3. Tolerance

The tolerance for CP-60,300 is 5.5 ppm as described in the original approval of NADA 141-244 dated May 24, 2005.

4. Withdrawal Period

The Agency calculated a withdrawal period of 13 days. As requested by the sponsor, in order to be consistent with the withdrawal period assigned to other classes of cattle, an 18-day withdrawal period is assigned for preruminating calves following a single subcutaneous injection at a maximum dose of 2.5 mg/kg body weight.

G. Analytical Method for Residues:

1. Description of Analytical Method

a. Determinative 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 supplemental approval, the standard curve was matrix-based and validated.

b. Confirmatory Method

The sample extraction and preparation for the confirmatory procedures 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 in preruminating calves 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:

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, when used according to the label, is safe and effective for the treatment of BRD associated with *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis*. Additionally, data demonstrate that residues in food products derived from species treated with DRAXXIN 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 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 and effectiveness 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.