Date of Approval: May 24, 2005

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-244

DRAXXIN Injectable Solution

(tulathromycin)

For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* (*Haemophilus somnus*); for the control of respiratory disease in cattle at high risk of developing BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*; and for the treatment of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, and *Haemophilus parasuis.*

Sponsored by:
Pfizer, Inc.

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1. GENERAL INFORMATION:

a. File Number:	NADA 141-244
b. Sponsor:	Pfizer Inc. 235 East 42d St. New York, NY 10017 Drug Labeler Code: 000069
c. Established Name:	Tulathromycin
d. Proprietary Name:	DRAXXIN Injectable Solution
e. Dosage Form:	Sterile injectable solution
f. How Supplied:	100 mL, 250 mL, and 500 mL glass vials
g. How Dispensed:	Rx
h. Amount of Active Ingredients:	100 mg/mL
i. Route of Administration:	Subcutaneous (cattle) or intramuscular (swine) injection in the neck
j. Species/Class:	Beef and non-lactating dairy cattle, and swine
k. Recommended Dosage:	2.5 mg/kg body weight (BW), administered once
l. Pharmacological Category:	Antimicrobial
m. Indications:	Cattle: DRAXXIN Injectable Solution is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* (*Haemophilus somnus*), and for the control of respiratory disease in cattle at high risk of developing BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* (*Haemophilus somnus*). Swine: DRAXXIN Injectable Solution is indicated for the treatment of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, and *Haemophilus parasuis*.

2. EFFECTIVENESS:

a. Dosage Characterization

Because the relationship exhibited by macrolide antibiotics between the pharmacokinetic (PK), pharmacodynamic (PD), and clinical variables is inconsistent, minimum inhibitory concentration (MIC) data cannot be reliably combined with pharmacologic criteria to produce a predictive model of clinical effectiveness. For this reason, dosage characterization for tulathromycin injectable solution in cattle and swine was primarily based on non-pivotal clinical effectiveness studies.

Cattle:

The effectiveness of tulathromycin was evaluated for the treatment of naturally-occurring BRD at doses of 1.25 mg/kg body weight (BW) and 2.5 mg/kg BW administered once by subcutaneous (SC) injection in five studies conducted in Europe. The formulation used differed from the commercial formulation only by the inclusion of phenol as a preservative. A total of 390 calves with pyrexia (rectal temperature ≥ 40 °C), abnormal respiration, and mild to moderate depression were randomly assigned to one of three treatment groups – tulathromycin at 1.25 mg/kg BW (156 calves) or 2.5 mg/kg BW (159 calves), or a positive control (75 calves). Following treatment, calves were evaluated for clinical signs of respiratory disease once daily for 15 days. Calves successfully completing the studies were those that following treatment did not meet the withdrawal criteria related to BRD.

Mannheimia haemolytica, *Pasteurella multocida,* and *Histophilus somni* (*Haemophilus somnus*) were isolated in all five studies. A higher percentage of calves (86.8%) treated with tulathromycin at 2.5 mg/kg BW successfully completed the studies compared to the calves (76.9%) treated with 1.25 mg/kg BW. There was no significant difference in the number of BRD-related mortalities between treatments.

A series of earlier non-pivotal studies conducted in cattle in the U.S. with very similar formulations did not show any additional benefit of increasing the dosage of tulathromycin to 5.0 mg/kg BW.

Swine:

The effectiveness of tulathromycin was evaluated for the treatment of swine respiratory disease (SRD) at dosages of 2.5 and 5.0 mg/kg BW. These dosages were compared in an induced *Actinobacillus pleuropneumoniae* infection study conducted in Nebraska. Two hundred crossbred, castrated male pigs were commingled with 40 pigs previously challenged intranasally with *A. pleuropneumoniae*. Fifty commingled pigs showing signs of SRD were treated with tulathromycin at a dose of either 2.5 or 5.0 mg/kg BW as a single intramuscular (IM) injection. Tulathromycin at both dosages reduced the percentage of total lung with lesions and the number of clinically ill pigs compared to a saline-treated control group. There were no significant differences between the two dosages of tulathromycin.

These dosages were subsequently compared in four clinical field studies conducted using a common protocol, in the Netherlands, the United Kingdom (2), and France. A total of 330 pigs with SRD were treated with tulathromycin at either 2.5 or 5.0 mg/kg BW as a single IM injection. No significant differences were found between the two dosages.

No adverse reactions or events attributable to the test article administration were observed during the dosage characterization studies.

The dosage of 2.5 mg/kg BW was selected for further testing.

b. Substantial Evidence

Cattle:

1. Field Studies of Tulathromycin for the Treatment of Cattle with Bovine Respiratory Disease (BRD). Study Numbers 1133C-60-99-305, 1133C-60-99-306, 1133C-60-99-307, and 1133C-60-99-308. October 1999 to November 1999.

a. Type of Study: Multi-site field dose confirmation study

b. Investigators:

David T. Bechtol, D.V.M. Agri Research Center, Inc., Canyon, TX
E.G. Johnson, D.V.M. Johnson Research, Parma, ID
Kelly F. Lechtenberg, D.V.M., Ph.D., Midwest Veterinary Services, Inc., Oakland, NE
Terry N. TerHune, D.V.M., Ph.D., HMS Veterinary Development, Inc., Tulare, CA

c. Study Design:

1) *Objective*: To evaluate the effectiveness of tulathromycin injectable solution for the treatment of naturally occurring BRD, when administered subcutaneously as a single dose of 2.5 mg/kg BW.

2) *Animals*: Castrated, male, beef-crossbred calves, 4 to 11 months old, weighing 150 to 320 kg.

3) *Experimental Design*: The study was conducted at four sites. At each site, calves were randomly assigned to treatment groups and allocated to pens. At each site, approximately 80 tulathromycin-treated calves (a total of 314 tulathromycin-treated calves) and 40 negative control (saline-treated) calves were enrolled (a total of 160 saline-treated calves). Each pen contained two saline-treated calves and four tulathromycin-treated calves. A positive control group was also included in the study, but was not analyzed statistically.

Calves were enrolled in the study when they were diagnosed with BRD and met the enrollment criteria of attitude score $\geq$ 1, respiratory score = 1, and

rectal temperature of ≥ 104 °F. The following clinical scoring scales were used:

Attitude: 0 = normal; 1 = mild depression; 2 = moderate/marked depression; 3 = severe depression

Respiration: 0 = normal rate and character for recently transported cattle; and 1 = abnormal, notable increase in rate or abnormal character of respiration

To characterize the BRD outbreak, nasopharyngeal swabs were collected from each calf prior to treatment and cultured for the presence of BRD pathogens.

4) *Test Article Administration*: Tulathromycin injectable solution (100 mg/mL) was administered as a single dosage of 2.5 mg/kg BW. Saline at an equivalent volume (0.025 mL/kg) was used as the negative control article. Test and control articles were administered subcutaneously in the neck at enrollment (Day 0).

5) *Measurements and Observations*: The primary variable was the determination of treatment success (cure rate) on Day 14. A calf was classified as a success if it survived through Day 14 without being classified as a non-responder or Day 14 treatment failure. A calf was classified as a non-responder on Days 3-13 if it had an attitude score ≥ 1, and respiration score = 1, and a rectal temperature of ≥ 104 °F. Non-responders were removed from the study, and analyzed as treatment failures. A calf was defined as a treatment failure on Day 14 if it had an attitude score ≥ 2, or respiration score = 1, or a rectal temperature of ≥ 104 °F. Calves were weighed on Days 0 and 14, when classified as a non-responder, or when found dead. Mortalities were recorded and necropsied. Cultures were performed on lung swabs and lung samples from saline-treated mortalities.

6) *Statistical Analysis*: The cure rate was analyzed using logistic regression in SAS Proc GLIMMIX with a logit link function. The individual calf was the experimental unit. Treatment was included as a fixed effect, and site, site by treatment, and pen were included as random effects.

d. Results: Refer to Table 2.1 below. The cure rate was statistically significantly higher ($p = 0.002$) in the tulathromycin-treated calves compared with the saline-treated calves. Across all sites, model-based estimates demonstrate that 78.3% of the calves treated with tulathromycin were cured, compared to 23.5% of the saline-treated calves. While no significant differences in moralities were seen, the overall percent mortality was reduced in the tulathromycin-treated group.

Table 2.1. Comparison of results of tulathromycin and saline effectiveness for the treatment of BRD.

Treatment Group	Number Treated	Number of Mortalities	Number Cured	Percent Cured*
Tulathromycin	314	2	245	78.3%
Saline	160	9	38	23.5%

* Percentage calculated as Least Squares Mean (LSM). The LSM percentage is based on the analysis model and is not identical to the percentage calculated from the raw data.

Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and *Mycoplasma* spp. were isolated from study animals. Minimum Inhibitory Concentration (MIC) data are summarized in the Microbiology section below (2.d).

e. Adverse Reactions: No adverse reactions were reported.

f. Conclusion: The results demonstrate that tulathromycin, when administered to cattle as a single SC dosage of 2.5 mg/kg BW was effective for the treatment of BRD associated with *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni.*

2. Field Studies of Tulathromycin for the Control of BRD in Cattle at High Risk of Developing BRD. Study Numbers 1133C-60-99-309, 1133C-60-99-310, 1133C-60-99-311, and 1133C-60-99-312. October 1999 to December 1999.

a. Type of Study: Multi-site field dose confirmation study

b. Investigators:

David T. Bechtol, D.V.M. Agri Research Center, Inc., Canyon, TX
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Kelly F. Lechtenberg, D.V.M., Ph.D., Midwest Veterinary Services, Inc., Oakland, NE
Terry N. TerHune, D.V.M., Ph.D., HMS Veterinary Development, Inc., Tulare, CA

c. Study Design:

1) *Objective*: To evaluate the effectiveness of tulathromycin injectable solution for the control of BRD in cattle at high risk of developing BRD, when administered subcutaneously as a single dose of 2.5 mg/kg BW.

2) *Animals*: Castrated, male, beef-crossbred calves, 4 to 11 months old, weighing 150 to 326 kg.

3) *Experimental Design*: The study was conducted at four sites. At each site, calves were randomly assigned to treatment groups and allocated to pens. At each site, approximately 100 tulathromycin-treated calves (a total of 399 tulathromycin-treated calves) and 100 negative control (saline-treated)

calves were enrolled (a total of 402 saline-treated calves). Treatment groups were commingled in pens. A positive control group was also included in the study, but was not analyzed statistically.

Calves with above average risk of developing BRD were obtained at auction and transported by truck to the study sites and enrolled if they were free of clinical signs of disease or injury. Test and control articles were administered at the time of processing (Day 0), one to two days after arrival.

4) *Test Article Administration*: Tulathromycin injectable solution (100 mg/mL) was administered as a single dosage of 2.5 mg/kg BW. Saline at an equivalent volume (0.025 mL/kg) was used as the negative control article. Test and control articles were administered subcutaneously in the neck.

5) *Measurements and Observations*: On Days 1 through 14, calves were assigned respiration and attitude scores, using the same scales described for the study summarized above (2.b.1.). Any calf developing BRD (a respiration score = 1 and attitude score ≥ 1 and rectal temperature ≥ 104 °F) was classified as a non-responder and removed from the study. The primary variable was the determination of failure rate through Day 14. Failures included calves classified as non-responders and calves that died or were euthanized for severe BRD. Mortalities were recorded and necropsied. Cultures were performed on lung swabs from saline-treated non-responders, and lung swabs and lung samples from saline-treated mortalities.

6) *Statistical Analysis*: The failure rate was analyzed using logistic regression in SAS Proc GLIMMIX with a logit link function. The individual calf was the experimental unit. Treatment was included as a fixed effect, and site, site by treatment, and pen were included as random effects.

d. Results: Refer to Table 2.2 below. The failure rate was statistically significantly lower ($p = 0.011$) in the tulathromycin-treated calves compared to the saline-treated calves. Across all sites, model-based estimates demonstrate that 10.6% of the calves treated with tulathromycin were failures, compared to 59.4% of the saline-treated calves.

Table 2.2. Comparison of results of tulathromycin and saline effectiveness for the control of BRD in cattle at high risk of developing BRD.

Treatment Group	Number Treated	Number of Mortalities	Number of Failures	Percent of Failures*
Tulathromycin	399	0	53	10.6%
Saline	402	2	236	59.4%

* Percentage calculated as LSM. The LSM percentage is based on the analysis model and is not identical to the percentage calculated from the raw data.

Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and *Mycoplasma* spp. were isolated from study animals. MIC data are summarized in the Microbiology section below (2.d.).

e. Adverse Reactions: No adverse reactions were reported.

f. Conclusion: The results demonstrate that tulathromycin, when administered to cattle as a single SC dosage of 2.5 mg/kg BW was effective for the control of BRD in cattle at high risk of developing BRD associated with *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni.*

Swine:

1. Six Clinical Field Studies Conducted Following a Common Protocol for the Use of Tulathromycin in Swine for the Treatment of SRD. Study Numbers 1123C-60-00-190, 1123C-02-01-192, 1123C-60-01-193, 1123C-60-01-195, 1123C-60-01-196, and 1123C-60-01-198. March 2001 to January 2002.

a. Type of Study: A multi-site field dose confirmation study

b. Investigators:

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c. Study Design:

1) *Objective*: To evaluate the effectiveness of tulathromycin injectable solution against naturally occurring SRD when administered as a single IM injection of 2.5 mg/kg BW.

2) *Animals*: Female and castrated male crossbred swine, six to 24 weeks of age and weighing 10 to 62.7 kg at treatment. Pigs were obtained from locations experiencing SRD, or they originated from the study site at the time of the study.

3) *Experimental Design*: Approximately 48 pigs were randomly assigned to each treatment group at each of the five U.S. sites and 30 pigs to each treatment group at the Ontario site for a total of 266 tulathromycin-treated pigs and 267 saline-treated negative (placebo) control pigs.

A positive (treated) control group was used at four of six sites, but did not contribute to study conclusions and was not included in the statistical analysis. Additional pigs from the candidate pool at each site were allocated to a non-

treated ("NTX") group. NTX pigs were euthanized and necropsied to help characterize the disease outbreak, and were not included in the statistical analysis.

Pigs were enrolled if they had a respiration score >1 and/or an attitude score >1 and a rectal temperature of ≥ 104 °F. Pigs meeting enrollment criteria were randomly allocated to treatment groups and treated on Day 0. The following clinical scoring scales were used:

Respiration: 0 = normal; 1 = mild increase in respiratory effort and/or occasional cough; 2 = moderate increase in respiratory effort and/or obvious cough (several coughing episodes within a few minutes); 3 = dyspnea and/or cyanosis.

Attitude: 0 = normal; 1 = mild depression, pigs appear mildly depressed or lethargic prior to stimulation, but upon stimulation appear normal; 2 = moderate depression, pigs will rise upon stimulation, but appear lethargic; 3 = severely depressed or moribund; unable to rise, resistant to stimulation but will rise, continues to look depressed, or seeks to lie down.

4) *Test Article Administration*: Tulathromycin injectable solution (100 mg/mL) was administered as a single dosage of 2.5 mg/kg BW. Saline at an equivalent volume (0.025 mL/kg) was used as the negative control article. Test and control articles were administered as a single IM injection at enrollment (Day 0).

5) *Measurements and Observations*: General health observations (not related to SRD) were recorded twice daily from the beginning of the pre-treatment period through Day 7 post-treatment. Post-treatment observations were conducted approximately one and four hours following dosing. On Days 5 and 7, all pigs remaining in the study were clinically scored and had rectal temperatures measured. The primary assessment of effectiveness was cure rate. A pig was considered a cure if, on Day 7 after treatment, it was alive and had a respiration score ≤ 1, an attitude score of ≤ 1, and a rectal temperature of <104 °F. Mortality was also recorded. Pigs that died or were euthanized during the study were necropsied. Lung samples were obtained from saline-treated pigs with significant gross SRD lung lesions and submitted for pathogen identification.

6) *Statistical Analysis*: The multi-site analysis of cure rate was analyzed with a generalized linear mixed effects model (GLIMMIX). The model included the fixed effect of treatment, and the random effects of study site, pen nested in study site, treatment by study site and treatment by pen nested in study site.

d. Results: Refer to Table 2.3 below. There was a statistically significant difference between tulathromycin and the saline control in the percent of cures across all six sites ($p = 0.0214$). Across all six sites, model-based estimates demonstrate that

70.54% of the pigs treated with tulathromycin were cured, compared to 46.09% of the saline-treated pigs.

Table 2.3. Comparison of results of tulathromycin and saline effectiveness against SRD.

Treatment Group	Number Treated	Number of Mortalities	Number Cured	Percent Cured*
Tulathromycin	266	7	189	70.54%
Saline	267	24	124	46.09%

* Percentage calculated as LSM. The LSM percentage is based on the analysis model and is not identical to the percentage calculated from the raw data.

Actinobacillus pleuropneumoniae, Pasteurella multocida, Bordetella bronchiseptica, and *Haemophilus parasuis* were isolated from study pigs. MIC data are summarized in the Microbiology section below (2.d.).

e. Adverse Reactions: No adverse reactions were reported.

f. Conclusion: The results demonstrate that tulathromycin, when administered to swine as a single IM injection of 2.5 mg/kg BW was effective for the treatment of SRD associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Bordetella bronchiseptica,* and *Haemophilus parasuis.*

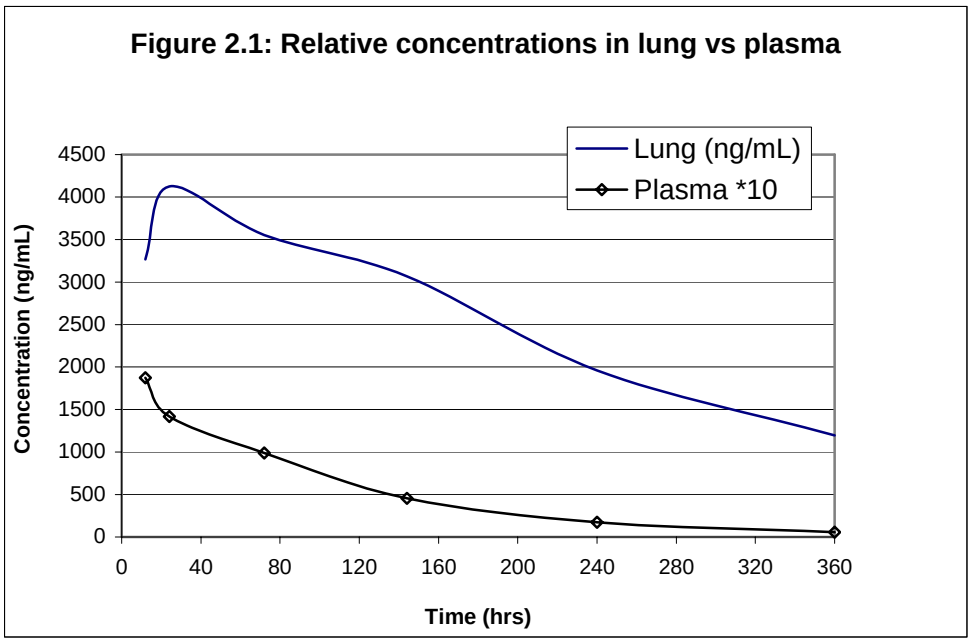
c. Pharmacokinetic Studies

Cattle:

1. Plasma and Lung Pharmacokinetics of a Single 2.5 mg/kg Dose of Subcutaneously Administered CP-472,295 in Cattle. Study Number 1530N-60-00-359.

Forty-two crossbred beef cattle ranging in weight from 182 to 260 kg were given tulathromycin (CP-472,295) injectable solution (100 mg/mL) as a single 2.5 mg/kg BW SC injection in the right lateral neck. Calves were randomly assigned (three males and three females per group) to one non-medicated group or one of six tulathromycin-treated groups. Tulathromycin-treated groups differed only with respect to the duration of plasma sampling and the time of lung sample collections (12 to 360 hours post-injection). Samples were assayed for unchanged tulathromycin by a high performance liquid chromatographic method with tandem mass spectrometry detection (LC-MS/MS).

Although there were some differences in plasma and lung concentrations across treatment groups, there were no statistically significant differences in tulathromycin pharmacokinetics across genders. As shown in Figure 2.1, total drug concentrations in the lung were higher than plasma concentrations. The terminal elimination half life was longer in lungs ($T_{1/2}$ ~ 8.75 days) as compared to plasma ($T_{1/2}$ ~ 2.75 days). The clinical significance of these higher lung concentrations has not been determined.



2. Bioavailability of CP-472,295(e) via Subcutaneous Administration in Ruminant Calves. Study Number 1530N-60-00-363.

In this study, the bioavailability of tulathromycin (CP-472,295(e)) was determined in the lungs and plasma of calves following SC or intravenous (IV) injection of 2.5 mg/kg BW. A total of 16 castrated male and female beef calves, five to six months old, weighing 181 to 247 kg were used in this study. Calves were divided into four treatment groups, based on route of administration (SC or IV) and euthanasia date (Day 7 or 15). Two additional calves served as non-medicated controls. Blood samples were collected 0.25, 4, 8, 24, 48, 72, 96, 120, and 144 hours post-injection (Groups 1, 2, 3, and 4), and 168, 192, 216, 240, 264, 288, 312, 336, and 360 hours post-injection (Groups 2 and 4). Tulathromycin concentrations were quantified using an LC-MS/MS procedure.

Relative bioavailability of tulathromycin following SC administration was approximately 94%. After SC administration, C_{max}, observed at the first sampling time (0.25 hours) was 239 ng/mL (Day 7 group) or 377 ng/mL (Day 15 group). After IV administration, the average volume of distribution was 11 L/kg and the total systemic clearance was 179 mL/hr/kg. The mean residence time was 68 hours in plasma. Lung concentrations following SC administration were similar to concentrations seen in Study Number 1530N-60-00-359.

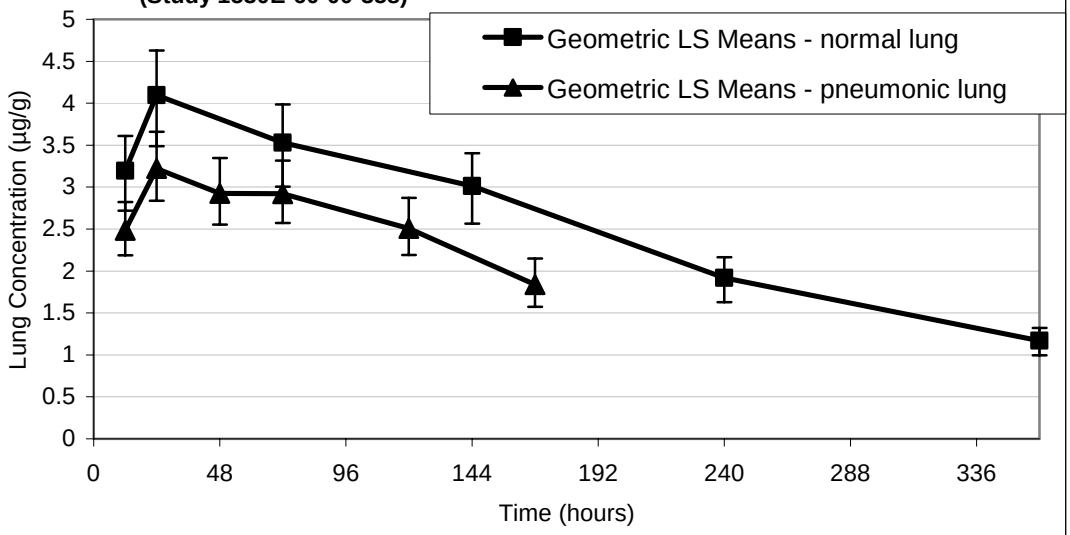
3. Concentrations of CP-472,295(e) in Pneumonic Lung Tissue of Cattle Following a Single Dose of the Drug Administered Subcutaneously at 2.5 mg/kg Body Weight. Study Number 1530E-60-00-358.

Calves approximately 4 to 11 months of age and weighing 150 to 252 kg were selected for this study. After on-arrival processing, 50 calves that exhibited clinical

signs of BRD (abnormal respiration and a rectal temperature ≥ 104 °F) were enrolled and randomly assigned to one of six treatment groups (based on necropsy time) containing eight animals each (4 castrated males, 4 females). Calves received tulathromycin (CP-472,295(e)) as a single SC injection of 2.5 mg/kg BW and were sacrificed at either 12, 24, 48, 72, 120, or 168 hours post-injection. Lung samples were analyzed for tulathromycin concentrations using the validated LC-MS/MS method.

The lung concentrations observed in healthy lungs (Study #1530N-60-00-359) and diseased lungs (Study 1530E-60-00-358) are provided in Figure 2.2 (where the graph represents the geometric least square means ± 90% confidence intervals about the mean value at each time point). The design of these studies did not allow for an evaluation of relative concentrations of free versus bound compound in the pulmonary tissues, nor was it possible to directly measure drug concentrations at the site of infection in the diseased pulmonary tissues. Therefore, the clinical relevance of the observed lung concentrations relative to organism MIC values was not determined.

Figure 2.2: Comparison of tulathromycin concentrations in normal cattle lung (Study 1530N-60-00-359) versus pneumonic lung concentrations (Study 1530E-60-00-358)



4. Protein Binding of CP-472,295(e) in Cattle, Swine, Dog, and Rat Plasma. Study Number 1670E-60-00-220.

Plasma protein binding was determined in cattle, swine, dog, and rat plasma using equilibrium dialysis techniques. Plasma samples were adjusted to pH 7.4 using 10% phosphoric acid. Tulathromycin (CP-472,295(e)) concentrations were 100, 500, and 1000 ng/mL. All tests were conducted at 37 °C.

The unbound fraction of drug was found to be similar across all target animal species examined, ranging from 0.53 to 0.68. Fraction unbound (F_u) did not substantially differ across concentrations ranging from 100 to 1000 ng/mL, nor did F_u vary among the different animal species.

Swine:

1. Plasma and Lung Pharmacokinetics of a Single 2.5 mg/kg Dose of Tulathromycin Intramuscularly Administered to Pigs. Study Number 1520N-03-00-189.

Tulathromycin was administered as a sterile injectable solution, 10% w/v (100 mg tulathromycin/mL) to 36 Landrace-Large White male and female crossbred pigs, two to three months of age, weighing 20 to 25.5 kg. All pigs received a 2.5 mg/kg BW dose as a single IM injection administered caudal to the right ear. Pigs were monitored twice daily throughout the course of the study.

There were six treatment groups, which differed only in the time to the final collection of blood samples and lung tissue collection (12 to 360 hours post-injection). Blood samples were collected from each pig by venipuncture from the jugular vein or cranial vena cava into 20 mL syringes. Tulathromycin concentrations in lung and blood were quantified using an LC-MS/MS procedure.

Peak concentrations occurred rapidly, generally by the first sampling time (0.5 hour). Blood levels declined rapidly thereafter (distribution phase), but the terminal elimination half-life was long (approximately 60 to 90 hours). Multiple peaks were observed in most pigs, suggesting the presence of a recycling of the parent compound. This recycling can prolong the residence time of drug in the body. The plasma pharmacokinetic data are summarized in Table 2.4.

Table 2.4. Summary of mean plasma pharmacokinetic data after a single IM administration of tulathromycin at a dosage of 2.5 mg/kg BW.

Group (T_{last})	Number of Pigs	$AUC_{0\text{-last}}$ [1] (ng*hr/mL)	T_{max} [2] (hrs)	C_{max} [3] (ng/mL)	$T_{1/2}$ [4] (hrs)
T01 (12 hr)	6	2390	0.50	434	--
T02 (24 hr)	6	4680	2.42	466	--
T03 (72 hr)	6	9770	0.50	613	--
T04 (144 hr)	6	8860	0.50	556	49.4
T05 (240 hr)	6	1140	1.08	551	60
T06 (360 hr)	6	12200	0.50	868	91

[1] $AUC_{0\text{-last}}$ = the area under the plasma concentration vs. time curve from time of injection to the last sample at or above the limit of quantification (LOQ) of the analytical method

[2] T_{max} = the time after injection when C_{max} occurs

[3] C_{max} = maximum plasma concentration

[4] $T_{½}$ = terminal phase biological half life

Lung concentrations were consistently higher than those observed in plasma, but the clinical relevance of these concentrations is undetermined. Lung tissue had a longer

terminal elimination half-life (approximately 140 hours) compared to plasma, indicating that the very slow depletion from the peripheral tissues is a rate-controlling step in the elimination of tulathromycin from the body.

2. The Bioavailability of Tulathromycin after Intramuscular Administration in Pigs. Study Number 1520N-03-00-188.

In this study, the bioavailability of tulathromycin (as the commercial prototype) was determined in pigs following IM or IV injection of 2.5 mg/kg BW. A total of 20 Landrace/Large White crossbred pigs, two to three months old, weighing 21 to 30 kg were used in this study. Pigs were divided into four treatment groups - two groups receiving an IM dose and sacrificed at 168 (T01) or 360 (T02) hours post-injection, and two receiving an IV dose and sacrificed at 168 (T03) or 360 (T04) hours post-injection. The IM dose was administered into the neck, and the IV dose was administered into an ear vein. Tulathromycin concentrations in plasma and lung samples were quantified using a validated LC-MS/MS procedure.

Administration of tulathromycin did not result in any change in the health status of any of the pigs. There were no gender differences associated with the pharmacokinetics of tulathromycin. Following IV injection, plasma tulathromycin concentrations exhibited a rapid initial decline (distribution phase). Despite a rapid rate of systemic clearance ($CL_{systemic}$), the very large volume of distribution (Vss) resulted in a prolonged terminal elimination half-life. The IV pharmacokinetic parameter values are summarized in Table 2.5.

Table 2.5. Pharmacokinetic parameters based upon intravenous data.

	Group Mean Values[1]			
	T01 (IM)	**T02 (IM)**	**T03 (IV)**	**T04 (IV)**
$AUC_{0\text{-last}}$[2] ng*hr/mL	10900	15200	11000	13667
$AUC_{0\text{-inf}}$[3] ng*hr/mL	11500	15600	13000	13983
$T_{1/2}$[4] (hrs)	40	76	69	69
$CL_{systemic}$ (mL/kg*hr)	--	--	194	182
MRT[5] (hrs)	--	--	85	69
Vss (L/kg)	--	--	16	14
Lung (ng/g)	1380	778	1440	797

[1]The mean values including both males and females.

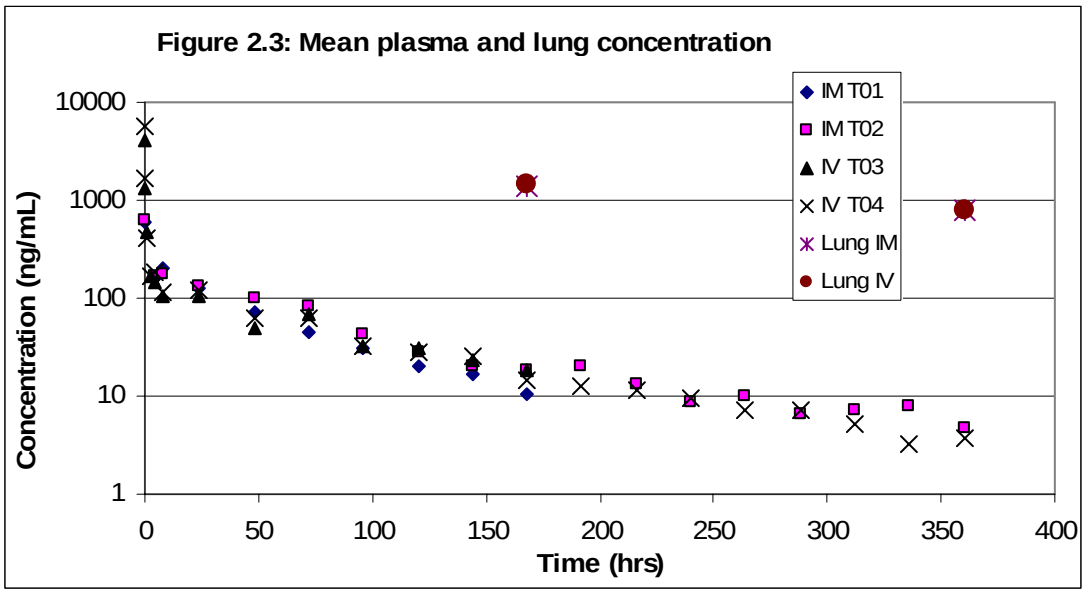
[2]$AUC_{0\text{-last}}$ = the area under the plasma concentration vs. time curve from time of injection to the last sample at or above the LOQ of the analytical method

[3] $AUC_{0\text{-inf}}$ = the area under the plasma concentration vs. time curve from time of injection to time infinity. $AUC_{0\text{-inf}}$ is estimated as $AUC_{0\text{-last}} + C_{last}/\lambda_z$ where C_{last} is the last concentration $\geq$ LOQ and λ_z is the terminal elimination rate constant.

[4]$T_{½}$ = terminal phase biological half life

[5]MRT = mean residence time

Pulmonary drug concentrations were markedly higher than plasma concentrations. However, the clinical relevance of these concentrations is undetermined. The terminal elimination half-life was effectively the same after IM and IV administration, indicating that the duration of exposure is a function of a recycling phenomenon (observed in all three pharmacokinetic studies in swine described in this FOI Summary) and a slow depletion from the peripheral tissues. This slow depletion was not attributable to an extended duration of absorption (Figure 2.3). The relative bioavailability of an IM dose, based upon the ratio of IM/IV values for $AUC_{0\text{-inf}}$, is 1.00 if we compare values across all four treatments or 1.11 if we compare T02 to T04. Since this is an intersubject comparison, these data indicate that the IM dose is effectively 100% bioavailable.



3. Plasma and Lung Pharmacokinetics of Tulathromycin Intramuscularly Administered to Swine. Study Number 1520E-60-00-191.

This study was conducted to determine whether or not plasma and lung tulathromycin concentrations increase in a manner that is proportional to the administered dose. The study was conducted in pigs approximately 8 to 10 weeks of age that weighed between 15.6 to 19.85 kg prior to dosing. Twenty crossbred pigs (10 castrated males and 10 females) were allocated to one of four treatment groups: a non-medicated group (two pigs), or tulathromycin administered by IM injection at a targeted dose of either 1.25, 2.5, or 5.0 mg/kg BW (six pigs per dose group). Blood samples were collected at 0.5, 1, 2, 4, 8, 24, 48, 72, 120, and 168 hours after dosing. Lung samples were collected after the 168-hour blood sample. Tulathromycin concentrations in plasma and lung samples were quantified using a validated LC-MS/MS procedure.

Systemic drug exposure increased in proportion to the administered dose for amounts up to 2.5 mg/kg BW. Blood levels associated with the 5.0 mg/kg BW dose group increased in a less-than-dose-proportional manner. While there was no difference in

the terminal elimination half-life across the three tulathromycin treatment groups, there was a less-than-proportional increase in drug exposure as the administered dose increased above 2.5 mg/kg BW. This was particularly evident in comparison of the dose-normalized values of AUC. Similarly, while there was no change in the time to peak concentrations (0.29, 0.33, and 0.25 hours for dosages of 1.25, 2.5, and 5.0 mg/kg BW, respectively), the peak concentration of the 5.0 mg/kg BW dose increased in a less-than-dose-proportional manner.

d. Microbiology

Tulathromycin is a semi-synthetic macrolide antibiotic of the subclass triamilide. Tulathromycin is primarily bacteriostatic, but may be bactericidal against some pathogens. It acts by binding to a bacterial ribosome sub-unit thereby inhibiting protein synthesis. *In vitro* activity of tulathromycin has been demonstrated against commonly isolated bacterial pathogens involved in BRD and SRD.

Cattle:

1. Determination of Minimum Inhibitory Concentrations of CP-472,295(e) Against Bacteria Associated with Bovine Respiratory Disease (BRD). Study Number 1671C-60-00-207.

The MICs of tulathromycin (CP-472,295(e)) were determined against isolates obtained from calves enrolled in the BRD field studies described in Section 2.b. above. In the field studies described in 2.b.1, isolates were obtained from nasopharyngeal swabs taken prior to treatment from all study animals exhibiting clinical signs of BRD, or from lung swabs or lung tissue of saline-treated animals that died. In the other field study (2.b.2), isolates were obtained from nasopharyngeal swabs of non-responders, and from lung swabs or lung tissue of saline-treated animals that developed BRD during the study. Susceptibility testing of bacterial isolates followed the methods of the Clinical Laboratory Standards Institute/National Committee for Clinical Laboratory Standards (CLSI/NCCLS). The MICs of tilmicosin against two quality control organisms, *Staphylococcus aureus* ATCC 29213 and *Histophilus somni* (*Haemophilus somnus*) ATCC 700025, were also determined; MIC values were within the CLSI/NCCLS acceptable range.

Table 2.6. Tulathromycin MIC values from field studies evaluating BRD in the U.S.

Organism	Number of Isolates	MIC_{90}* (µg/mL)	MIC range (µg/mL)
*Mannheimia haemolytica***	642	2.0	0.5 to 64.0
*Pasteurella multocida***	221	1.0	0.25 to 64.0
*Histophilus somni (Haemophilus somnus)***	36	4.0	1.0 to 4.0

* The minimum inhibitory concentration for 90% of the isolates.

** Clinical isolates supported by clinical data and indications for use.

2. Determination of Minimum Inhibitory Concentrations of CP-472,295(e) Against *Mycoplasma* spp. Associated with Bovine Respiratory Disease (BRD). Study Number 1671C-60-00-206.

The MICs of tulathromycin (CP-472,295(e)) were determined against *Mycoplasma* spp. isolates obtained from calves enrolled in the BRD field studies described in Section 2.b. above. The *Mycoplasma* organisms isolated were identified as to genus at regional laboratories before being shipped frozen to a central laboratory for MIC determination. For those pathogens that were viable, the species of each isolate was determined. *M. bovis*, *M. bovirhinis*, *M. bovoculi*, and other *Mycoplasma* spp. were identified. For each pure culture obtained, MIC determination was conducted by broth microdilution. *Mycoplasma bovis* ATCC 25523 was utilized as a reference strain in this study. The results of MIC determination for *M. bovis* are shown in Table 2.7.

Table 2.7. Tulathromycin MIC values against *Mycoplasma bovis* isolates* from field studies evaluating BRD in the U.S.

Organism	Number of Isolates	MIC_{90}** (µg/mL)	MIC range (µg/mL)
Mycoplasma bovis	35	1.0	≤ 0.063 to 2.0

* The correlation between *in vitro* susceptibility data and clinical response has not been confirmed.

** The minimum inhibitory concentration for 90% of the isolates.

Swine:

1. Determination of Minimum Inhibitory Concentrations of CP-472,295(e) Against Bacteria Associated with Swine Respiratory Disease (SRD). Study Number 1671C-60-00-213.

The MICs of tulathromycin were determined against isolates obtained from swine enrolled in the SRD field studies described in Section 2.b. above. Isolates were obtained from lung tissue and lung swab samples from saline-treated pigs. Susceptibility testing of bacterial isolates followed CLSI/NCCLS methods. The MICs of ceftiofur against quality control organisms (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Actinobacillus pleuropneumoniae* ATCC 27090) were also determined; MIC values were within the CLSI/NCCLS acceptable range.

Table 2.8. Tulathromycin MIC values from field studies evaluating SRD in the U.S. and Canada.

Organism	Number of Isolates	MIC_{90}* (µg /mL)	MIC range (µg/mL)
Actinobacillus pleuropneumoniae	135	32.0	16.0 to 32.0
Haemophilus parasuis	31	2.0	0.25 to >64.0
Pasteurella multocida	55	2.0	0.5 to >64.0
Bordetella bronchiseptica	42	8.0	2.0 to 8.0

* The minimum inhibitory concentration for 90% of the isolates.

3. TARGET ANIMAL SAFETY:

Cattle:

a. Acute Tolerance of Tulathromycin 10% Injectable Solution in Ruminant Cattle. Study Number 1431N-60-99-303. March 2001.

1. Type of Study: Target animal safety (tolerance) study. The study was performed in accordance with Good Laboratory Practice (GLP) requirements.

2. Study Director: Terry N. TerHune, D.V.M., Ph.D. HMS Veterinary Development, Inc., Tulare, CA

3. Study Design:

    a. Objective: To evaluate the toxic effects of tulathromycin 10% injectable solution when administered as a single SC injection to cattle at 25 mg/kg BW (10X the label dosage).

    b. Animals: Eight healthy crossbred calves (four non-pregnant female and four castrated males), approximately eight months old, weighing between 195 and 255 kg at the beginning of the study.

    c. Experimental Design: Four calves (two per gender) each were assigned to the tulathromycin and negative control groups. Test and control articles were administered on Day 0. All calves were euthanized and necropsied on Day 7.

    d. Test Article Administration: Tulathromycin injectable solution (100 mg/mL) was injected subcutaneously at a dosage of 25 mg/kg BW in the lateral neck on Day 0. Saline at an equivalent volume to the tulathromycin dose was used as the negative control article. The maximum injection volume was 10.0 mL per injection site.

    e. Measurements and Observations: Blood samples for hematology and serum chemistry were collected on Days -7, -1, 2, and 7. Individual feed intake was measured daily from Days -7 to 7. Clinical observations were made twice daily throughout the study starting from Day -7. On Day 0, clinical observations were made 0.25, 0.5, 1, 2, 4, 8, and 12 hours following treatment. Gross pathology and histopathology were evaluated at necropsy.

    f. Statistical Analysis: Clinical observations, average daily feed intake, hematology and serum chemistry results, and gross pathology and histopathology findings were summarized descriptively.

4. Results:

    a. Clinical Observations: Clinical signs of toxicity were injection site swelling, transient head-shaking, pawing at the ground and jumping immediately post-dosing, and decreased feed intake. One calf had a transient urine color change four hours post-dosing; at eight hours post-dosing, urine color appeared normal.

b. Mortality: All calves survived to scheduled euthanasia.

c. Hematology and Serum Chemistry: Variations from the normal reference range were noted for some parameters. Variations were present in both tulathromycin and saline-treated calves and in some cases, were present prior to Day 0. No tulathromycin-related abnormalities were identified.

d. Gross and Histopathologic Observations: Histopathologic findings were observed in the subcutaneous injection sites of all four tulathromycin-treated calves and included congestion, edema, hemorrhage, subacute inflammation, and at one site, vascular thrombosis. There were no other tulathromycin-related clinical or pathological findings.

5. Conclusions: Subcutaneous administration of tulathromycin administered to calves once at 10X the label dosage can induce injection site swellings and inflammation, transient signs of discomfort following injection, and a transient decrease in average daily feed intake.

b. Margin of Safety of Tulathromycin 10% Injectable Solution in Ruminant Cattle. Study Number 1432N-60-00-304. March 2001.

1. Type of Study: Target animal safety (toxicity) study. The study was performed in accordance with GLP requirements.

2. Study Director: Terry N. TerHune, D.V.M., Ph.D. HMS Veterinary Development, Inc., Tulare, CA

3. Study Design:

a. Objective: To assess the safety of tulathromycin 10% injectable solution when administered to cattle by SC injection of 2.5, 7.5, and 12.5 mg/kg BW (1X, 3X, and 5X the label dosage) three times at seven day intervals (3X the label duration).

b. Animals: Twenty-four healthy crossbred calves (twelve non-pregnant females and twelve castrated males), approximately seven months old, weighing between 194 and 274 kg at the beginning of the study.

c. Experimental Design: Calves were randomly allocated to one of four treatment groups, as shown in Table 3.1. Test and control articles were administered on Days 0, 7, and 14. All calves were euthanized and necropsied on Day 21 or 22.

Table 3.1. Treatment assignments, study number 1432N-60-00-304.

Group	Dosage	Animals
T01	Saline, 0.125 mL/kg BW	6 (3 male, 3 female)
T02 (1X)	Tulathromycin, 2.5 mg/kg BW	6 (3 male, 3 female)
T03 (3X)	Tulathromycin, 7.5 mg/kg BW	6 (3 male, 3 female)
T04 (5X)	Tulathromycin, 12.5 mg/kg BW	6 (3 male, 3 female)

d. Test Article Administration: Tulathromycin injectable solution (100 mg/mL) was injected subcutaneously at a dosage of 2.5, 7.5, or 12.5 mg/kg BW once every seven days for a total of three dosages. Injections were administered in the left lateral neck on Day 0, right lateral neck on Day 7, and left lateral shoulder on Day 14. Saline at an equivalent volume to the 5X dose (0.125 mL/kg BW) was used as the negative control article. A maximum of 10 mL was injected at each site.

e. Measurements and Observations: Blood samples for hematology and serum chemistry were collected on Days -7, -1, 2, 7, 14, and 21. Individual feed intake was measured daily from Days -7 to 21. On test article administration days, clinical observations were made approximately 0.25, 0.5, 1, 2, 4, 8, and 12 hours after injection. On all other days of the study clinical observations were made twice daily. Gross pathology and histopathology were evaluated at necropsy.

f. Statistical Analysis: Hematology and serum chemistry variables were analyzed using a repeated measures analysis of covariance with the main effects treatment and day and the interaction treatment by day included in the model. All three of these effects were treated as fixed. Day was the repeated effect; compound symmetry was selected as the covariance structure. Each effect was tested at the 10% significance level. If for a particular variable treatment by day was significant, the pairwise difference between the control group mean and each treatment group mean was tested for every day at the 10% significance level. If treatment by day was not significant but treatment was significant, the pairwise difference between the control group mean and each treatment group mean, with the means taken over days as well as animals, was tested at the 10% significance level.

   Daily feed intake was analyzed using an analysis of variance with the main effects treatment and sex and the interaction treatment by sex included in the model. All three of these effects were treated as fixed. Treatment and treatment by sex were tested at the 10% and 5% significance levels, respectively.

4. Results:

   a. Clinical Observations: Injection site swelling was observed in five of the six calves in the 1X group, and all calves in the 3X and 5X groups. Transient head shaking (up to four hours) was observed in one of six calves in the 1X group, four of six calves in the 3X group, and five of six calves in the 5X group. Average daily feed intake was decreased in the 3X and 5X groups.

   b. Mortality: All calves survived to scheduled euthanasia.

   c. Hematology and Serum Chemistry: Variations from the normal reference range were noted for some parameters, and some statistically significant differences were found. However, no trends or patterns were found, and none of the differences were considered clinically relevant. No tulathromycin-related abnormalities were identified.

d. Gross and Histopathologic Observations: Tulathromycin-related histopathologic findings were observed in the subcutaneous injection sites and included: congestion, edema, fibrosis/fibroplasia, hemorrhage, granulomatous inflammation, subacute inflammation, and in one site, vascular thrombosis.

5. Conclusions: Tulathromycin injectable solution is safe and has an adequate margin of safety in cattle when injected subcutaneously as a single dosage of 2.5 mg/kg BW.

c. Injection Site Tolerance of Tulathromycin 10% Injectable Solution Administered Subcutaneously in Ruminant Cattle. Study Number 1433N-60-99-32. March 2001.

1. Type of Study: Injection site irritation study. The study was performed in accordance with GLP requirements.

2. Study Director: Terry N. TerHune, D.V.M., Ph.D. HMS Veterinary Development, Inc., Tulare, CA

3. Study Design:

a. Objective: To evaluate the injection site tolerance of tulathromycin injectable solution administered to cattle as a single SC injection of 10 mL.

b. Animals: Twenty four healthy crossbred castrated male calves weighing 220 to 250 kg at the beginning of the study.

c. Experimental Design: Calves were randomly allocated to one of three treatment groups, as shown in Table 3.2. Test and control articles were administered on Day 0, 7, or 21, according to treatment group. All calves were euthanized and necropsied on Day 35.

Table 3.2. Treatment assignments, study number 1433N-60-99-32.

Group	Treatment Day	Days Before Necropsy	Animals
T01	0	35	8
T02	7	28	8
T03	21	14	8

d. Test Article Administration: Tulathromycin injectable solution (100 mg/mL) was injected subcutaneously in the neck as a single injection of 10 mL. Saline at an equivalent volume was used as the negative control article, and was injected subcutaneously in the opposite side of the neck of each calf.

e. Measurements and Observations: Calves were observed prior to and two hours following treatment administration, then once daily for the remainder of the study. Injection site reactions were assessed daily by visual inspection and weekly by palpation. Gross and histopathologic examination was limited to the site of injection.

f. Statistical Analysis: None.

4. Results:

    a. Clinical Observations: No abnormal clinical observations were noted during the study. Injection site reactions and visible swelling on the tulathromycin-treated side of the neck were present in all treatment groups. No calves dosed on Day 0 had palpable injection site reactions 35 days post-dosing. Two of eight calves dosed on Day 7 had palpable injection site reactions on Day 35 (28 days post-injection). Five of eight calves dosed on Day 21 had palpable injection site reactions on Day 35 (14 days post-injection).

    b. Mortality: All animals survived to scheduled euthanasia.

    c. Gross and Histopathologic Observations: At necropsy, gross lesions were present at the tulathromycin injection site in one of eight calves 35 days post-injection, two of eight calves 28 days post-injection, and seven of eight calves 14 days post-injection. Tulathromycin-related histopathologic findings were observed in the subcutaneous injection sites and included: edema, fibrosis/fibroplasia, hemorrhage, subacute inflammation, the presence of *Sarcocystis* spp. parasites, and a vascular thrombosis. The severity and frequency of lesions decreased as the post-injection interval increased.

5. Conclusions: Tulathromycin injectable solution, when administered to cattle as a SC injection at the maximum recommended dose volume of 10 mL per injection site, can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter.

d. Adverse Effects Observed in Non-Pivotal Studies

In one field study, two calves treated with tulathromycin at 2.5 mg/kg BW exhibited transient hypersalivation. One of these calves also exhibited transient dyspnea, which may have been related to pneumonia.

Swine:

a. Acute Tolerance of Tulathromycin 10% Injectable Solution in Swine. Study Number 1422N-60-00-182. July to August 2000.

1. Type of Study: Target animal safety (tolerance) study. The study was performed in accordance with Good Laboratory Practice (GLP) requirements.

2. Study Director: Daniel C. Ronning, M.S., Colorado Animal Research Enterprises, Inc., Fort Collins, CO

3. Study Design:

    a. Objective: To evaluate the toxic effects of tulathromycin injectable solution in swine when administered as single IM injection of 25 mg/kg BW (10X the label dosage).

b. Animals: Eight healthy crossbred pigs (four females and four castrated males) weighing 13 to 16 kg at the start of the study.

c. Experimental Design: Pigs were randomly allocated to one of two treatment groups, as shown in Table 3.3. Test and control articles were administered on Day 0. All pigs were euthanized and necropsied on Day 7.

Table 3.3. Treatment assignments, study number 1422N-60-00-182.

Group	Dosage	Animals
T01	Saline, 0.25 mL/kg BW	4 (2 male, 2 female)
T02 (10X)	Tulathromycin, 25 mg/kg BW	4 (2 male, 2 female)

d. Test Article Administration: Tulathromycin injectable solution (100 mg/mL) was injected intramuscularly in the lateral neck on Day 0. Saline at an equivalent volume to the tulathromycin dose was used as the negative control article. The maximum injection volume was 2.5 mL per injection site.

e. Measurements and Observations: Blood samples for hematology and serum chemistry were collected on Days -7, -1, 2, and 7. Individual feed intake was measured daily from Days -7 to 7. Clinical observations were made twice daily throughout the study starting from Day -7. On Day 0, clinical observations were made 0.25, 0.5, 1, 2, 4, 8, and 12 hours following treatment. Gross pathology and histopathology were evaluated at necropsy.

f. Statistical Analysis: Clinical observations, feed intake, hematology and serum chemistry results, and gross pathology and histopathology findings were summarized descriptively.

4. Results:

a. Clinical Observations: Vocalization and restlessness were observed for two hours after treatment in three of the four tulathromycin-treated pigs. The tulathromycin-treated group experienced a transient decrease in feed consumption post-injection.

b. Mortality: All pigs survived to scheduled euthanasia.

c. Hematology and Serum Chemistry: There were no inter-group differences for any of the hematology parameters.

Group means for AST in both the tulathromycin and saline control group were above the reference range of 0 to 32 U/L at all time points, but there was a difference in trends between groups. AST levels for the saline-treated animals decreased on Days 2 and 7. AST levels in the tulathromycin-treated animals showed a marked increase on Day 2 (104.5 U/L) that returned to below baseline level (47 U/L) by Day 7. The elevated AST levels observed in the tulathromycin-treated pigs may be attributed to muscle tissue damage associated with IM injection of the test article.

d. Gross and Histopathologic Observations: Red, tan, and yellow discoloration was observed at all tulathromycin-treated injection sites. Tulathromycin-related histopathologic findings were observed only at the injection sites, and included edema, hemorrhage, fibrosis/fibroplasia, mineralization, and Zenker's degeneration.

5. Conclusions: Tulathromycin injectable solution, when administered to swine intramuscularly as a single dosage of 25 mg/kg BW, can induce a transient elevation of serum AST, transient signs of discomfort following injection, and macroscopic and microscopic injection site changes.

b. Margin of Safety of Tulathromycin 10% Injectable Solution in Swine. Study Number 1422N-60-99-173. November to December 2000.

1. Type of Study: Target animal safety (toxicity) study. The study was performed in accordance with GLP requirements.

2. Study Director: Diane J. Fagerberg, Ph.D., Colorado Animal Research Enterprises, Inc., Fort Collins, CO

3. Study Design:

a. Objective: To evaluate the safety of tulathromycin 10% injectable solution in swine when administered as an IM injection at 2.5, 7.5, or 12.5 mg/kg BW (1X, 3X, or 5X the label dosage) given once every seven days for three doses (3X the label duration).

b. Animals: Twenty-four healthy crossbred pigs (twelve females and twelve castrated males) weighing 7 to 10 kg at the beginning of the study.

c. Experimental Design: Pigs were randomly allocated to one of four treatment groups, as shown in Table 3.4. Test and control articles were administered on Days 0, 7, and 14. All pigs were euthanized and necropsied on Day 21.

Table 3.4. Treatment assignments, study number 1422N-60-99-173.

Group	Dosage	Animals
T01	Saline, 0.125 mL/kg BW	6 (3 male, 3 female)
T02 (1X)	Tulathromycin, 2.5 mg/kg BW	6 (3 male, 3 female)
T03 (3X)	Tulathromycin, 7.5 mg/kg BW	6 (3 male, 3 female)
T04 (5X)	Tulathromycin, 12.5 mg/kg BW	6 (3 male, 3 female)

d. Test Article Administration: Tulathromycin injectable solution (100 mg/mL) was injected intramuscularly at a dosage of 2.5, 7.5, or 12.5 mg/kg BW once every seven days for three injections. Saline at an equivalent volume to the 5X dose (0.125 mL/kg BW) was used as the negative control article.

Injections were administered intramuscularly in the left hind leg on Day 0, right neck on Day 7, and left neck on Day 14. The maximum injection volume was 2.5 mL per injection site.

e. Measurements and Observations: Blood samples for hematology and serum chemistry were collected on Days -7, -1, 2, 6, 13, and 20. Individual feed intake was measured daily from Days -7 to 20. On test article administration days, clinical observations were made approximately 0.25, 0.5, 1, 2, 4, 8, and 12 hours after injection. On all other days of the study clinical observations were made twice daily. All pigs were euthanized and necropsied on Day 21. Gross pathology and histopathology were evaluated at necropsy.

f. Statistical Analysis: Hematology and serum chemistry variables were analyzed using analysis of variance with repeated measures in SAS Proc Mixed with the following fixed effects: treatment, sex, day, and two- and three-way interactions. Day was the repeated effect; the autoregressive covariance model of order 1 was used. The individual pig was the experimental unit.

   The organ weight/body weight ratio was analyzed for the kidneys, liver, and heart. A one-way analysis of variance was used with the fixed effect treatment. If treatment was significant at the 10% significance level for an organ, the pairwise difference between the control group mean and each treatment group mean was tested at the 10% significance level.

4. Results:

   a. Clinical Observations: Tulathromycin-related clinical signs were lameness, excessive vocalization, and discomfort. Lameness was observed after left hind leg injection in four of six pigs in the 3X group and five of six pigs in the 5X group. Two pigs in the 5X group remained lame on Day 21. Excessive vocalization occurred after neck injections on Day 7 in two pigs in the 5X group and on Day 14 in one pig in the 5X group. Other clinical signs of discomfort were observed after injection in five tulathromycin-treated pigs (two pigs in the 3X group and three pigs in the 5X group) and one saline-treated pig. Signs of discomfort generally resolved by two hours after injection, but persisted through 12 hours post-injection in one 3X pig. Tremors were seen in one pig in the 3X group eight hours after treatment.

   b. Mortality: All pigs survived to scheduled euthanasia.

   c. Hematology and Serum Chemistry: AST, SDH, total protein, and globulin were observed to exceed the reference range in pigs from all groups. Differences between groups were not statistically significant and no treatment-related trend was observed.

   d. Gross and Histopathologic Observations: Red, tan, yellow, or pale discoloration and occasional edema were observed at the tulathromycin-treated injection sites. Tulathromycin-related histopathologic findings were observed only at the injection sites, and included fibrosis/fibroplasia, hemorrhage, Zenker's degeneration, inflammation, and mineralization.

5. Conclusions: Tulathromycin injectable solution is safe and has an adequate margin of safety in swine when injected intramuscularly as a single dosage of 2.5 mg/kg BW.

c. Injection Site Tolerance of Tulathromycin 10% Injectable Solution Administered Intramuscularly in Swine. Study Number 1423N-60-00-181. August 2001.

1. Type of Study: Injection site irritation study. The study was performed in accordance with GLP requirements.

2. Study Director: Diane J. Fagerberg, Ph.D., Colorado Animal Research Enterprises, Inc., Fort Collins, CO

3. Study Design:

   a. Objective: To evaluate the injection site tolerance of tulathromycin injectable solution administered to swine as a single IM injection of 2.5 mL.

   b. Animals: Twenty four healthy castrated male pigs weighing 59 to 71 kg at the beginning of the study.

   c. Experimental Design: Pigs were randomly allocated to one of four treatment groups, as shown in Table 3.5. Test and control articles were administered on Day 0, 7, 14, or 21, according to treatment group. All pigs were euthanized and necropsied on Day 35.

Table 3.5. Treatment assignments, study number 1423N-60-00-181.

Group	Treatment Day	Days Before Necropsy	Animals
T01	0	35	6
T02	7	28	6
T03	14	21	6
T04	21	14	6

   d. Test Article Administration: Tulathromycin injectable solution (100 mg/mL) was injected intramuscularly in the neck as a single injection of 2.5 mL. Saline at an equivalent volume was used as the negative control article, and was injected intramuscularly in the opposite side of the neck of each pig.

   e. Measurements and Observations: Pigs were observed prior to and one to three hours following treatment administration, then once daily for the remainder of the study. Injection site reactions were assessed by visual inspection and palpation for erythema, heat, pain, and swelling on Days -15, -1, 0, 7, 8, 14, 15, 21, 22, and 35. Evaluation of injection site swelling was scored on a scale of 0 to 3, where 0 was the absence of swelling and 3 was severe swelling ($\geq$ 15 cm diameter). Pigs were weighed on Days -15, -1, and 35. Gross and histopathologic examination was limited to the site of injection.

   f. Statistical Analysis: None.

4. Results:

    a. Clinical Observations: No abnormal clinical observations were noted during the study. All injection sites were scored as "0" for swelling at all observations prior to necropsy. Erythema, heat, and pain were scored as "absent" for all pigs at all observations prior to necropsy.

    b. Mortality: All pigs survived to scheduled euthanasia.

    c. Gross and Histopathologic Observations: The pathology results for one pig (T04) were not evaluated because of data collection uncertainty. Gross and microscopic pathology at the tulathromycin-treated side of the neck was present in one of six animals 35 days post-injection (T01), four of six animals 28 days post-injection (T02), four of six animals 21 days post-injection (T03), and five of five animals evaluated 14 days post-injection (T04). Combinations of red, tan, and yellow discoloration of the muscle tissue were observed grossly in tulathromycin-treated injection sites. Fresh-appearing red discoloration was observed grossly in the saline-treated injection site of one pig (T04), but was not evaluated histologically. Average lesion size at necropsy ranged from 4.5 cm^3 (T01) to 56.5 cm^3 (T04). Tulathromycin-related histopathologic findings at injection sites included fibrosis/fibroplasia, necrosis, hemorrhage, mineralization, a foreign body tissue response, subacute inflammation, and/or Zenker's degeneration of surrounding muscle fibers. The severity and frequency of lesions decreased as the post-injection interval increased.

5. Conclusions: Tulathromycin injectable solution, when administered to swine as an IM injection at the maximum recommended dose volume of 2.5 mL per injection site, can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter.

d. Adverse Effects Observed in Non-Pivotal Studies

Three additional clinical field studies were conducted between October and November 2000, following a common protocol, at three locations in Nebraska and Iowa. A total of 278 pigs, weighing 15.6 to 82.7 kg, were treated with tulathromycin injected intramuscularly at a dosage of 2.5 mg/kg BW. These studies were not used to support the determination of effectiveness in swine. However, in one study, one of the tulathromycin-treated pigs exhibited mild salivation that resolved in less than four hours.

4. HUMAN FOOD SAFETY:

All pivotal testing was conducted in full compliance with the Good Laboratory Practice (GLP) Regulations (21 CFR 58). In all studies, the reported dose levels or concentrations tested are expressed in terms of the free base form of tulathromycin. Tulathromycin is an equilibrating mixture of two isomers: CP-472,295 (15-membered macrocyclic ring) and CP-547,272 (translactonized 13-membered macrocyclic ring). Although CP-472,295 is manufactured as a solid material comprising primarily one isomer, it has been demonstrated that, under a broad range of conditions, both isomers are present in solutions prepared from single isomer solids and, given sufficient time, those isomers will form a dynamic equilibrium at a ratio of 9:1 (CP-472,295:CP-547,272). This equilibration occurs rapidly under physiological conditions, thus it is the equilibrated mixture, CP-472,295(e), which is considered to be the active ingredient of the drug product.

a. Toxicology

1. Studies

a. Three Month Oral Toxicity Study in Sprague-Dawley Rats. Report Number 99-1507-15. February 2000 to March 2001.

1) Investigator and Laboratory: Cynthia Davenport, Ph.D., Drug Safety Evaluation Department, Pfizer Global Research & Development, Pfizer, Inc., Groton, CT

2) Identity of Substance and Dosage Form: Tulathromycin; citrate buffered deionized water as an oral solution

3) Species and Strain of Test Animals: Crl:CD®(SD)BR VAF/Plus® Sprague-Dawley rats

4) Number of Animals of Each Sex in Each Group: 20/sex/dose

5) Levels and Duration of Dosing: 0, 5, 15, and 100 mg tulathromycin/kg BW/day for three consecutive months

6) Route of Drug Administration: Oral gavage

7) Parameters Studied and Discussion of Results:

Clinical Signs and Survival: All rats survived the dosing period and no drug-related clinical signs were observed.

Body Weights and Food Intake: No treatment-related effects on body weight or food consumption were observed.

Ophthalmology: No treatment-related ocular changes were observed.

Hematology: There were no treatment-related effects on hematology parameters.

Serum Chemistry: Minimal elevations (≤ 2.0X concurrent control means) in serum liver enzymes (ALT and SDH in females; AST in both sexes) were noted at 100 mg/kg BW/day, and very slight decreases in total protein (females, Day 57), albumin and globulin were observed in 100 mg/kg BW/day males only. These changes were without histologic correlate.

Urinalysis: There were no treatment-related changes in urine parameters in any dose group.

Lung Drug Concentrations: Mean concentrations of drug in lung tissue increased with increasing dose, with the greatest increases occurring between Days 1 and 30.

Microsomal Enzyme Analysis: No treatment-related effects on the hepatic drug metabolizing systems were noted.

Organ Weights: There were no treatment-related effects on organ weights.

Necropsy Findings: There were no treatment-related findings at necropsy.

Microscopic Findings: No treatment-related effects on the hepatic drug metabolizing systems were noted.

8) Significant Toxicities Observed: None

9) No-Observed-Adverse-Effect Level: 15 mg tulathromycin/kg BW

10) Conclusion(s): Based upon statistically significant dose- and time-related mean elevations in hepatic enzymes (AST, AST, SDH) at 100 mg/kg BW, 15 mg/kg BW was identified as a "no-observed-adverse-effect level" (NOEL) in this three month oral toxicity in rats treated with tulathromycin.

b. Three Month Oral Toxicity Study in Beagle Dogs. Report Number 99-1507-14. July 1999 to March 2001.

1) Investigator and Laboratory: Cynthia Davenport, Ph.D., Drug Safety Evaluation Department, Pfizer Global Research & Development, Pfizer, Inc., Groton, CT.

2) Identity of Substance and Dosage Form: Tulathromycin; citrate buffered deionized water as an oral solution

3) Species and Strain of Test Animals: Beagle dogs

4) Number of Animals of Each Sex in Each Group: 4/sex/group

5) Levels and Duration of Dosing: 0, 5.7, 17.0, and 56.7 mg tulathromycin/kg BW/day for three consecutive months

6) Route of Drug Administration: Oral gavage

7) Parameters Studied and Discussion of Results:

Clinical Signs and Mortality: All animals survived the 3-month dosing period except for one male in the 56.7 mg/kg BW/day group, which died from dosing trauma. The incidence of loose stools and emesis was greater in 56.7 mg/kg BW/day animals.

Body Weight and Food Intake: Mean body weights and food intake were not affected by treatment.

Physical Examination/Vital Signs: There were no drug-related effects on heart rate, respiration rate, or body temperature. Physical examinations were within normal limits.

Electrocardiograms/Blood Pressure: No drug-related electrocardiographic alterations or consistent alteration in blood pressure measurements were noted.

Ophthalmology: No drug-related ophthalmologic alterations were noted on Day 45. Multiple small, focal, unilateral, silver foci were noted ophthalmoscopically near the tapetal junction of the retina in two 17.0 mg/kg BW/day dogs (one animal on Day 70 and both on Day 88). The foci were reduced in size on the second observation (Day 88) in one dog and had no histological correlate in either animal. These findings were not dose-related since they were not observed in the high dose animals.

Hematology: No drug-related changes in hematological parameters were noted.

Serum Chemistry: Starting at around one month, serum transaminase (ALT, AST) concentrations in 56.7 mg/kg BW/day animals increased with duration of exposure (2.6-3.6X control, Day 85), and were without histological correlate.

Urinalysis: There were no drug-related changes in urinalysis parameters.

Plasma and Lung Drug Concentrations: All dogs were systemically exposed to drug, and concentrations in plasma and lung generally increased with increasing dose. No gender difference in exposure was noted.

Microsomal Enzyme Analysis: Based upon microsomal enzyme analysis, tulathromycin is not a hepatic microsomal enzyme inducer in the dogs.

Organ Weights: Organ weights were unremarkable.

Necropsy Findings: There were no drug-related necropsy findings. The single 56.7 mg/kg BW male that was found dead had heavy, mottled, dark red lungs and froth in the trachea, consistent with dosing trauma.

Microscopic Findings: There were no drug-related histopathological abnormalities observed. The single male in the 56.7 mg/kg BW/day group, which died had marked, focal hemorrhage in the lungs consistent with dosing trauma.

8) Significant Toxicities Observed: Unilateral, focal silver foci were seen in the retinas of two 17 mg/kg BW/day dogs on Days 70 and/or 88 and elevated AST was found in one 17 mg/kg BW/day female on Day 85. Serum transaminase concentrations were increased at the high dose.

9) No-Observed-Adverse-Effect Level: 5.7 mg tulathromycin/kg BW

10) Conclusion(s): The NOEL identified in this three month oral toxicity study in dogs was conservatively set at 5 mg tulathromycin/kg BW/day, based upon ophthalmoscopic observations of unilateral, focal silver foci in the retinas of two 17 mg kg BW/day dogs on Days 70 and/or 88 and upon elevated AST in one 17 mg/kg BW/day female on Day 85.

c. Effects of Tulathromycin on Embryo/Fetal Development in Rats. Report Number 00-1507-30 Pfizer; WIL-344021. August 2000 to May 2001.

1) Investigator and Laboratory: Mark D. Nemec, B.S., D.A.B.T., WIL Research Laboratories, Inc., Ashland, OH

2) Identity of Substance and Dosage Form: Tulathromycin; citrate buffered deionized water as an oral solution spiked with approximately 1% CP-60,300

3) Species and Strain of Test Animals: Crl:CD®(SD)IGS BR rats

4) Number of Animals of Each Sex in Each Group:

Maternal and developmental toxicity: 22 bred females
Toxicokinetic phase: 6 bred females

5) Levels and Duration of Dosing:

Maternal and developmental toxicity: 0, 15, 100, and 200 mg/kg BW as a single daily dose from gestation days 6 – 17.
Toxicokinetic phase: 0, 15, 100, and 200 mg/kg BW as a single daily dose from gestation days 6 – 20.

6) Route of Drug Administration: Oral gavage

7) Parameters Studied and Discussion of Results:

Clinical Signs and Mortality: All maternal animals survived to the scheduled necropsy on gestation day 20. No treatment-related clinical signs were noted in the 15, 100, or 200 mg/kg BW/day groups.

Body Weights, Body Weight Gain, and Food Consumption: Food consumption and net body weight gain were significantly reduced in the 100 and 200 mg/kg BW/day groups during the gestation period. Mean fetal body weights (males, females and combined sexes) were significantly reduced in the 15, 100, and 200 mg/kg BW/day groups.

Necropsy Findings: No test article-related findings were observed.

Fetal Development: The mean litter proportion (% per litter) of viable fetuses was significantly reduced in the 100 and 200 mg/kg BW/day groups. The mean litter proportions (% per litter) of early resorptions, total resorptions, and post-implantation loss were significantly increased in the 100 and 200 mg/kg BW/day groups. Fetal sex ratios for all doses were similar to control group ratios. Fetal malformations were observed in 1(1), 2(1), and 1(1) fetuses (litters) from the 15, 100, and 200 mg/kg BW/day dose groups, respectively, and were considered to be spontaneous in origin; no fetal malformations were observed in the control group. No test article-related fetal developmental variations were observed in this study. No treatment-related visceral effects or skeletal malformations were observed at any dose level.

Lung Drug Concentrations: All dams receiving doses of tulathromycin were exposed to circulating drug. Systemic exposure (plasma concentrations), as assessed by C_{max} and $AUC_{(0-24)}$, increased with increasing dose. Mean concentrations of tulathromycin in maternal rat lung samples and in the fetal rat samples also increased with increasing dose.

8) No-Observed-Adverse-Effect Level: 15 mg tulathromycin/kg BW/day for maternal toxicity and fetal toxicity

d. Effects of Tulathromycin on Embryo/Fetal Development in Rabbits. Report Number 99-1507-17 Pfizer; WIL-344018. January 2000 to April 2001.

1) Investigator and Laboratory: Donald G. Stump, Ph.D., D.A.B.T., WIL Research Laboratories, Inc., Ashland, OH

2) Identity of Substance and Dosage Form: Tulathromycin; citrate buffered deionized water as an oral solution

3) Species and Strain of Test Animals: New Zealand White rabbits

4) Number of Animals of Each Sex in Each Group:

Maternal and developmental toxicity: 22 bred females
Toxicokinetic phase: 6 bred females

5) Levels and Duration of Dosing:

   Maternal and developmental toxicity: 0, 5, 15, and 50 mg/kg BW day as a single daily dose from gestation days 7 - 20
   Toxicokinetic phase: 0, 5, 15, and 50 mg/kg BW as a single daily dose from gestation days 7 - 21

6) Route of Drug Administered: Oral gavage

7) Parameters Studied and Discussion of Results:

   No treatment-related effects on maternal survival or clinical signs were observed. Mean maternal body weight, body weight gain, net body weight, net body weight gain, gravid uterine weight, and placental weight were unaffected by administration of tulathromycin. At the scheduled necropsy, no test article-related internal changes were observed in the does.

   The number of viable fetuses, early and late resorptions, implantation sites, and corpora lutea were not affected by treatment. No test article-related increases in external, visceral, or skeletal malformations or developmental variations were observed for fetuses in this study.

   Maternal plasma, lung, and fetal concentrations of tulathromycin in pregnant rabbits increased with increasing dose levels. Mean T_{max} was 1, 1, and 1.25 hours post-dosing in the 5, 15, and 50 mg/kg BW/day group, respectively. The mean concentration of tulathromycin was much lower (87.5 to 512 ng/g) in the fetal samples than in the maternal lung samples (5.46 to 124 μg/g).

8) Significant Toxicities Observed: None

9) No-Observed-Effect Level: ≥ 50 mg tulathromycin/kg BW/day

10) Conclusion(s): At the dose levels tested, tulathromycin had no effect on dams and fetuses at any of the dose levels tested.

e. Two-Generation Reproductive Toxicity Study of Tulathromycin in Rats. Report Number 99-1507-16 Pfizer; WIL-344019. October 1999 to June 2001.

1) Investigator and Laboratory: Mark D. Nemec, B.S., D.A.B.T., WIL Research Laboratories, Inc., Ashland, OH

2) Identity of Substance and Dosage Form: Tulathromycin; citrate buffered deionized water as an oral solution

3) Species and Strain of Test Animals: Crl:CD®(SD)IGS BR rats

4) Number of Animals of Each Sex in Each Group: 30/sex/group

5) Levels and Duration of Dosing: 0, 15, 50, and 100 mg/kg BW/day as a single daily dose for at least 70 consecutive days prior to mating and continued throughout mating, gestation, and lactation, until euthanasia for F_0 and F_1 parental animals.

6) Route of Drug Administration: Oral gavage

7) Parameters Studied and Discussion of Results:

Clinical Signs and Mortality: No test article-related mortalities or clinical findings were observed in the F_0 or F_1 generations.

Reproductive Parameters: Pre-coital intervals in F_1 parental animals greater than five days increased slightly with treatment. All mean values were within the WIL Research Laboratories historical range (2.0-6.0 days), except for the value in the 50 mg/kg BW dose group (6.8 days). The number of pairings with no evidence of mating was 2/30, 3/30, 6/30, and 5/30 animals in the 0, 15, 50, and 100 mg/kg BW dose groups, respectively. In the high dose group, 2 of 5 animals with no evidence of mating actually delivered. The mating and fertility indices were similar across all treatment groups for both males and females.

Body Weights and Body Weight Gains: Mean body weight gains in the 100 mg/kg BW/day group F_0 males were reduced between weeks 4-5, 9-10, and 11-12. Similarly, mean body weight gains in the F_1 males in the 100 mg/kg BW/day group were reduced between weeks 27-28 and 36-37. Mean body weight gains in the 50 and 100 mg/kg BW/day group F_1 females were reduced during gestation days 0-4 and 0-20.

Food Consumption and Feed Efficiency: Food consumption in the 100 mg/kg BW/day F_0 males was reduced during weeks 17-18 and 18-19. Feed efficiency in this group was reduced during weeks 4-5, 9-10 (pre-breeding), and weeks 11-12. In the F_1 males, food consumption was slightly reduced in the 100 mg/kg BW/day group for weeks 31-32 through 36-37 of the post-mating period. Feed efficiency in this group was reduced for weeks 24-25 through 36-37.

Hematology and Serum Chemistry: No effects of treatment were noted on hematology parameters. The mean AST level was increased in the 100 mg/kg BW/day group F_0 males at the week 18 evaluation. Reductions in mean urea nitrogen levels were noted in F_0 males in the 50 and 100 mg/kg BW/day groups and in F_0 females in the 100 mg/kg BW/day group at the week 9 and 18 evaluations. At the week 18 evaluation, reductions in mean urea nitrogen levels were also noted in the F_0 males in the 15 mg/kg BW/day group and in F_0 females in the 50 mg/kg BW/day group. Test article-related reductions in total protein were noted in the 50 and 100 mg/kg BW/day F_0 males at weeks 9 and 18 and in the 100 mg/kg BW/day F_0 females at week 18, due predominantly to decreased albumin and/or globulin levels.

Necropsy: At the scheduled necropsies of the F_0 and F_1 parental animals, no treatment-related macroscopic internal findings were observed at any dose level. No microscopic lesions attributed to test article administration were observed in the 100 mg/kg BW/day group F_0 and F_1 males and females. No test article-related macroscopic findings were noted in the F_1 or F_2 pups.

Organ Weights: Mean liver weights (absolute and/or relative to final body weights) were reduced in F_0 males, F_1 males, and F_0 females at all tulathromycin dose levels and in F_1 females in the 50 and 100 mg/kg BW/day groups. Although the reductions in mean liver weights were considered treatment-related, no macroscopic or microscopic findings were noted in either generation to correlate with the weight reductions observed. Also, relative-to-final body weight value for the liver did not demonstrate a dose response despite a seven-fold increase in dose. Mean absolute and relative adrenal gland weights were increased in F_0 males, F_1 males, and F_1 females in the 100 mg/kg BW/day group. Changes in adrenal weights were considered test article-related. No effects of the test article were observed on F_1 and F_2 weanling organ weights.

Histopathology: No microscopic lesions attributed to test article administration were observed in the 100 mg/kg BW/day group F_0 and F_1 males and females.

Offspring Body Weights: Mean F_1 offspring body weight gains were increased in males and females in the 50 and 100 mg/kg BW/day groups for post-natal day (PND) 14-21; mean body weights in these animals were increased on PND 21 (including the 15 mg/kg BW/day F_1 males).

Pup Sex Ratios: Mean F_1 and F_2 pup sex ratios, live litter sizes, and postnatal survival indices were unaffected by test article administration at all dose levels.

Gross Appearance of Fetuses: The mean days on which balanopreputial separation and vaginal patency were observed in the F_1 pups were similar between the control and treated groups. The general physical condition of the F_1 and F_2 pups was similar in the control and treated groups throughout the postnatal period.

Lung and Milk Concentrations: Tulathromycin concentration in lungs from F_0 dams increased with increasing dose. Both F_1 and F_2 rat pups were exposed to the compound; exposure route may have been *in utero* and/or from dams' milk. Mean milk concentrations in F_1 and F_2 neonates increased with increasing dose. Mean lung concentrations for F_1 neonates increased in an approximate manner with increasing dose, while the majority of individual lung concentrations for F_2 neonates were below the limit of quantification for the assay.

8) Significant Toxicities Observed: None

9) No-Observed-Effect Level:

Parental toxicity: 15 mg tulathromycin /kg BW/day
Reproductive and neonatal toxicity: not determined

10) Conclusion(s): A number of changes in parameters (body weights, organ weights and serum chemistry) were indicative of parental toxicity supporting the 15 mg/kg BW/day NOEL.

f. One Year Oral Toxicity Study in Dogs. Report Number 00-1507-29. August 2000 to May 2002.

1) Investigator and Laboratory: Cynthia Davenport, Ph.D., Drug Safety Evaluation Department, Pfizer Global Research & Development, Pfizer, Inc., Groton, CT

2) Identity of Substance and Dosage form: Tulathromycin; citrate buffered deionized water as an oral solution spiked with approximately 1% CP-60,300

3) Species and Strain of Test Animals: Beagle dogs

4) Number of Animals of Each Sex in Each Group: 4/sex/group

5) Levels and Duration of Dosing: 0, 2, 5, and 25 mg/kg BW for 12 consecutive months (372 consecutive days)

6) Route of Drug Administration: Oral gavage

7) Parameters Studied and Discussion of Results:

Clinical Signs: Sporadic salivation was noted in all treatment groups (excluding control) with a slightly greater incidence in the 5 and 25 mg/kg BW groups, most notably for females.

Body Weight, Food Intake, Vital Signs, Electrocardiograms, Heart Rate, Blood Pressure, Physical Examinations, Ophthalmology, Urinalysis, Hematology, and Necropsy: No treatment-related changes were observed.

Serum Chemistry: Slight elevations (1.2-2.9X control mean) in mean serum transaminase values (ALT, AST) occurred in males and/or females at 25 mg/kg BW.

Organ Weights: Testis weights (mean absolute and relative) were increased (1.4X control mean) in the 25 mg/kg BW males, but were without histologic correlate.

Plasma and Lung Drug Concentrations: All compound-treated dogs were systemically exposed to drug, and concentrations in plasma and lung generally increased with increasing dose. No gender difference in exposure was noted.

8) Significant Toxicities Observed: Slight elevations of serum transaminase (ALT, AST) concentrations were found at 25 mg/kg BW. Increased incidence of salivation was observed in the 5 and 25 mg/kg BW/day females.

9) No-Observed-Effect Level: 2 mg tulathromycin /kg BW/day

10) Conclusion(s): Tulathromycin was generally well tolerated by Beagle dogs at dose levels up to 25 mg/kg BW when administered by oral gavage for one year. Plasma and lung drug concentrations were similar for the 3 and 12 month studies and suggest no additional drug accumulation occurred in the additional 9 months of drug exposure.

g. Microbial Reverse Mutation Assays (Ames Tests). Report Number 97-1507-06. May 1998 to May 1999.

1) Study Director and Laboratory: Warren W. Ku, Ph.D., Drug Safety Evaluation Dept., Central Research Division, Pfizer, Inc., Groton, CT

2) Identity of Substance and Dosage Form: Tulathromycin (CP-472,294(e)) in citrate buffer

3) Species and Strain: *Salmonella typhimurium* tester strains TA 1535, TA 1537, TA 98, and TA 100 and *Escherichia coli* strain WP2 uvrA pKM101.

4) Number of Animals of Each Sex in Each Group: Not applicable

5) Levels and Duration of Dosing:

For each *Salmonella typhimurium* strain: The concentration range was from 0.050 to 5.0 μg/plate and 0.15 to 15 μg/plate without and with metabolic activation, respectively.

For the *E. coli* strain: the concentration range was from 0.15 to 15 μg/plate and 0.50 to 50 μg/plate without and with metabolic activation, respectively. All cells were incubated for approximately 48 to 72 hours at 37 °C.

6) Route of Drug Administration: Agar/plate inoculation

7) Parameters Studied: Induction of *in vitro* bacterial mutations from four histadine-auxotrophs *Salmonella typhimurium* strains and one tryptophan-auxotrophic *Escherichia coli* strain in the presence and absence of S9 metabolic activator.

8) Significant Toxicity Observed: In the absence of S9 metabolic activation, dose-related cytotoxicity was observed at 5.0 μg/plate with each of the *Salmonella typhimurium* strains and at 15 μg/plate with *E. coli*. In the presence of S9 metabolic activation, dose-related cytotoxicity was observed at concentrations ≥ 5.0 μg/plate with TA 1535 and TA 100, at concentrations ≥ 15 μg/plate with TA 1537, TA 98, and *E. coli*.

9) Conclusion(s): Tulathromycin does not induce bacterial mutagens either directly or with metabolic activation in *Salmonella typhimurium* or *E. coli* strains when tested up to cytotoxic concentrations with each of the strains.

h. CHO/HGPRT Mammalian Mutation Assay. Report Number 00-1507-31. August 2000 to April 2001.

1) Study Director and Laboratory: Robert J. Mauthe, Ph.D., Drug Safety Evaluation Dept., Central Research Division, Pfizer, Inc., Groton, CT

2) Identity of Substance and Dosage Form: Tulathromycin (CP-472,294(e)) with 1% CP-60,300

3) Species and Strain of Test Animals: Chinese hamster ovary (CHO) cells (HGPRT subclone CHO-K_1-BH_4)

4) Number of Animals of Each Sex in Each Group: Not applicable

5) Levels and Duration of Dosing: In the nonactivation phase, concentrations of 500 to 5000 μg/mL were used; in the S9 activation phase, concentrations of 500 to 6000 μg/mL were used. Five-hour exposure.

6) Route of Drug Administration: Infusion into cell culture

7) Parameters Studied: Induction of forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGRPT) locus in the presence and absence of S9, as assayed by colony growth of Chinese hamster ovary cells in the presence of 6-T; cytotoxicity (based on cell counts, cell lysis, and cell morphology).

8) Significant Toxicity Observed: In the definitive assay without metabolic activation, substantial cytotoxicity was observed at concentrations $\geq$ 4000 mg/mL. Moderate cytotoxicity was also noted at 2000 and 3000 mg/mL, respectively. In the definitive assay in the presence of metabolic activation, no substantial cytotoxicity was observed at any concentration tested.

9) Conclusion(s): Tulathromycin did not produce a mutagenic response in the presence or absence of rat S9 in the CHO/HGPRT mammalian mutation assay.

i. Mouse Lymphoma L5178Y $TK^{+/-}$ Forward Mutation Assay. Report Number 01-1507-32. October 2001 to April 2002.

1) Study Director and Laboratory: Maria A. Cifone, Ph.D., Covance Laboratories, Inc., Vienna, VA

2) Identity of Substance and Dosage Form: Tulathromycin (CP-472,294(e)) in water

3) Species and Strain of Test Animals: L5178Y 3.7.2C mouse lymphoma cells heterozygous at the thymidine kinase locus (TK ±)

4) Number of Animals of Each Sex in Each Group: Not applicable

5) Levels and Duration of Dosing: The levels and duration of dosing in the initial non-activation mutation assay doses of 12.5 to 200 μg/mL were analyzed for mutants and in the repeat confirmatory non-activation assay (24-hour exposure) doses of 300 to 500 μg/mL were analyzed for mutations. In the initial activation assay (4-hour exposure), doses of 200 to 1000 μg/mL were used for analysis of mutation and doses of 400 to 1000 μg/mL were used in the confirmatory mutation assay (4-hour exposure).

6) Route of Drug Administration: Cell culture perfusion

7) Parameters Studied: Induction of forward mutations at the thymidine kinase (TK) locus in the presence and absence of S9, as assayed by colony growth of L5178Y/TK mouse lymphoma cells in the presence of 5-trifluorthymidine (TFT).

8) Significant Toxicity Observed: The cell viability results for the clonable treatment conditions indicated that exposure to tulathromycin resulted in a range of growth from 14.6% Relative Total Growth (RTG) to 86.8% RTG over a dose range of 300 to 500 μg/mL (-S9), and 13.9% RTG to 139.1% RTG over a dose range of 400 to 1000 μg/mL (+S9).

9) Conclusion(s): No mutagenicity was observed at any dose with or without activation. In the nonactivated test, the eight treatments induced no cytotoxicity to high cytotoxicity (86.8% to 14.6% relative growths). None of the analyzed treatments produced a mutant frequency greater than 127.8×10^{-6}, the level determined as mutagenic. In the activated test, the eight treatments induced no cytotoxicity to high cytotoxicity (139.1% to 13.9% relative growths). None of the analyzed treatments induced a mutant frequency that exceeded the minimum criteria of 120.5×10^{-6} (48.0 to 72.5×10^{-6}). This system was sensitive to the positive controls as evidenced by the positive responses observed with methyl methane sulfonate and methylcholanthrene, without or with metabolic activation, respectively. Tulathromycin was not mutagenic to L5178Y ($TK^{+/-}$) cells, with or without metabolic activation.

j. *In vitro* Human Lymphocyte Cytogenetics Assays. Report Number 98-1507-10. May 1998 to May 1999.

1) Study Director and Laboratory: Paula A. Muehlbauer, B.A., Drug Safety Evaluation Dept., Central Research Division, Pfizer, Inc., Groton, CT

2) Identity of Substance and Dosage Form: Tulathromycin (CP-472,294(e)) in citrate buffer

3) Species and Strain: Human lymphocytes

4) Number of Animals of Each Sex in Each Group: Not applicable

5) Levels and Duration of Dosing: Initial direct assay: (3 and 24-hour treatment) 90.5 to 2200 μg/mL; second 3-hour direct assay: 608 to 1810 μg/mL; second 24-hour direct assay: 198 to 1084 μg/mL. Initial S9 activation assay: (3-hour treatment) 90.5 to 2200 μg/mL; second 3-hour activation assay: 1450 to 3520 μg/mL.

6) Route of Drug Administration: Infusion into cell culture

7) Parameters Studied: Clastogenic activity *in vitro* in the human lymphocyte cultures with or without exogenous metabolic activation.

8) Significant Toxicity Observed: In the initial direct assay, the mitotic suppression (%) ranged from 4% to 53% over a dose range of 812 to 1450 μg/mL in the absence of a metabolic activation and 0 to 72% over a dose-range of 1450 to 2820 μg/mL. The 3520 μg/mL was toxic to the human lymphocytes used in the assay.

9) Conclusion(s): Tulathromycin does not induce chromosome aberrations in human lymphocyte cultures *in vitro* either with or without metabolic activation when tested up to concentrations that produce a substantial reduction of the mitotic index.

k. *In vivo* Rat Bone Marrow Cytogenetic Assay. Report Number 98-1507-11. August 1998 to May 1999.

1) Study Director and Laboratory: Warren W. Ku, Ph.D., Drug Safety Evaluation Dept., Central Research Division, Pfizer, Inc., Groton, CT

2) Identity of Substance and Dosage Form: Tulathromycin (CP-472,294(e)) in citrate buffer

3) Species and Strain of Test Animals: Sprague Dawley rat strain Crl:CD®(SD)IGS BR

4) Number of Animals of Each Sex in Each Group: 6/sex/group: three treatment groups sacrificed at about 24 hours after dosing

5) Levels and Duration of Dosing: 500, 1000, or 2000 mg/kg BW; single treatment for three days

6) Route of Drug Administration: Oral gavage

7) Parameters Studied: Induction of chromosomal aberrations in metaphase cells of rat bone marrow.

8) Significant Toxicity Observed: No evidence of toxic effect related to the treatment was observed in either the bone marrow or the target animal.

9) Conclusion(s): Orally administered tulathromycin does not induce micronuclei in the polychromatic bone marrow erythrocytes of male or female rats when tested up to a maximum practical oral dose of 2000 mg/kg BW.

l. Microbiological Safety

CVM currently requires sponsors to submit an assessment concerning the effects of antimicrobial residues present in the edible tissues of food animals on the intestinal flora of the consumer. The assessment submitted by the sponsor to comply with the human food safety requirements of antimicrobial drugs showed that the consumption of tulathromycin residues present in edible tissues of cattle and swine and ingested at a maximum ADI of 3 mg/person/day are not expected to have any adverse effect on human intestinal flora.

2. No Observed Effect Level (NOEL)

Based on these toxicology studies, the developmental toxicity study in rats was determined to be the most appropriate study upon which to base the NOEL. The NOEL from this study was 15 mg/kg BW/day.

3. Acceptable Daily Intake (ADI)

Based on these toxicology studies, a NOEL of 15 mg/kg BW/day, and a safety factor of 1000, the Agency has determined that the Acceptable Daily Intake (ADI) for ingested residues of tulathromycin is 15 µg/kg BW/day or 0.9 mg/person/day.

4. Safe Concentration of Residues

The safe concentrations (SC) for total residues are calculated from the ADI, assuming the average weight of a man to be 60 kg and the daily human intake of tissues as follows:

Muscle	300 g
Liver	100 g
Kidney	50 g
Fat	50 g

Therefore:

MUSCLE: Safe Concentration$_{(muscle)}$ = (15 µg/kg/day) **x** 60 kg / 300 g = **3 ppm**
LIVER: Safe Concentration$_{(liver)}$ = (15 µg/kg/day) **x** 60 kg / 100g = **9 ppm**
KIDNEY: Safe Concentration$_{(kidney)}$ = (15 µg/kg/day) **x** 60 kg / 50 g = **18 ppm**
FAT: Safe Concentration$_{(fat)}$ = (15 µg/kg/day) **x** 60 kg / 50 g = **18 ppm**

b. Residue Chemistry

1. Studies

Cattle:

a. Radiotracer Residue Depletion Study in Edible Tissues and Injection Site of Cattle Treated Subcutaneously with [^{14}C]-CP-472,295(e). Study Number 1535N-60-99-294. August 1999 to May 2001.

1) Study Director and Laboratory:

In life: Mark Nowakowski, Ph.D., Pfizer, Inc., Terre Haute, IN
Analytical: Robert R. Robinson, Ph.D., XenoBiotic Laboratories, Plainsboro, NJ

2) Identity of Substance: Tulathromycin, [^{14}C]-CP-472,295(e) in citrate buffer with propylene glycol

3) Test Animals: Mixed breed beef cattle, 5-7 months old, 172.8-209.0 kg BW

4) Number of Animals: 24 (12 male castrates, 12 females)

5) Dose: One dose at 2.5 mg/kg BW subcutaneously.

6) Results:

Table 4.1. Average total radiolabeled residues (ppm) following administration of [^{14}C]-CP-472,295(e)

Withdrawal (days)	Tissue				
	Injection Site	Liver	Kidney	Muscle	Fat
0.5	200 ± 40	6.4 ± 1.9	7.3 ± 0.6	1.8 ± 0.1	0.56 ± 0.13
5	13 ± 6	13.0 ± 3.0	7.5 ± 0.6	1.12 ± 0.18	0.5 ± 0.16
15	6 ± 2	6.4 ± 0.8	2.7 ± 0.4	0.18 ± 0.04	0.21 ± 0.06
25	2.5 ± 0.7	5.0 ± 2.0	1.3 ± 0.3	0.067 ± 0.009	0.104 ± 0.015
36	1.8 ± 0.7	3.6 ± 0.8	0.62 ± 0.14	NE	0.05 ± 0.02
48	0.70 ± 0.3	1.2 ± 0.4	0.25 ± 0.03	NE	NE

NE: not estimated

Mean total residues determined by combustion were compared to residues of the common fragment, CP-60,300, to determine the marker: total ratio. Results for liver and kidney are summarized in Tables 4.2 and 4.3.

Table 4.2. Marker: total ratio for the common fragment, CP-60,300 in liver

Withdrawal (days)	Total Residue (ppm)	Marker Residue (ppm)	Marker: Total Ratio
5	13.0 ± 3.0	7.7 ± 1.7	0.59
15	6.4 ± 0.8	4.1 ± 0.9	0.64
25	5.0 ± 2.0	2.9 ± 0.6	0.58
36	3.6 ± 0.8	2.3 ± 0.7	0.64
48	1.2 ± 0.4	0.7 ± 0.2	0.58

Table 4.3. Marker: total ratio for the common fragment, CP-60,300 in kidney

Withdrawal (days)	Total Residue (ppm)	Marker Residue (ppm)	Marker: Total Ratio
5	7.5 ± 0.6	5.6 ± 0.5	0.75
15	2.7 ± 0.4	2.3 ± 0.4	0.85
25	1.3 ± 0.3	1.1 ± 0.3	0.85
36	0.62 ± 0.14	0.53 ± 0.08	0.85
48	0.25 ± 0.03	0.19 ± 0.03	0.76

b. Analysis of Total [^{14}C] Residues in Bile, Blood, Intestinal Samples, Mesenteric Lymph Nodes, Intestinal Contents and Excreta and Metabolic Profiling of Selected Excreta from Calves Medicated with a Single Subcutaneous Dose of CP-472,295(e) at 2.5 mg/kg Body Weight. Study Number 1535N-60-99-296. November 1999 to May 2001.

1) Study Director and Laboratory:

In life: Mark Nowakowski, Ph.D. Pfizer, Inc., Terre Haute, IN
Analytical: Robert R. Robinson, Ph.D., XenoBiotic Laboratories, Plainsboro, NJ

2) Identity of Substance: Tulathromycin, [^{14}C]-CP-472,295(e) in citrate buffer with propylene glycol

3) Test Animals: Mixed breed beef cattle, 5-7 months old, 172.8-209.0 kg BW

4) Number of Animals: 24 (12 male castrates, 12 females)

5) Dose: One dose at 2.5 mg/kg BW subcutaneously.

6) Results: A large portion of the administered dose was excreted in the urine and feces in the majority of animals during the first 14 days post dose. The total radioactive residues excreted in urine peaked during the first 24 hours post-dose collection period (all $\geq$ 25 µg/mL). Subsequently, the residues excreted in the urine declined to less than 0.01 µg/mL by 47 days post-dose. The total radioactive residues excreted in feces peaked during the first

48 hours post-dose collection period – some in the first 24-hour collection period and some in the second 24-hour collection period. Subsequently, the residues excreted in the fecal material declined to less than 0.4 ppm by 14 days post-dose. Residues of [^{14}C]-CP-472,295(e) declined rapidly in the intestinal contents and intestinal tissues where as the levels in the mesenteric lymph node did not change significantly.

c. The Metabolic Profile of [^{14}C]-CP-472,295(e) in Cattle and Swine Bile, Urine, Feces and Edible Tissues. Study Number 1576N-60-00-209. June 2000 to May 2001.

1) Study Director and Laboratory:

In life: Mark Nowakowski, Ph.D., Pfizer, Inc., Terre Haute, IN
Analytical: Robert R. Robinson, Ph.D., XenoBiotic Laboratories, Plainsboro, NJ

2) Identity of Substance: Tulathromycin, [^{14}C]-CP-472,295(e) in citrate buffer with propylene glycol

3) Test Animals: Mixed breed beef cattle, 5-7 months old, 172.8-209.0 kg BW

4) Number of Animals: 24 (12 male castrates, 12 females)

5) Dose: One dose at 2.5 mg/kg BW subcutaneously (SC).

6) Results:

Table 4.4. Total residues for cattle administered 2.5 mg/kg BW SC injection of [^{14}C]-CP-472,295(e)

Tissue	**CP-472,295**	**CP-547,272**	**CP-60,300**	***M2***	***M3***	***M4***	***M5***	***M6***	***M7***	***M8***	***M9***	***M10***
Urine	+	+	+	+	+	+	+	X	X	+	+	+
Bile	+	+	+	+	+	+	+	+	+	+	+	+
Feces	+	+	+	X	+	X	+	X	+	+	+	+
Liver	+	+	X	+	+	X	+	X	+	+	+	+
Kidney	+	+	+	X	+	+	+	X	+	X	+	+
Injection Site	+	+	+	X	X	X	X	X	+	X	+	X
Muscle	+	+	+	X	X	X	X	X	X	+	X	+

+: observed
X: not observed

d. A Comparative Metabolism Study of [^{14}C]-CP-472,295(e) in Rats and Dogs. Study Number 1576N-60-00-211. August 2000 to May 2001.

1) Study Director and Laboratory: Richard Schneider, Pfizer, Inc., Groton, CT

2) Identity of Substance: Tulathromycin, [^{14}C]-CP-472,295(e)

3) Test Animals:

Sprague Dawley rats; 3 males, 3 females; 162-198 grams BW
Beagle dogs; 2 males, 2 females; 7.70-9.30 kg BW

4) Dose:

Rats: 50 mg/kg BW/day for 2 days
Dogs: 15 mg/kg BW/day for 2 days

5) Results:

Table 4.5. Summary of metabolites identified in rat tissues

Tissue	**Sex**	**CP-472,295**	**CP-547,272**	**CP-60,300**	***M2***	***M3***	***M4***	***M5***	***M6***	***M7***	***M8***	***M9***	***M10***
Urine	M	84.8	11.9	X	X	X	MS	MS	X	X	MS	3.2	MS
	F	90.2	9.8	X	X	X	MS	MS	X	X	X	0.9	MS
Feces	M	88.2	9.6	0.8	X	X	X	MS	X	X	MS	0.4	1
	F	85.3	11.5	1.2	X	X	MS	MS	X	X	MS	0.7	1.3
Liver	M	86	4.3	X	X	X	X	MS	X	X	MS	2.6	1.8
	F	81.1	3.2	X	X	X	X	MS	X	X	X	2.2	MS

X: not observed
MS: Seen by mass spectrometric methods only

Table 4.6. Summary of metabolites identified in dog tissues

Tissue	**Sex**	**CP-472,295**	**CP-547,272**	**CP-60,300**	***M2***	***M3***	***M4***	***M5***	***M6***	***M7***	***M8***	***M9***	***M10***
Urine	M	82.1	12	0.1	X	X	MS	1.3	X	X	MS	2.5	1.5
	F	82.1	10.5	X	X	X	MS	0.7	X	X	MS	1.1	1
Feces	M	85.6	10.1	MS	X	X	MS	1.6	X	X	MS	2.6	MS
	F	85.9	10.5	0.5	X	X	0.8	0.9	X	X	MS	1	0.5
Bile	M	89.9	6.5	0.4	X	X	X	MS	X	MS	X	0.5	0.9
	F	92.8	3.8	0.6	X	X	X	MS	X	X	X	0.4	MS
Liver	M	86.1	2.9	X	X	X	X	1.1	X	X	X	3.8	1.8
	F	84.4	1.9	X	X	X	X	1.5	X	X	MS	0.9	1.8

X: not observed
MS: Seen by mass spectrometric methods only

e. Marker Residue Depletion Study in Edible Tissues of Cattle Treated Subcutaneously with CP-472,295(e). Study Number 1531N-60-99-330. January 2000 to May 2001.

1) Study Director and Laboratory:

In life: Mark Nowakowski, Ph.D., Pfizer, Inc., Terre Haute, IN
Analytical: BAS Analytical, West Lafayette, IN

2) Identity of Substance: Tulathromycin in the commercial formulation

3) Test Animals: Mixed breed beef cattle, 6-7 months old, 171-249 kg BW

4) Number of Animals: 42 (21 male castrates, 21 females)

5) Dose: One dose at 2.5 mg/kg BW subcutaneously

6) Results:

Table 4.7. Average CP-60,300 (common fragment) residues (ppm)

Withdrawal (days)	Tissue				
	Injection Site	**Liver**	**Kidney**	**Muscle**	**Fat**
5	3.6 ± 1.2	4.0 ± 0.6	3.3 ± 0.4	0.39 ± 0.05	0.19 ± 0.04
12	2.3 ± 0.8	2.8 ± 0.6	1.8 ± 0.3	0.12 ± 0.03	0.10 ± 0.04
18	1.6 ± 0.5	2.3 ± 0.3	0.9 ± 0.3	0.063 ± 0.011	0.07 ± 0.06
25	0.6 ± 0.3	1.7 ± 0.8	0.5 ± 0.2	0.034 ± 0.02	0.03 ± 0.01
36	0.6 ± 0.6	0.9 ± 0.4	0.3 ± 0.2	0.014 ± 0.006	0.015 ± 0.01
48	0.4 ± 0.1	0.46 ± 0.10	0.15 ± 0.06	0.006 ± 0.002	0.005 ± 0.002

Swine:

a. Radiotracer Total Residue Study in Edible Tissues of Swine Treated Intramuscular with [^{14}C]-CP-472,295(e). Study Number 1525N-60-99-175. February 2000 to May 2001.

1) Study Director and Laboratory:

In life: Charles E. Heird, Ph.D., Southwest Bio-Labs, Inc., Las Cruces, NM
Analytical: Pamela L. Boner, Ph.D., XenoBiotic Laboratories, Plainsboro, NJ

2) Identity of Substance: Tulathromycin, [^{14}C]-CP-472,295(e) in citrate buffer with propylene glycol

3) Test Animals: Mixed breed swine, 3 months old, 43-62 kg BW

4) Number of Animals: 18 (9 gilts, 9 barrows)

5) Dose: One dose at 2.5 mg/kg BW intramuscularly

6) Results:

Table 4.8. Average total radiolabeled residues (ppm) following administration of [^{14}C]-CP-472,295(e)

Withdrawal (days)	Tissue				
	Injection Site	Liver	Kidney	Muscle	Skin/Fat
4	4.73 ± 0.69	2.85 ± 0.42	6.61 ± 0.55	0.61 ± 0.04	0.48 ± 0.06
12	2.44 ± 0.61	1.39 ± 0.23	2.50 ± 0.84	0.12 ± 0.03	0.18 ± 0.04
24	1.40 ± 0.31	0.57 ± 0.10	0.79 ± 0.16	0.06 ± 0.01	0.1
36	0.76 ± 0.41	0.20 ± 0.06	0.27 ± 0.08	<LOQ	<LOQ

LOQ = limit of quantification

Mean total residues determined by combustion were compared to residues of the common fragment, CP-60,300 to determine the marker: total ratio. Results for liver and kidney are summarized in Tables 4.9 and 4.10.

Table 4.9. Marker: total ratio for the common fragment, CP-60,300 in liver

Withdrawal (days)	Total Residue (ppm)	Marker Residue (ppm)	Marker: Total Ratio
4	2.85 ± 0.42	2.54 ± 0.25	0.89
12	1.39 ± 0.23	1.32 ± 0.24	0.95
24	0.57 ± 0.10	0.54 ± 0.07	0.95
36	0.20 ± 0.06	0.19 ± 0.06	0.95

Table 4.10. Marker: total ratio for the common fragment, CP-60,300 in kidney

Withdrawal (days)	Total Residue (ppm)	Marker Residue (ppm)	Marker: Total Ratio
4	6.61 ± 0.55	5.34 ± 0.64	0.81
12	2.50 ± 0.84	2.03 ± 0.70	0.81
24	0.79 ± 0.16	0.70 ± 0.13	0.89
36	0.27 ± 0.08	0.22 ± 0.07	0.81

b. Analysis of Total [^{14}C] Residues in Bile, Blood, Intestinal Samples, Mesenteric Lymph Nodes, Intestinal Contents and Excreta and Chromatographic Profiling of Metabolites in Excreta from Pigs Medicated with a Single Subcutaneous Dose of CP-472,295(e) at 2.5 mg/kg Body Weight. Study Number 1525N-60-00-177. April 2000 to May 2001.

1) Study Director and Laboratory:

In life: Charles E. Heird, Ph.D., Southwest Bio-Labs, Inc., Las Cruces, NM
Analytical: Michael Hosea, MS, Southwest Bio-Labs, Inc., Las Cruces, NM

2) Identity of Substance: Tulathromycin, [^{14}C]-CP-472,295(e) in citrate buffer with propylene glycol

3) Test Animals: Mixed breed swine, 3 months old, 43-62 kg BW

4) Number of Animals: 24 (12 gilts, 12 barrows)

5) Dose: One dose at 2.5 mg/kg BW intramuscularly.

6) Results: A large portion of the administered dose was excreted in the urine and feces in the majority of animals during the first five days post dose. The total radioactive residues excreted in urine represented 27-38% of the administered dose. The total radioactive residues excreted in feces represented 54-74% of the administered dose. Residues of [^{14}C]-CP 472,295(e) declined rapidly in the intestinal contents, intestinal tissues, and bile whereas the levels in the mesenteric lymph node did not change significantly.

c. The Metabolic Profile of [^{14}C]-CP-472,295(e) in Cattle and Swine Bile, Urine, Feces and Edible Tissues. Study Number 1576N-60-00-209. June 2000 to May 2001.

1) Study Director and Laboratory:

In life: Mark Nowakowski, Ph.D., Pfizer, Inc., Terre Haute, IN
Analytical: Robert R. Robinson, Ph.D., XenoBiotic Laboratories, Plainsboro, NJ

2) Identity of Substance: Tulathromycin, [^{14}C]-CP-472,295(e) in citrate buffer with propylene glycol

3) Test animals: Mixed breed swine, 3 months old, 43-62 kg BW

4) Number of Animals: 18 (9 gilts, 9 barrows)

5) Dose: One dose at 2.5 mg/kg BW intramuscularly

6) Results:

Table 4.11. Total residues for swine administered 2.5 mg/kg BW IM injection of [^{14}C]-CP-472,295(e)

Tissue	**CP-472,295**	**CP-547,272**	**CP-60,300**	***M2***	***M3***	***M4***	***M5***	***M6***	***M7***	***M8***	***M9***	***M10***
Urine	+	+	X	X	X	+	MS	X	X	X	+	+
Bile	+	+	+	X	X	X	+	X	X	MS	+	+
Feces	+	+	+	X	X	MS	+	X	X	MS	+	+
Liver	+	+	MS	X	X	X	MS	X	X	X	+	+
Kidney	+	+	MS	X	X	X	+	X	X	MS	+	+
Injection Site	+	+	X	X	X	X	X	X	X	MS	+	+
Skin/Fat	+	+	X	X	X	+	X	X	X	MS	+	+
Muscle	+	+	+	X	X	X	X	X	X	X	+	+

+: observed
X: not observed
MS: mass spectral evidence only

d. A Comparative Metabolism Study of [^{14}C]-CP-472,295(e) in Rats and Dogs. Study Number 1576N-60-00-211. August 2000 to May 2001.

See 4.b.1.d., above.

e. Marker Residue Depletion Study in Edible Tissues of Swine Treated Intramuscularly with CP-472,295(e). Study Number 1521N-60-99-176. January 2000 to May 2001.

1) Study Director and Laboratory:

In life: Mark Nowakowski, Ph.D., Pfizer, Inc., Terre Haute, IN
Analytical: BAS Analytical, West Lafayette, IN

2) Identity of Substance: Tulathromycin in the commercial formulation

3) Test Animals: Mixed breed swine, 3-4 months old, 43.0-53.5 kg BW

4) Number of Animals: 30 (15 gilts, 15 barrows)

5) Dose: One dose at 2.5 mg/kg BW intramuscularly

6) Results:

Table 4.12. Average CP-60,300 (common fragment) residues (ppm)

Withdrawal (days)	Tissue				
	Injection Site	Liver	Kidney	Muscle	Skin/Fat
5	1.7 ± 0.24	1.2 ± 0.2	2.1 ± 0.3	0.31 ± 0.11	0.17 ± 0.04
12	1.0 ± 0.45	0.7 ± 0.1	0.87 ± 0.17	0.07 ± 0.01	0.08 ± 0.04
18	0.81 ± 0.19	0.5 ± 0.1	0.55 ± 0.23	0.053 ± 0.032	0.042 ± 0.024
25	0.39 ± 0.26	0.20 ± 0.03	0.22 ± 0.05	0.025 ± 0.014	0.014 ± 0.007
36	0.40 ± 0.15	0.11 ± 0.03	0.12 ± 0.04	0.013 ± 0.005	0.011 ± 0.006

2. Target Tissue and Marker Residue Assignments

Based on the results of the total residue study (1535N-60-99-294) and the tissue metabolism study (1576N-60-00-209), liver is assigned as the target tissue in cattle.

Based on the results of the total residue study (1525N-60-99-175) and the tissue metabolism study (1576N-60-00-209), kidney is assigned as the target tissue in swine.

The marker residue in cattle liver and swine kidney is the common fragment, CP-60,300.

3. Tolerance for the Marker Residue

Based on the results of the marker: total residue analysis, a tolerance of 5.5 ppm is established for residues of the common fragment, CP-60,300, in cattle liver. The assigned tolerance represents 61% of the liver safe concentration of 9 ppm.

Based on the results of the marker: total residue analysis, a tolerance of 15 ppm is established for residues of the common fragment, CP-60,300, in swine kidney. The assigned tolerance represents 81% of the kidney safe concentration of 18 ppm.

4. Withdrawal Period

In cattle, mean total radiolabeled residues in the target tissue are less than the liver safe concentration by 15 days withdrawal. Radiolabeled residues at the injection site are less than 10X the muscle safe concentration by 5 days withdrawal.

Using the residue depletion data from the residue depletion study, 1531N-60-99-330, a tolerance of 5.5 ppm for the marker residue (common fragment CP-60,300) in the target tissue (liver), and our statistical tolerance algorithm for the 99th percentile with 95% confidence, a withdrawal period of 18 days is assigned for the use of tulathromycin in cattle. A withdrawal period of 18 days is consistent with depletion of residues at the injection site.

In swine, mean total radiolabeled residues in the target tissue are less than the kidney safe concentration at all sampling times. Radiolabeled residues at the injection site are less than 10X the muscle safe concentration at all sampling times.

Using the residue depletion data from the residue depletion study, 1521N-60-99-176, a tolerance of 15 ppm for the marker residue (common fragment CP-60,300) in the target tissue (kidney), and our statistical tolerance algorithm for the 99th percentile with 95% confidence, a withdrawal period of 5 days is assigned for the use of tulathromycin in swine. A withdrawal period of 5 days is consistent with depletion of residues at the injection site.

c. Microbial Food Safety:

Microbial food safety data were submitted for tulathromycin (CP-472,295(e)) which attempted to study a broad range of relevant microbiological parameters that characterized the mechanism of action, activity against macrolide resistant and sensitive pathogens, and resistance development in a variety of key zoonotic and commensal bacteria. The use of tulathromycin will not adversely affect the development of macrolide resistance in zoonotic human pathogens. The experiments were logical and generally demonstrated that tulathromycin will not exert any more pressure on the generation of macrolide resistance in zoonotic pathogens than already approved antimicrobials. Macrolides such as erythromycin are not active against many Gram-negative [Gm(-)] organisms, and would not be used in treating infections in humans caused by *E. coli* or *Salmonella*. Any resistance to tulathromycin that develops in *E. coli* or *Salmonella*, as a result of the use of tulathromycin in animals, would not be expected to have much impact on human medicine. The impact would be limited to gene transfer to a pathogenic organism that would be treated with erythromycin in a clinical setting. Currently, there is little data to suggest that this scenario occurs with any frequency. Macrolides are not used for treatment of enterococcal infections in humans. However, they are one of the drugs of choice for treatment of human infections caused by *Campylobacter*. Data were submitted that addressed the development of macrolide resistance in *Campylobacter* spp. The data supporting the microbial food safety of tulathromycin have been submitted in their original applications. The summary of the studies conducted for this product is presented below.

Tulathromycin is a chemically novel macrolide, which shares a common binding site on the ribosome with erythromycin but which differs from it by the presence of three ionizable amine groups (compared to a single amine group in erythromycin). Bacterial strains resistant to erythromycin are also resistant to tulathromycin. Inducible forms of resistance to tulathromycin were not identified in naïve field isolates. The frequency of resistance selection, determined by a direct-plating method, was generally less than 1×10^{-9} per cell plated, and plasmid mediated transfer of resistance genes between *E. faecalis* strains occurred at comparable frequency when tulathromycin, erythromycin, or tilmicosin was a selection agent. No tendency toward rapid development of resistance was observed *in vitro* with tulathromycin.

1. Studies

a. *In vitro* Activity of Triamilide CP-472,295(e) Against Ribosomes Isolated from *E. coli*

1) Type of Study: Mechanism of action

2) Investigator: Dr. Thomas D. Gootz, Pfizer Global Research and Development-Veterinary Medicine, Safety and Metabolism, Groton, CT

3) Results and Conclusions:

The studies tested tulathromycin equilibrated solution and tulathromycin drug substance in an *in vitro* transcription-translation assay. The mean inhibitory concentrations (IC_{50} (SD)) against macrolide-sensitive ribosomes (*E. coli*) were 0.37 (0.02) μM and 0.44 (0.10) μM, respectively. The mean IC_{50s} for erythromycin and tilmicosin were 0.57 (0.17) μM and 0.39 (0.04) μM respectively. These data indicate that the inhibitory potential for tulathromycin against erythromycin sensitive ribosomes is comparable to that of structurally conventional macrolides.

The binding of tulathromycin to macrolide sensitive ribosomes was further characterized by conducting competitive binding assays. A study measuring the displacement of ^{14}C-erythromycin from the ribosome by tulathromycin indicated that the tulathromycin had potent binding activity. Tulathromycin had an EC_{50} of 0.4 μM, compared with the values of 1.5 and 0.78 μM for unlabeled erythromycin and tilmicosin, respectively. This relationship demonstrates that tulathromycin shares a binding site on the ribosome with erythromycin and tilmicosin.

b. *In vitro* Activity of Triamilide CP-472,295(e) Against Human Pathogens Containing Characterized Mechanisms of Macrolide Resistance

1) Type of Study: Macrolide resistant human pathogens

2) Investigator: Dr. Thomas D. Gootz, Pfizer Global Research and Development-Veterinary Medicine, Safety and Metabolism, Groton, CT

3) Results and Conclusions:

Tulathromycin was eightfold less active than erythromycin against macrolide-susceptible, Gram positive strains. The resistance determinants *erm*B, *erm*C, and *mef*A conferred complete cross-resistance to all the drugs tested. Tulathromycin was generally more potent than erythromycin against Gm(-) strains. A 32-fold drop in minimum inhibitory concentration (MIC) was observed for all macrolides in an AcrAB knockout mutant compared with an isogenic wild type *Haemophilus influenzae* strain, suggesting that efflux decreases the intrinsic activity of macrolides against some Gm(-) organisms. Tulathromycin is not active against macrolide-resistant organisms.

c. Macrolide Resistance Gene Survey of Field Isolates by PCR Methodology

1) Type of Study: PCR screen of field isolates

2) Investigators: Dr. Shigeru F. Hayashi and Dr. Cheryl L. Santoro, Pfizer Global Research and Development-Veterinary Medicine, Biological Discovery, Groton, CT

3) Results and Conclusions:

Fifty-seven field isolates (14 *Salmonella*, 16 *E. coli*, 12 *Campylobacter jejuni/coli*, and 15 *Enterococcus faecalis/faecium*) were screened using the polymerase chain reaction (PCR) for *erm*A, *erm*B, and *erm*C (methylases), *mph*A, *mph*B, and *ere*A (macrolide inactivating enzymes), and the *msr*A and *mef*A efflux pumps. There were no *erm*A, *erm*B, *erm*C, *ere*A, *mph*B, *msr*A, or *mef*A macrolide resistance determinants among the 57 field isolates. Three *E. faecium* strains produced a PCR-amplified band from the multiplex set 1 primers (*erm*A, *erm*B, *erm*C, and *msr*A) which were produced from the *msr*A primers. One *E. faecalis* strain also produced a strong band of 1300 base pairs (bp) from the set 1 primers. This band was a much larger amplicon than expected from these primers. *E. faecium* contains the *msr*C gene, which is the same size as the product produced from *msr*A using this primer set. *msr*C is 62% identical to *msr*A at the DNA level and has not been found to be involved in macrolide resistance. The 1300 bp product obtained from the *E. faecalis* strain is a most likely product of the *msr*A primer, and actually the *msr*C gene, although it is twice the size expected. The susceptibilities of the three *E. faecium* strains and the *E. faecalis* strain were the same as those of the other isolates, indicating that they do not contain constitutively expressed macrolide resistance genes. Two *E. coli* strains appeared to contain *mph*A resistance determinant. Tulathromycin retains comparable activity against both of these strains and other susceptible *E. coli* strains. Acquired resistance determinants were not observed in the *Campylobacter* isolates.

d. Sequence Comparisons of *mph*A from *E. coli* Field Isolates

1) Investigator: Dr. Thomas D. Gootz, Pfizer Global Research and Development-Veterinary Medicine, Safety and Metabolism, Groton, CT

2) Results and Conclusions:

Two *E. coli* strains screened by PCR for macrolides resistance genes produced a very strong PCR product from the *mph*A primers. The MICs of tulathromycin for these two strains were one dilution higher compared to other *E. coli* strains. These *E. coli* strains contain sequences identical to the published sequence for *mph*A. *mph*A is not a resistant determinant that is commonly found and not much is known about its regulation and its expression. The clinical relevance of *mph*A is uncertain.

e. *In vitro* Induction of the MLS_B Phenotype

1) Investigators: Dr. Shigeru F. Hayashi and Dr. Laura J. L. Norcia, Pfizer Global Research and Development-Veterinary Medicine, Biological Discovery, Groton, CT

2) Results and Conclusions:

Because the expression of MLS_B genes in Gram positive bacteria can be inducibly expressed, and this inducible expression is not always detected in standard MIC testing, selected strains have been tested in an induction assay. None of the selected test strains had an inducible MLS_B phenotype. Tulathromycin is a weak inducer of the MLS_B phenotype. Results also showed that tulathromycin is bactericidal for *Campylobacter* spp.

f. Frequency of Spontaneous Resistance to Triamilide CP-472,295 against Foodborne Pathogens *Salmonella*, *E. coli*, *Enterococcus*, and *Campylobacter*

1) Type of Study: *In vitro* selection for resistance

2) Investigators: Dr. Shigeru F. Hayashi and Dr. George A. Kopitas, Pfizer Global Research and Development-Veterinary Medicine, Biological Discovery, Groton, CT

3) Results and Conclusions:

Analysis of the *in vitro* spontaneous resistance development to tulathromycin was conducted as part of the antibiotic resistance emergence studies. Fifty-seven field isolates were tested for the frequency of spontaneous mutation conferring macrolide resistance. The frequency of mutation conferring resistance to tulathromycin for all *E. coli*, *Salmonella*, and *Enterococcus* was less than 1×10^{-9} per cell plated. The mutation frequencies observed for the *Campylobacter* isolates were $<1 \times 10^{-8}$ to 1×10^{-9} per cell plated.

g. Macrolide Resistance Transfer Frequency Study

1) Type of Study: Plasmid mediated transfer

2) Investigator: Dr. Shigeru F. Hayashi, Pfizer Global Research and Development-Veterinary Medicine, Biological Discovery, Groton, CT

3) Results and Conclusions:

The frequency of cell to cell transfer of resistance to tulathromycin and other macrolides was tested in the *Enterococcus* filter mating system. Tulathromycin, erythromycin, or tilmicosin, at selecting concentrations of 50 μg/mL, generated similar macrolide resistance transfer frequencies of $1\text{-}2 \times 10^{-2}$. Tulathromycin does not increase the frequency of macrolide transfer compared with erythromycin and tilmicosin.

2. Category Designation

Based on the information submitted, it is determined that tulathromycin is a Category I drug.

d. Analytical Methods for Residues:

1. Determinative Method

Cattle:

The regulatory method for determination of tulathromycin in bovine liver is an LC-MS/MS assay which successfully completed a sponsor monitored multilaboratory method trial.

Swine:

The regulatory method for determination of tulathromycin in swine kidney is an LC-MS/MS assay which successfully completed a sponsor monitored multilaboratory method trial.

2. 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.

3. Availability of the Methods

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

5. USER SAFETY:

Human warnings are provided on the product labeling as follows:

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

To request a material safety data sheet, call 1-800-733-5500.

6. 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 of the implementing regulations. The data demonstrate that DRAXXIN Injectable Solution (tulathromycin), when administered as a subcutaneous (cattle) or intramuscular (swine) injection, is safe and effective for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* (*Haemophilus somnus*); for the control of respiratory disease in cattle at high risk of developing BRD

associated with *M. haemolytica*, *P. multocida*, and *H. somni*; and for the treatment of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, and *Haemophilus parasuis.*

Labeling restricts this drug to use by or on 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 BRD or SRD, (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, and (c) the rate of emergence of tulathromycin-resistant organisms may be reduced by the involvement of veterinarians in product use.

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.

Tulathromycin is under the following U.S. patent numbers:

U.S. Patent Number	Date of Expiration
6,329,345	November 18, 2019
6,420,536	May 29, 2018
6,514,945	January 24, 2021
6,583,274	May 2, 2020
6,777,393	May 29, 2018

7. ATTACHMENTS:

Facsimile labeling is attached as indicated below.

a. DRAXXIN Injectable Solution – 100 mL vial label and insert
b. DRAXXIN Injectable Solution – 100 mL carton
c. DRAXXIN Injectable Solution – 100 mL shipper label
d. DRAXXIN Injectable Solution – 250 mL vial label and insert
e. DRAXXIN Injectable Solution – 250 mL carton
f. DRAXXIN Injectable Solution – 250 mL shipper label
g. DRAXXIN Injectable Solution – 500 mL vial label and insert
h. DRAXXIN Injectable Solution – 500 mL carton
i. DRAXXIN Injectable Solution – 500 mL shipper label