Date of Approval: May 14, 2012

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-334

ZUPREVO

Tildipirosin
18% Injectable Solution
Beef and Non-Lactating Dairy Cattle

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

Sponsored by:

Intervet, Inc.

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I. GENERAL INFORMATION:

A. File Number:	NADA 141-334
B. Sponsor:	Intervet, Inc. 556 Morris Ave. Summit, NJ 07901 Drug Labeler Code: 000061
C. Proprietary Name:	ZUPREVO
D. Established Name:	Tildipirosin
E. Pharmacological Category:	Antimicrobial
F. Dosage Form:	18% Injectable solution
G. Amount of Active Ingredient:	180 mg/mL
H. How Supplied:	50, 100, and 250 mL glass vials
I. How Dispensed:	Rx
J. Dosage:	4 mg/kg body weight (BW) once
K. Route of Administration:	Subcutaneous (SC) injection in the neck
L. Species/Classes:	Cattle/beef and non-lactating dairy
M. Indications:	For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in beef and non-lactating dairy cattle, and for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*.

II. EFFECTIVENESS:

A. Dosage Characterization:

Similar to other macrolides, tildipirosin (referred to as 20, 23-di-piperidinyl-mycaminosyl-tylonolide or "PMT" during product development) inhibits essential bacterial protein biosynthesis with selective binding to ribosomal subunits in a bacteriostatic and time-dependent manner. Tildipirosin may be bactericidal against certain isolates of *Mannheimia haemolytica* and *Pasteurella multocida.*

1. Pharmacokinetic Study

a. Study title: "Determination of 20, 23-di-piperidinyl-mycaminosyl-tylonolide (tildipirosin) in blood plasma (pharmacokinetic profile), bronchial fluid, and lung tissue of cattle after a single subcutaneous administration of 18% w/v tildipirosin solution for injection at 3 doses (2 mg, 4 mg, and 6 mg tildipirosin/kg BW)"; Study number V-0045-0072.

b. Study design: Twenty-four (12 male and 12 female) Holstein Friesian calves, weighing 212 to 259 kg were randomly assigned to receive tildipirosin (180 mg/ml) at 2 mg/kg (n=5), 4 mg/kg (n=14), or 6 mg/kg BW (n=5) as a single subcutaneous (SC) injection. Plasma was obtained from blood samples collected from all animals before and at several time points after treatment; bronchial fluid (collected *in vivo* and *post mortem*) and lung homogenate samples were collected at several time points after treatment (from cattle treated with 4 mg tildipirosin/kg BW). Plasma, bronchial fluid, and lung tissue homogenate samples were analyzed for tildipirosin content using validated High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (HPLC-MS/MS) methods.

c. Study results: Tildipirosin was rapidly absorbed after SC administration and the maximum drug plasma concentration was reached on average at 45 (± 25 SD) minutes post-treatment. The prolonged presence of tildipirosin in plasma was demonstrated by plasma concentrations >LOQ (limit of quantification) for up to 504 hours (= 21 days) and a long terminal half-life (approximately 6.5 days). Mean PK parameters of tildipirosin in plasma after a single SC administration of 4 mg tildipirosin/kg BW in cattle are listed in Table II.1.

Table II.1: Summary of plasma pharmacokinetics of tildipirosin administered subcutaneously to calves at a dose of 4 mg/kg BW.

Parameter	Average	SD
C_{max} (ng/mL)	767*	284
T_{max} (hr)	0.75*	0.43
$AUC_{0\text{-last}}$ (hr·ng/mL)	21017**	3499
$AUC_{0\text{-inf}}$ (hr·ng/mL)	24934**	3508

Parameter	Average	SD
$t_{1/2}$ (hr)	210**	53

* Value based on all 14 animals dosed at 4 mg/kg BW

** Value based on 8 animals that were slaughtered at 504 hours post-treatment

C_{max}: Maximum observed plasma concentration

T_{max}: Time at which C_{max} was observed

$AUC_{0\text{-last}}$: Area under the plasma concentration versus time curve measured from time zero to the last sample with tildipirosin concentrations exceeding the limit of quantification of the analytical method

$AUC_{0\text{-inf}}$: AUC estimated from time zero to time infinity

t½: Terminal elimination half-life

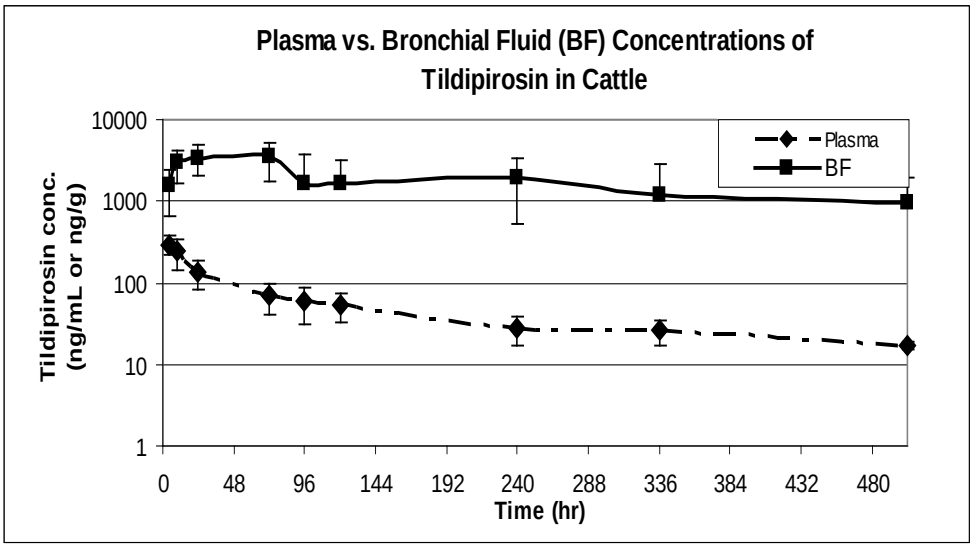
Dose linearity was observed within the dose range of 2 to 6 mg/kg. Plasma concentration data indicated that the 4 mg/kg dose could potentially be used in support of the proposed claim. However, because plasma levels of macrolides are not reflective of the drug exposure at the site of action, this dose was further tested by measuring tildipirosin concentrations in bronchial fluid.

In vivo tildipirosin concentration in bronchial fluid far exceeded the tildipirosin concentrations in plasma (Table II.2 and Figure II.1). The 5:1 ratio of mean tildipirosin concentrations in bronchial fluid (*in vivo*) vs. blood plasma at 4 hours indicates fast tissue distribution and the ratio peaked at 240 hours.

Table II.2: Mean (± standard deviation) concentration of tildipirosin in bronchial fluid (*in vivo*) and plasma in cattle, and bronchial fluid-to-plasma (BF/P) ratio of tildipirosin following a subcutaneous injection at a dose of 4 mg/kg body weight in the neck.

Time (hours)	Average (±SD) Bronchial fluid (BF) concentration (ng/g)	Average (±SD) Plasma (P) concentration (ng/mL)	BF/P Ratio
4	1543 (895)	297 (81.8)	5.2
10	2975 (1279)	242 (96.7)	12.3
24	3448 (1433)	136 (53.9)	25.4
72	3489 (1712)	70.7 (29)	49.3
96	1644 (2024)	60.2 (29)	27.3
120	1619 (1629)	52.3 (19.9)	30.9
240	1937 (1416)	27.1 (10.8)	71.5
336	1225 (1682)	26.1 (9.2)	47
504	935 (1032)	16.8 (1.7)	55.6

Figure II.1: Mean (± standard deviation) tildipirosin concentrations in bronchial fluid (BF) and plasma after a single SC administration of tildipirosin at a dose rate of 4 mg tildipirosin/kg BW in cattle.



d. Conclusions: Results of this study demonstrate that the bronchial fluid concentrations of tildipirosin in cattle are consistently and significantly higher than plasma concentrations (for up to 21 days) when administered subcutaneously at the label dose. Based on the tissue levels of tildipirosin that are maintained above the MICs of major BRD pathogens for an extended period of time, the dose of 4 mg/kg BW was used for the development of tildipirosin in cattle for the treatment of BRD.

2. Pilot Effectiveness Studies

Dose selection for tildipirosin was further confirmed in clinical effectiveness studies because, for macrolides, there is an inconsistent relationship between plasma pharmacokinetics (PK), pharmacodynamics (PD), and clinical effectiveness.

The effectiveness of tildipirosin was evaluated for the treatment of naturally-occurring bovine respiratory disease (BRD) in two studies conducted under field conditions in the United States. In both studies, entrance criteria included abnormal respiration, abnormal attitude, and a rectal temperature of 104 °F or higher. In the first study, a total of 150 cross-bred beef calves (steers) meeting entrance criteria were randomized to one of five treatment groups: saline control, tildipirosin at 1 mg/kg body weight (BW), tildipirosin at 2 mg/kg BW, tildipirosin at 4 mg/kg BW, or a positive control. In the second study, a total of 300 cross-bred beef calves (bulls and steers) meeting entrance criteria were randomized to one of five treatment groups: saline control, tildipirosin at 2 mg/kg BW, tildipirosin at 4 mg/kg BW, tildipirosin at 6 mg/kg BW, or a positive control. Pre-treatment nasopharyngeal swab samples were obtained during both studies. Study animals were observed daily for 14 days after treatment administration for clinical signs of BRD. Beginning on Day 3 (72 hours post-

treatment), animals were evaluated for treatment success/failure determination.

The primary effectiveness criterion in both studies was treatment success rate. In the first study, the treatment success rate for the group treated with 4 mg/kg BW tildipirosin (93.3%) was statistically significantly greater ($p<0.05$) than the saline-treated group (66.7%). The treatment success rates for the 1 mg/kg BW tildipirosin-treated group (73.3%) and the 2 mg/kg tildipirosin-treated group (80%) were not statistically significantly different ($p>0.05$) from the saline-treated group (66.7%). Two mortalities were reported in the study (one in the saline-treated group and one in the group treated with 1 mg/kg BW tildipirosin).

In the second study, the treatment success rates for the 6 mg/kg BW tildipirosin-treated group (61%), 4 mg/kg BW tildipirosin-treated group (71.7%), and the 2 mg/kg BW tildipirosin-treated group (60%) were statistically significantly greater ($p<0.05$) than the treatment success rate for the saline-treated group (18.6%). The mortality rate in the saline-treated group (10.2%) was statistically significantly higher than the tildipirosin-treated groups (0 to 1.7%).

In the first study, *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* isolates were obtained from the pre-treatment nasopharyngeal swab samples and the lung tissue samples collected from the mortalities. In the second study, *Mannheimia haemolytica* and *Pasteurella multocida* isolates were obtained from the pre-treatment nasopharyngeal swab samples and the lung tissue samples collected from the mortalities.

Based on the results of these studies, a single subcutaneous dose of 4 mg tildipirosin/kg BW was selected for use in clinical field studies conducted to provide substantial evidence of effectiveness.

B. Substantial Evidence:

1. Natural Infection Clinical Field Study

a. Title: "Multi-center field dose confirmation study of the therapeutic efficacy of a single injection of 4 mg/kg body weight of 20, 23-di-piperdinyl-mycaminosyl-tylonolide (tildipirosin) in cattle undergoing a naturally occurring outbreak of bovine respiratory disease." Study Number 2052-010-00. October 2007 to November 2007.

b. Study Investigators and Locations:

David T. Bechtol, D.V.M., Agri Research Center, Canyon, TX
Edward G. Johnson, D.V.M., Johnson Research, LLC, 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
Karen C. Rogers, D.V.M., VRCS, LLC, Greeley, CO

c. Study Design:

i. *Objective:* To demonstrate the effectiveness of tildipirosin injectable solution for the treatment of bovine respiratory disease (BRD).

ii. *Test Animals:* Cross-bred and pure-bred beef steers, bulls, and heifers, six to twelve months of age, weighing 292 to 751 lbs.

iii. *Experimental Design:* The study was conducted at five sites. At each site, calves were enrolled in the study when they were diagnosed with BRD and met the enrollment criteria of respiratory score ≥1, attitude score of 2 or 3, and rectal temperature ≥104.0 °F. Animals that were moribund (attitude score of 4), other concurrent systemic disease, or a severe injury were not enrolled. The following clinical scoring scales were used:

Respiratory Scoring Scale:

0 = Normal: no abnormal respiratory symptoms are present; respiratory rate and effort are appropriate for the environment.

1 = Mild respiratory distress: serous nasal or ocular discharge and/or cough.

2 = Moderate respiratory distress: mucous or mucopurulent nasal or ocular discharge and/or increase in respiratory rate or effort.

3 = Severe respiratory distress: marked increase in respiratory rate or effort, including one or more of the following: open-mouth breathing, abdominal breathing, and/or extended head.

Attitude Scoring Scale:

0 = Normal: bright, alert, and responsive.

1 = Mildly depressed: may stand isolated with its head held down or ears drooping, but is responsive to stimulation.

2 = Moderately depressed: may remain recumbent or stand isolated with head down, may show signs of muscle weakness (standing cross-legged, knuckling or swaying when walking), depression obvious when stimulated.

3 = Severely depressed: may be recumbent and reluctant to rise, or if standing, is isolated and reluctant to move; when moving, is ataxic, knuckling or swaying evident; head carried low with ears drooping; eyes dull, possible excess salivation/lacrimation, obvious gauntness.

4 = Moribund: Unable to stand; approaching death; highly unlikely to respond to any antimicrobial therapy.

At each site, enrolled calves were randomly assigned to one of three treatments (tildipirosin, saline, or positive control) and allocated to pens in groups of three (one calf per treatment group) until study pens were filled to capacity. The housing of the calves reflected standard feedlot practices. The individual calf was the experimental unit. A positive control group was also included in the study, but was not analyzed statistically.

Nasopharyngeal swabs were collected for bacterial culture from each enrolled animal pre-treatment and, if applicable, at treatment failure. Lung tissue samples were taken from each animal found dead or euthanized during the course of the study. All samples were cultured for BRD pathogens.

iv. *Test Article Administration:* The test article was tildipirosin injectable solution (180 mg/mL, commercial formulation). The control article was saline (0.9% sodium chloride) injectable solution. Treatments were administered subcutaneously (SC) in the neck once on the day of enrollment (Day 0). The maximum injection volume did not exceed 10 mL for either tildipirosin or saline. Across the five study sites, dose volumes administered during the study ranged from 3.3 mL to 6.8 mL for tildipirosin and 2.9 mL to 7.5 mL for saline.

v. *Treatment groups:* The treatment groups are described below in Table II.3.

Table II.3. Treatment Groups.

Treatment	Dosage	Number of Animals
Saline	0.022 mL/kg BW[1] SC once	300
Tildipirosin	4 mg tildipirosin/kg BW (0.022 mL/kg BW) SC once	300

[1]Volume equivalent to tildipirosin injectable solution dosages.

vi. *Measurements and Observations:* Cattle were evaluated daily and scored for attitude and respiration. From Day 1 through Day 14, any animal assigned an attitude score of 4 was designated as a treatment failure, weighed, had its rectal temperature measured, and was euthanized and necropsied. Animals that died due to BRD while on study were also designated as treatment failures. Starting on Day 3 after treatment and continuing through Day 13, any calf meeting the following criteria was designated a treatment failure: respiratory score ≥2, and attitude score of ≥2, and rectal temperature ≥104.0 °F or respiratory score of 3 regardless of attitude score or rectal temperature, or attitude score of ≥3 regardless of respiratory score or rectal temperature. On Day 14 any calf not previously determined to be a treatment failure and meeting the following criteria, was considered a treatment success: respiratory score of ≤1, and attitude score of ≤1, and rectal temperature <104.0 °F.

The effectiveness of tildipirosin for the treatment of BRD was evaluated by comparing the proportion of treatment successes in the tildipirosin-treated group to the saline-treated control group.

vii. *Statistical Analysis:* The study was conducted as a randomized complete block design. The treatment success rate on Day 14 was analyzed using an α = 0.05 two-sided significance level using the GLIMMIX procedure of SAS Version 9.1.3. Treatment was included as a fixed effect and location, pen-within-location, and location-by-treatment were included as random effects. To model overdispersion, the residual term was included as a separate random statement.

d. Results:

i. *Treatment Success*: The percentage of calves classified as a treatment success was statistically significantly higher (p=0.003) in the tildipirosin-treated group (229/300, 76%) compared to the saline-treated control group (96/300, 32%).

ii. *Mortality:* There was no mortality in the tildipirosin-treated group compared to a 7% mortality rate (21/300) in the saline-treated control group.

iii. *Microbiology:* Sufficient numbers of isolates of *M. haemolytica*, *P. multocida*, and *H. somni* were recovered across the study for inclusion in the BRD treatment indication.

e. Adverse Reactions: There were no systemic adverse reactions observed from administration of tildipirosin during the study. The only adverse events documented during the study were mild to moderate injection site swellings.

f. Conclusions: The results of this study demonstrate that tildipirosin, when administered as a single subcutaneous injection of 4 mg/kg BW, is effective for the treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in beef and non-lactating dairy cattle.

2. Natural Infection Clinical Field Study

a. Study Title: "Multi-center field dose confirmation study of a single injection of 4 mg/kg body weight of 20, 23-di-piperdinyl-mycaminosyl-tylonolide (tildipirosin) in cattle at high risk for developing bovine respiratory disease." Study Number 2052-009-00. October 2007 to November 2007.

b. Study Investigators and Locations:

David T. Bechtol, D.V.M., Agri Research Center, Canyon, TX
Edward G. Johnson, D.V.M., Johnson Research, LLC, Parma, ID
Kelly F. Lechtenberg, D.V.M., Ph.D., Midwest Veterinary Services, Inc., Oakland, NE

Breck Hunsaker, D.V.M., Ph.D., Horton Feedlot and Research Center, Wellington, CO
Karen C. Rogers, D.V.M., VRCS, LLC, Greeley, CO

c. Study Design:

i. *Objective:* To demonstrate the effectiveness of tildipirosin in cattle at high risk of developing BRD.

ii. *Test Animals:* Cross-bred and pure-bred beef steers, bulls, and heifers, six to 12 months of age, weighing 404 to 782 lbs.

iii. *Experimental Design:* The study was conducted at five sites. Only healthy calves with respiratory score ≤ 1, attitude score equal to 0, rectal temperature <104.0 °F, and no concurrent systemic disease or severe injury were enrolled in the study. The following clinical scoring scales were used:

Respiratory Scoring Scale:

0 = Normal: no abnormal respiratory symptoms are present; respiratory rate and effort are appropriate for the environment.

1 = Mild respiratory distress: serous nasal or ocular discharge and/or cough.

2 = Moderate respiratory distress: mucous or mucopurulent nasal or ocular discharge and/or increase in respiratory rate or effort.

3 = Severe respiratory distress: marked increase in respiratory rate or effort, including one or more of the following: open-mouth breathing, abdominal breathing, and/or extended head.

Attitude Scoring Scale:

0 = Normal: bright, alert, and responsive.

1 = Mildly depressed: may stand isolated with its head held down or ears drooping, but is responsive to stimulation.

2 = Moderately depressed: may remain recumbent or stand isolated with head down, may show signs of muscle weakness (standing cross-legged, knuckling or swaying when walking), depression obvious when stimulated.

3 = Severely depressed: may be recumbent and reluctant to rise, or if standing, is isolated and reluctant to move; when moving, ataxic, knuckling or swaying evident; head carried low with ears drooping; eyes dull, possible excess salivation/lacrimation, obvious gauntness.

4 = Moribund: Unable to stand; approaching death; highly unlikely to respond to any antimicrobial therapy.

At each site, enrolled calves were randomly assigned to one of three treatments (tildipirosin, saline, or positive control) and were allocated to pens in groups of three (one calf per treatment group) until study pens were filled to capacity, except for one pen at the Nebraska site which was filled to 91% capacity. The housing of the calves reflected standard feedlot practices. The individual calf was the experimental unit. A positive control group was also included in the study, but was not analyzed statistically.

Nasopharyngeal swabs were collected for bacterial culture from each enrolled animal pre-treatment and, if applicable, at treatment failure. Lung tissue samples were taken from each animal found dead or euthanized during the course of the study. All samples were cultured for BRD pathogens.

iv. *Test Article Administration:* The test article was tildipirosin injectable solution (180 mg/mL, commercial formulation). The control article was saline (0.9% sodium chloride) injectable solution. Treatments were administered subcutaneously (SC) in the neck once on the day of enrollment (Day 0). The maximum injection volume did not exceed 10 mL for either tildipirosin or saline. Across the five study sites, dose volumes administered during the study ranged from 4.1 to 7.6 mL for tildipirosin and 4.2 to 7.7 mL for saline.

Treatment groups: The treatment groups are described below in Table II.4.

Table II.4. Treatment Groups.

Treatment	Dosage	Number of Animals
Saline	0.022 mL/kg BW[1] SC once	387
Tildipirosin	4 mg tildipirosin/kg BW (0.022 mL/kg BW) SC once	386

[1]Volume equivalent to tildipirosin injectable solution dosages.

v. *Measurements and Observations:* Cattle were evaluated daily and scored for attitude and respiration. Starting on Day 1 after treatment and continuing through Day 14, any calf meeting the following criteria was designated a treatment failure: attitude score =1 or 2 and rectal temperature ≥104.0 °F, or respiratory score =2 and rectal temperature ≥104.0 °F, or respiratory score =3 regardless of rectal temperature, or attitude score of ≥3 regardless of rectal temperature. Any animal assigned an attitude score of 4 was designated a treatment failure, weighed, had its rectal temperature measured, and was euthanized and necropsied. Animals that died due to BRD while on study were also designated as treatment failures. Calves that completed the study (Day 14) and were not designated treatment failures were designated treatment successes.

The effectiveness of tildipirosin for the treatment of BRD was evaluated by comparing the proportion of treatment successes in the tildipirosin treatment group to the saline-treated control group.

vi. *Statistical Analysis:* The study was conducted as a randomized complete block design. The treatment success rate for BRD was analyzed using $\alpha=0.05$ two-sided significance level using the GLIMMIX procedure of SAS Version 9.1.3. Treatment was included as a fixed effect and location, pen-within-location, and location-by-treatment were included as random effects. To model overdispersion, the residual term was included as a separate random statement.

d. Results:

i. *Treatment Success*: The percentage of calves classified as a treatment success was statistically significantly higher ($p=0.001$) in the tildipirosin-treated group (305/386, 79%) compared to the saline-treated control group (197/387, 51%).

ii. *Mortality:* There were three calves that died or were euthanized due to BRD during the study (one tildipirosin-treated calf and two saline-treated calves).

iii. *Microbiology:* Sufficient numbers of isolates of *M. haemolytica*, *P. multocida*, and *H. somni* were recovered across the study for inclusion in the control of high risk of BRD indication.

e. Adverse Reactions: There were no systemic adverse reactions observed from administration of tildipirosin during the study. The only adverse events documented during the study were mild to moderate injection site swellings.

f. Conclusions: The results of this study demonstrate that tildipirosin, when administered as a single subcutaneous injection of 4 mg/kg BW, is effective for the control of respiratory disease in beef and non-lactating dairy cattle at high risk for developing BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*.

III. TARGET ANIMAL SAFETY:

A. Systemic Target Animal Safety (Toxicity) Study

1. Study Title: "Target Animal Safety Study in Cattle Injected subcutaneously with 18% w/v PMT (20, 23-di-piperidinyl-mycaminosyl-tylonolide) Solution for Injection, at 1, 3, and 5X the Proposed Maximum Dose Level, Administered on 3 Occasions, 7 Days Apart." Study Number V-0045-0185. November 2008 to December 2008.

2. Study Director and Location:
Ciara Vance, BSc, Charles River Laboratories, Tranent, Edinburgh, UK

3. Study Design:

a. *Objective:* To demonstrate a margin of safety for tildipirosin when administered subcutaneously (SC) in cattle at 0, 4, 12, or 20 mg/kg BW on three occasions, 7 days apart.

b. *Test Animals:* A total of 32 (16 intact male, 16 intact female) healthy, weaned beef cattle (Aberdeen Angus cross and one Belgian Blue) were obtained from a single supplier and enrolled in the study. Animals were approximately 5 months of age and weighed between 124.0 and 183.5 kg at arrival (on or before Day -19). Animals were identified by duplicate individually numbered ear tags. Within 24 hours of arrival, all animals were administered a single oral dose of fenbendazole and cefquinome 2.5% suspension IM once daily at the labeled dose for 3 consecutive days. All IM doses were administered in the hindquarters.

Animals were randomly allocated to treatments and pens. Eight blocks were formed based on the facility diagram such that four adjacent pens formed a block. Same sex animals were grouped together (four to a group) based on similar body weights and randomly assigned to blocks and pens within blocks. Within-block animals were randomly assigned to treatment. The study was comprised of eight blocks (two male and two female animals per block). To facilitate necropsy, each treatment group was divided into two subgroups for staggered treatment administration days.

Animals were housed individually in indoor pens labeled with the animal number and study number. Animals had access to an appropriate ration of non-medicated feed pellets fed twice daily during the study and were fed hay for the first few days after arrival. Water was provided *ad libitum*. All study animals were euthanized at the end of the study.

c. *Test Article Administration:* The test article, tildipirosin 18% w/v injectable solution, was identical to the intended commercial formulation. The control article was sterile physiologic saline for injection. Treatment groups are summarized in Table III.1. Animals were dosed based on body weights obtained the day prior to treatment administration. A maximum injection volume of 10 mL per injection site was used. The left lateral neck was used as injection site for treatment 1 (Day 0), the region behind the left shoulder for treatment 2 (Day 7), and the right lateral neck for treatment 3 (Day 14). If multiple injections were required per treatment occasion, injections were administered cranially to caudally.

Table III.1. Summary of treatment groups.

Group	Treatment Regimen	No. of Animals
1	Saline (0X), volume equivalent to the 5X treatment group (0.5 mL/kg BW), administered SC on Days 0, 7, and 14	8 (4M & 4F)

Group	Treatment Regimen	No. of Animals
2	Tildipirosin, 4 mg/kg BW (1X), administered SC on Days 0, 7, and 14	8 (4M & 4F)
3	Tildipirosin, 12 mg/kg BW (3X), administered SC on Days 0, 7, and 14	8 (4M & 4F)
4	Tildipirosin, 20 mg/kg BW (5X), administered SC on Day 0, 7, and 14	8 (4M & 4F)

d. *Measurements and Observations:* General health observations were conducted twice daily from arrival to the end of the study (Day 21). On Day -2, animals were enrolled in the study if they were clinically healthy and had normal pre-treatment hematology, clinical chemistry, urinalysis, and fecal analysis results. Study animals were 5 months old and weighed between 100 kg and 180 kg at arrival. On each treatment day, animals were continuously observed for abnormal events from before administration to 6 hours post-treatment. Additional observations were performed at 2, 6, and 8 hours after each treatment, and at 48 and 72 hours after the first and second treatments. Clinical examinations and injection site evaluations were conducted on Days -2, 1, 8, 15, and 19. Body weights were recorded at enrollment, and on Days -12, -11, -1, 6, 13, and 20. Individual feed consumption (twice daily) and water consumption (once daily) were measured from approximately Day -7 through Day 20. Blood samples were collected from the jugular vein for hematology, coagulation, and clinical chemistry parameters on Days -12, -11, -3, 6, 13, and 20. Urine and fecal samples were collected for analysis on Days -3, 13, and 20. All observations and data collection were performed by masked, trained personnel.

   All study animals were euthanized on Day 21. Each animal was subjected to detailed necropsy and gross pathological investigation by a masked veterinary pathologist. Organ weights were obtained for the brain, pituitary gland, kidneys, heart, liver, adrenal glands, spleen, and gonads (testes or ovaries), and reported as [relative weights to body weight] a percent of Day 20 body weight. Representative tissues were collected for histopathology, including gross lesions and injection site tissue from the first injection site from all groups for each treatment day.

e. *Statistical Methods*: All continuous variables were analyzed using a mixed-model of covariance. Variables measured multiple times were analyzed using a repeated measures analysis of covariance with the following fixed effects: baseline value (covariate: Day -3), treatment group, sex, time, and two- and three-way interactions. Animal number was included in the model as a random effect. Statistical comparisons of treatment effects and comparison of treatment by time were performed at the 0.1 level of significance. Comparison of treatment by sex, and treatment by time by sex were performed at the 0.05 level of significance.

4. Results:

a. *Clinical Observations*: There were no statistically significant differences in water consumption, feed consumption, or body weight for any of the tildipirosin-treated groups compared to the controls. Abnormal clinical observations are described in Table III.2.

Table III.2. Summary of abnormal clinical observations (except injection site reactions) reported during the treatment phase of the study (Days 0 to 21).

Group	Observations
1 (0X)	Lip smacking [one animal, Day 2 and Day 14] Ringworm [one animal, present prior to treatment]
2 (1X)	Subdued behavior [one animal, 6 hours post-treatment, Day 0]
3 (3X)	Mild occasional cough and mild wounds on right hind leg [one animal, Day 14], not related to an injection site
4 (5X)	Posture and movement abnormalities noted with wound on right hind leg [one animal, Day 14], not related to an injection site

With the exception of subdued behavior observed in the 1X group, abnormal clinical observations in the study were considered not related to test article administration. The cause of subdued behavior is unknown but was considered likely related to injection site inflammation.

Injection site swelling was noted in all tildipirosin-treated animals in all groups. Heat (one animal in the 3X group) and signs of pain (all animals in all tildipirosin-treated groups) including mild lameness, pain on palpation of injection sites, decreased appetite and water consumption for 24 to 48 hours post injection, tail twitching, increased respiratory rates, tail switching, pawing at the ground, head shaking, and restlessness were reported in association with the swellings, which ranged in size up to 30 cm x 20 cm. On Day 19, injection site swelling was still evident in one animal in the 1X group, one animal in the 3X group, and three animals in the 5X group.

Control animals gained weight throughout the study. The 1X group gained weight from Day -1 to Day 13 and weight remained constant in the group from Day 12 to Day 20. The 3X group gained weight throughout the study. The 5X group gained weight from Day -1 to Day 13, but lost weight from Day 13 to Day 20. Differences between groups were not statistically significant and a dose-related trend was not clearly evident. In addition, all groups gained weight from Days -1 to 20 (Table III.3).

Table III.3. Mean body weight values (kg).

Group	Day -1	Day 6	Day 13	Day 20
1 (0X)	168.1	178.5	184.1	185.0
2 (1X)	168.4	178.4	184.8	184.8

Group	Day -1	Day 6	Day 13	Day 20
3 (3X)	166.4	177.5	184.1	184.6
4 (5X)	174.3	186.1	189.3	187.4

b. *Laboratory Analyses*: Statistically significant differences were seen in each of the tildipirosin-treated groups compared to the control group for some hematological variables (large unclassified cells, platelets, mean corpuscular hemoglobin, mean corpuscular volume, monocytes, eosinophils, and fibrinogen) and some clinical chemistry variables (aspartate aminotransferase, alanine aminotransferase, inorganic phosphorous, creatine phosphokinase, magnesium, blood urea nitrogen, amylase, and total bile acids). However, only one hematological variable (fibrinogen) and two clinical chemistry variables (aspartate aminotransferase and creatine phosphokinase) were considered clinically significant and are described below:

Fibrinogen values were statistically significantly lower at most time points in all of the tildipirosin-treated groups, showed a dose-dependent trend, and were more severe in males compared to females. However, the values appeared to improve or be improving by Day 20, and were considered related to injection site inflammation.

Aspartate aminotransferase (AST) values were statistically significantly increased in all of the tildipirosin-treated groups compared to the control group and showed a dose-dependent trend. The AST values in all tildipirosin-treated groups were increased above published reference ranges for AST in cattle, and were considered related to injection site inflammation.

Creatine phosphokinase (CPK) values were statistically significantly increased in the tildipirosin-treated animals on Day 2, showed a dose-dependent trend, and were considered primarily related to injection site inflammation. CPK increases were transient and returned to values similar to those of the control group by Day 6.

There were no clinically significant differences in urine and fecal analysis results between groups.

c. *Post-Mortem Findings*:

Gross Necropsy: The adjusted mean kidney weights were statistically significantly higher in all tildipirosin-treated male groups compared to the control group in males. For female animals, only the tildipirosin 5X group adjusted mean kidney weights were statistically significantly higher than the control group. There were no abnormal gross necropsy findings noted in the kidney tissues. Adjusted mean liver weights in the 5X group were statistically significantly higher compared to the control group. One animal in the 5X group had necrotic foci (multifocal) and inflammation (minimal to mild) in the liver. Necrotic foci and inflammation were not found in the other treated groups or the control group. These findings were not considered clinically significant.

Histopathology: Histopathologic examination was performed on tissues from the control and 5X groups, as well as injection site tissue from all groups. Subcutaneous injection sites in the 3X and 5X groups were associated with necrosis, inflammation, fibroplasia, and myofiber regeneration at 7, 14, and 21 days after dosing. In addition, subcutaneous injections in all tildipirosin-treated groups were associated with necrosis, fibroplasia, and myofiber regeneration at 7 and 14 days after dosing. One animal in the 1X group showed granulomatous panniculitis at 21 days post-dosing.

5. Conclusion: Tildipirosin 18% w/v injectable solution is safe in beef and non-lactating dairy cattle when administered subcutaneously once at 4 mg/kg BW (the labeled dose). Administration of tildipirosin 18% w/v injectable solution to cattle may cause injection site swelling and inflammation that may be severe.

B. Injection Site Tolerance Study

1. Study Title: "Injection Site Safety of a Single Subcutaneous Injection of PMT (20, 23-di-piperidinyl-mycaminosyl-tylonolide) 18% Injectable Solution Administered to Cattle." Study Number V-0045-0231. October 2008 to November 2008.

2. Study Director and Location:
Mary I. Wray, Ph.D., Intervet, Inc., De Soto Research Farm, De Soto, KS.

3. Study Design:

   a. *Objective:* To evaluate the potential clinical and injection site reactions associated with a single subcutaneous (SC) administration of tildipirosin 18% injectable solution at the maximum injection volume of 10 mL in cattle.

   b. *Test Animals:* A total of 27 healthy castrated male cattle were obtained from multiple sources and enrolled in the study. Animals were commercial Angus cross breed steers that were approximately 5 to 9 months old and weighed 482 to 684 lbs upon arrival. At arrival, each animal was administered an intranasal viral respiratory disease vaccine, a subcutaneous (left thorax) *Clostridium* bacterin, and an oral dose of fenbendazole.

   Steers were housed in a partially-covered building in pens of three steers each under ambient environmental conditions. Diets were formulated to meet the National Research Council nutritional requirements for beef cattle. Feed and water were available *ad libitum*. No concurrent medications, other than a coccidiostat in the feed, were administered during the study.

   c. *Test Article Administration:* The test article was tildipirosin 18% w/v injectable solution in the commercial formulation. Treatments were administered on the assigned day (Table III.4 below) by SC injection in

the right neck region. Injection sites were clipped and permanently marked prior to injection.

Table III.4. Summary of treatment groups.

Group	Treatment Regimen	Time to Necropsy	No. of Animals
T1	Tildipirosin, 10 mL, administered SC on Day 0	35 days	6
T2	Tildipirosin, 10 mL, administered SC on Day 7	28 days	6
T3	Tildipirosin, 10 mL, administered SC on Day 14	21 days	6
T4	Tildipirosin, 10 mL, administered SC on Day 21	14 days	6

d. *Measurements and Observations:* During the acclimation period (arrival to Day -6) and the baseline period (Days -6 to -1), steers were observed at least once daily for general health. On Day -7, animals were weighed for randomization and physical exams were conducted (including examination of intended injection site locations). Animals were observed for signs of abnormal behavior, locomotion, injection site irritation (visual observation of swelling and pain) from 1 to 4 hours after treatment and daily until Day 35. Injection sites were measured for volume of swelling and palpated for heat and pain on Days 7, 14, 21, 28, and 35. On Day 35, steers were euthanized and necropsied for gross examination and photography of injection sites. At necropsy, tissues were systematically examined at the levels of the external skin surface, subcutaneous tissue and underlying skin, surface neck musculature, and deep neck musculature.

e. *Statistical Methods*: None.

4. Results**:**

a. *Clinical Observations*: There were no serious systemic adverse events in the study. Evidence of pain immediately following injection was noted in one animal. No abnormalities in behavior or locomotion were observed, and no other observations of pain were noted following injection through Day 35.

Injection site swellings were observed during the live phase and abnormal tissue observations were observed at necropsy. Steers in the T2 group had injection site swellings up to 339 cm^3 in volume and up to 15.4 cm x 8.7 cm in area reported during the first 7 days after treatment with swellings measuring 8 cm x 16 cm during the first 4 hours after treatment. In treatment groups T1 and T2 there were no palpable lesions by 28 days post-treatment administration. In treatment group T3 there were no palpable lesions by 21 days post-treatment administration (end of study). In treatment group T4, four of six animals had palpable swellings 14 days post-treatment administration (end of study).

b. *Gross Necropsy Findings*: Group T1 had no animals with abnormalities in the deep or surface muscle. One animal had a lesion in the subcutaneous tissue attached to subcutaneous muscle and one animal had a surface skin change described as raised white foci.

Group T2 had no animals with abnormalities in the deep or surface muscle tissue. The subcutaneous tissue attached to the muscle from all Group T2 cattle had red or tan focal lesions or a thickened appearance. The dermis of five animals showed these lesions. Multiple white foci were noted on the external skin surface of one animal.

Group T3 had no visible deep muscle lesions. One animal had a red focus and thickened area on the surface muscle. One animal had abnormalities in the surface muscle consisting of a red focus and thickened area. The dermis in three steers, and the subcutaneous tissue in four steers, had red or tan focal lesions or thickened areas. One steer showed skin nodules that were multifocal in distribution with respect to the injection site and a second steer had a skin nodule located in the center of the injection site.

Group T4 had no visible deep muscle lesions. All cattle showed red or tan foci and/or thickened areas in the dermis and subcutaneous tissue. One animal exhibited fluid-filled and friable subcutaneous tissue. Three animals showed lesions in the surface muscle consisting of red or tan foci. One steer exhibited swelling that was firm on palpation and extended ventral and caudal to the injection site. The skin surfaces of the T4 cattle were affected with skin nodules that were typically small but one nodule measured 6 cm in one animal.

5. Conclusion: Tildipirosin 18% w/v injectable solution, when administered subcutaneously to beef and non-lactating dairy cattle at 10 ml per injection site, causes injection site swelling and inflammation that may be severe. Swelling and visible lesions in edible tissue persisted at least 35 days after injection.

IV. HUMAN FOOD SAFETY:

A. Microbial Food Safety (Antimicrobial Resistance)

The Agency evaluated a *hazard characterization* and qualitative risk assessment describing the microbial food safety associated with a single use of ZUPREVO (20, 23-di-piperidinyl-mycaminosyl-tylonolide [PMT] or tildipirosin) 18% injectable solution for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in beef and non-lactating dairy cattle, and for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*.

In the context of the conditions of use, the microbial food safety hazard was defined as human campylobacteriosis caused by macrolide-resistant *Campylobacter* spp. attributable to the consumption of beef derived from

tildipirosin-treated cattle, and subsequently treated with human-use macrolides. The hazardous agent was considered to be macrolide-resistant *Campylobacter* spp. in or on treated cattle (and subsequently cattle-derived food products), as a consequence of the proposed use of tildipirosin in cattle.

The firm's qualitative risk assessment included 1) a *release assessment* to describe the probability that the conditions of use for tildipirosin in cattle will not result in the emergence of resistant food-borne pathogens, 2) an *exposure assessment* to describe the likelihood of human exposure to resistant food-borne pathogens through consumption of cattle-derived food products, and 3) a *consequence assessment* to describe potential human health consequences arising from exposure to defined resistant pathogens by considering the human medical importance of macrolides in the treatment of human infectious diseases.

The *release assessment* was ranked medium, based on the integration of relevant individual parameters favoring resistance emergence in bacteria of cattle origin, particularly zoonotic, food-borne pathogens. The primary food-borne pathogen of interest for this application was *Campylobacter* spp. Enterococci were considered as commensal organisms of interest.

Human exposure to macrolide-resistant *Campylobacter* spp. was assessed and ranked medium. The *exposure assessment* was derived from two separate yet interrelated parts: 1) *per capita* consumption of beef in the U.S., which is high, and 2) prevalence of *Campylobacter* spp. contamination in cattle-derived food products, which is low. This medium ranking of *exposure* should be considered conservative, as National Antimicrobial Resistance Monitoring System (NARMS) retail meat surveillance data from 2002 to 2007 found *Campylobacter* contamination in retail beef to be low.

Macrolides are ranked as critically important drugs in human medicine; thus, by default, the *consequence assessment* yields a high ranking.

Decision Statement: The Agency's integration of the degree of risk derived from the three individual assessments (medium, medium, and high) gave an overall risk estimation of high. The conditions of use are compatible with the Agency's risk management strategies for a Category 1 drug, corresponding to the estimated high risk. The product is available as prescription (Rx) only, and drug delivery is by injection in individual animals for treatment of BRD or control of BRD in high risk cattle associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*, and thus, the extent of use is relatively low. Further, post-approval monitoring may be achieved from surrogate antimicrobials (erythromycin and azithromycin) in the current NARMS programs.

B. Impact of Residues on Human Intestinal Flora

1. Determination of the need for establishing a microbiological ADI (mADI)

A step-by-step approach was followed to determine whether there is a concern for effect of tildipirosin residues on human intestinal flora.

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

Yes, tildipirosin has activity against representative human intestinal flora. This conclusion was supported by an *in vitro* susceptibility study (summarized below) performed against representative bacterial groups from human subjects.

i. Study Title: "Activity of Tildipirosin Against Bacterial Strains Representing the Normal Human Intestinal Microbiota: Determination of Minimum Inhibitory Concentration (MIC)."

ii. Study Number: DWS Study No.: 062/05

iii. Study Director: Andrew Pridmore, BSc, PhD

iv. Study Location: Don Whitley Scientific Ltd., United Kingdom

v. Study Report Date: 25 August, 2006 (Experimental work performed between February 23 and March 31, 2006)

vi. Study design: The MIC of tildipirosin was measured in 100 bacterial strains (10 isolates from each of 10 bacterial groups) representing normal human intestinal flora. All bacterial strains were collected from the fecal microbiota of healthy, unmedicated human volunteers. The testing system was a standardized agar dilution method as described in Clinical and Laboratory Standards Institute (CLSI) guidelines. For each strain used in the MIC testing, the standardized inoculum was enumerated to demonstrate compliance with CLSI standards. Strains of *Bacteroides fragilis* ATCC 25285 and *Enterococcus faecalis* ATCC 29212 were used for quality control purposes, with MIC QC ranges between 0.5 and 2 µg/ml and between 4 and 16 µg/ml, respectively.

vii. Results and conclusions: Tildipirosin demonstrated variable *in vitro* activity against different *genera* or species of tested bacterial groups. Moderate activity was observed against *E. coli*, *Fusobacterium*, *Clostridium*, and *Enterococcus* isolates. Poor or no activity was found for *Lactobacillus*, *Peptostreptococcus*, *B. fragilis*, and other *Bacteroides* spp. Detailed MIC information is listed in Table IV.1 (below):

Table IV.1. *In vitro* susceptibility of tildipirosin against representative human intestinal flora.

Bacterial Group	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Geometric MIC (µg/ml)	MIC range (µg/ml)
B. fragilis	32	>128	48	8 - >128
Other *Bacteroides*	32	>128	48	8 - >128
Bifidobacterium	16	64	17	1 - >128
Clostridium	2	64	4.3	0.5 - >128

Bacterial Group	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Geometric MIC (µg/ml)	MIC range (µg/ml)
Enterococcus	8	8	7.5	4 - 8
E. coli	4	8	5.7	2 - 16
Eubacterium	32	64	37	16 - >128
Fusobacterium	2	64	5.3	1 - 64
Lactobacillus	128	>128	56	8 - >128
Peptostreptococcus	>128	>128	79	4 - >128
All strains (n=100)	16	>128	19	0.5 - >128

b. **Step 2**: Do residues enter the human colon?

Yes, the firm performed a study in rats using radiolabeled tildipirosin and demonstrated that tildipirosin residues enter the human colon. The study is described below in the section titled *Determination of the fraction of oral dose available for microorganisms.*

c. **Step 3**: Do residues entering the colon remain microbiologically active?

Yes, tildipirosin remains microbiologically active in the colon, as demonstrated in the study described below in the section titled *Determination of the fraction of oral dose available for microorganisms*.

d. **Step 4**: Is there any scientific justification to eliminate testing for either one or both endpoints of concern, i.e., colonization barrier disruption or resistance development?

Based on the firm's written assessment and conclusions, the Agency does not consider the development of resistant bacteria as an endpoint of concern for the following reasons:

- Many Gram-negative anaerobes are intrinsically resistant to macrolides, as supported by submitted MIC data;
- Macrolides are generally not the *drug of choice* for anaerobic infections;
- The high end of the MIC range and the MIC_{50} of the majority of bacterial groups tested are higher than the projected residue levels in edible muscle tissue of cattle consumed by humans;
- *In vitro* susceptibility study results (described in Step 1 above) suggest that non-susceptible populations already exist in most bacterial groups in the normal human population; therefore, it would be almost impossible to evaluate development of resistance to tildipirosin among human intestinal flora.

Therefore, determination of mADI will be based on the colonization barrier disruption endpoint.

2. Determination of the final mADI

   a. Determination of the "fraction of oral dose available to microorganisms"

      The firm conducted a study to demonstrate the fraction of tildipirosin available to microorganisms, and the study is summarized below. Based on the evaluation of the study data, it is concluded that the "fraction" to be used for calculation consists of three factors, yielding a final value of "fraction" available to microorganisms of 0.182% (i.e., 42.5% x 60% x 71.3%). The three factors are as follows.

      - An average of 42.5% tildipirosin-related residues enter the colon;
      - Of these, about 60% were tildipirosin (up to 7.7%) and its major metabolite (about 52.1%); The remaining 40% are considered inactive;
      - A maximum of 71.3% of tildipirosin and its metabolite were extractable from feces, representing the free or unbound portion.

      i. Study title: "A Chromatographic Investigation into the Absorption, Distribution, Metabolism, and Excretion of ^{14}C-tildipirosin Following Multiple Oral Administrations at a Dose Level of 25 µg and 25 mg tildipirosin/kg Body Weight to Rats."

      ii. Study number: Intervet Study V-0045-0047

      iii. Study Location: Charles River Laboratories, Tranent, Edinburgh, United Kingdom (Charles River Study No.: 209981, and Charles River Laboratories Report No.: 27448)

      iv. Study Director: Chris Lowrie, BSc

      v. Study Report date: December 15, 2006

      vi. Study design: The objective of the study was to determine the total radioactive residues (TRR) and metabolite profile (by HPLC) of ^{14}C- tildipirosin in selective tissues, urine, feces, and colon samples from orally dosed rats. ^{14}C- tildipirosin was given daily to three male and three female rats at 0.025 and 25 mg/kg BW for 7 days. One male and one female were dosed with vehicle only (control animals). Animals were housed individually in glass metabolism cages for daily collection of urine and feces. Following sacrifice, tissue samples (brown fat, muscle, liver, and kidney) were collected from the high dose animals, and colon contents from all animals. Samples were analyzed for radioactive residues. HPLC analysis and mass spectrometry were used on composite samples of liver, kidney, urine, feces, and colon contents.

      vii. Results and conclusions: The mean amount of total radioactive residues present in the colon on day 7 (including parent tildipirosin and its major metabolite M7 [consistent with *dihydro-PMT-SO3H*]) were 42.5% of the low dose and 40.8% of the high dose. The radio-HPLC results showed that, among residues in the colon, unchanged parent tildipirosin represented only between 4.6% and

7.7% of the TRR. Metabolite M7 represented up to 52.1% (range 32.3% to 52.1%) of the TRR in the rat colon. Extraction assays further demonstrated that up to 71.3% residues were extractable. Other radioactive metabolites found in the colon representing multiple structurally different chemicals.

b. Determination of the mADI using MIC_{calc}

For the purpose of calculation of MIC_{calc}, MIC_{50}s from *in vitro* susceptibility data derived from DWS Study No. 062/05 (described above under Step 1) were considered. According to their relevance to the activity of tildipirosin and the likelihood of being affected by residues, five groups or *genera* (i.e., *Clostridium, Fusobacterium, E. coli, Enterococcus, and Bifidobacterium*) were included in the determination of MIC_{calc}, which was set at 2.47 µg/ml.

Tildipirosin's mADI is 50 µg/kg BW/day (or 3 mg/person/day).

C. Toxicology:

1. Summary of Toxicology Studies

All pivotal testing was conducted in acceptable compliance with the Good Laboratory Practice (GLP) regulation (21 CFR 58). Toxicity tests determining the human food safety of tildipirosin (PMT) are summarized below:

a. **4-Week Oral (Gavage) Toxicity Study in Rodents**

Study Title: "PMT-Macrolide Rat Repeat Dose 28-Day Oral Toxicity."

Study No: PT04-0147; Intervet Reference Number: V-0045-0004

Report Date: November 19, 2004

Study Director: Dr. H. M. Kauffmann

Study Location (in-life): Aventis Pharma, Hatterheim, Germany

Purpose: This study was designed to address any potential systemic toxicological effects of PMT.

Experimental Design: Hsd:Sprague Dawley rats (5/sex/group) were dosed daily by oral gavage with PMT solution for 28 days at either 40, 160, or 640 mg/kg BW/day PMT tartrate, *i.e.* 25, 100, and 400 mg/kg BW/day free base, while control rats were fed a buffered aqueous solution (pH 7.4). The dose volume was 5 mL/kg BW/day. Clinical observations and body weight measurements were obtained. Blood chemistry, hematology, urine analysis, necropsy, and histopathology were performed on the final day of the study. Necropsy was performed on all animals, and tissues were collected, weighed, and preserved for histopathology.

Results and Conclusions: Females dosed with either 100 or 400 mg/kg BW/day PMT exhibited stilted gait and squatting posture. No effect on fore- or hind limb grip strength was observed. Locomotor activity was significantly increased in all PMT dosed females when compared to control. In males, this effect showed a similar, but non-statistically significant trend. Treatment with either 100 or 400 mg/kg BW/day PMT significantly decreased neutrophil count in both males and females, and monocyte counts were significantly decreased in females exposed to 400 mg/kg BW/day PMT. Further, exposure of males to either 100 or 400 mg/kg BW/day PMT significantly decreased serum alkaline phosphatase, total bilirubin, and total protein, whereas in females these same dose levels significantly decreased total protein and total globulin. In addition, females in the 400 mg/kg BW/day group had decreased serum calcium concentrations. In the 25 mg/kg BW/day PMT group, these effects were non-significantly decreased. Other major findings include a significant reduction of urine pH in females of the highest dosed group and a significant increase of testes weights in the 100 and 400 mg/kg BW/day PMT groups. Erythroid hyperplasia was found in the spleens of 80 percent of males, but not females, exposed to 400 mg/kg BW/day PMT. A no-observed-effect level (NOEL) could not be established in this study because the locomotor activity was increased in female animals of all treatment groups when compared to controls. Increased locomotor activity in females was the only significant effect observed at 25 mg/kg/day PMT; therefore, this dosage is considered as the lowest-observed-effect level (LOEL).

b. **13-Week Oral (Gavage) Toxicity Study in Rodents**

Study Title: "20, 23-Di-piperidinyl-mycaminosyl-tylonolide (PMT): 13-Week Oral Toxicity (Gavage) Study in Wistar Rats."

Study No: A43277; Intervet Reference Number V-0045-0048

Report Date: May 25, 2007

Study Director: St. Kaiser, Ph.D.

Study Location (in-life): RCC Ltd. CH-4452 Itingen and CH-4414 Füllinsdorf, Switzerland

Purpose: The study was designed to address any potential systemic toxicological effects following repeated oral exposure to PMT at various dose levels.

Experimental Design: Wistar rats were exposed daily by oral gavage to PMT solution at 20, 60, and 400 mg/kg BW/day, while control rats were fed vehicle (a buffered aqueous solution, pH 7.4). The dose volume was 10 mL/kg BW per day. Clinical observations were performed and body weights were measured throughout the study. On day 91, all rats were anesthetized with isoflurane, and blood was drawn for hematology and clinical chemistry evaluation. Necropsy was performed on all animals at the end of the study and tissues were collected, weighed, and preserved for histopathology.

Results and Conclusions: Exposure of rats for 90 days to 400 mg/kg BW/day induced toxic responses on nervous, immune, and vascular systems, as well as on the kidneys, liver, and heart. Forelimb grip strength was significantly reduced in males, whereas locomotor activity was not significantly affected. 20 and 60 mg/kg BW/day PMT produced lesser toxic effects on the skeletal muscle, pharynx, and relative kidney weights, and both sexes showed increased locomotor activity, whereas only males displayed a reduction of forelimb grip strength. Therefore, a NOEL could not be established, and 20 mg/kg BW/day of PMT was established as the LOEL.

c. **13-Week Oral (Capsule) Toxicity Study in Non-Rodents**

Study Title: "20, 23-Di-piperidinyl-mycaminosyl-tylonolide (PMT): 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog."

Study No: A43288; Intervet Reference Number: V-0045-0056

Report Date: June 14, 2007

Study Director: Dr. L. Braun

Study Location (in-life): RCC Ltd., Itingen/Switzerland and Füllinsdorf/Switzerland

Purpose: This study was designed to address any potential cumulative toxicity of PMT in a non-rodent animal model.

Experimental Design: Purebred beagle dogs (4/sex/group) were exposed repeatedly by daily oral gavage to PMT at 6, 20, and 60 mg/kg BW/day for 13 weeks. Control animals received empty gelatin capsules. Clinical signs, morbidity, and mortality were recorded. Additionally, a detailed clinical observation and ophthalmoscopic examination were performed. Food consumption and body weights were measured. Electrocardiograms (ECGs) of each animal were recorded at weeks 9 and 13. Blood samples for hematology and clinical chemistry, and urine samples for urinalysis were collected from overnight-fasted animals at pretest and weeks 6 and 13. On Day 1 and in week 6 and 13 of dosing, blood samples were collected from the jugular vein of all animals at time 0 (pretest), 0.5, 1, 2, 4, 8, and 24 hours after dosing to determine the blood plasma PMT concentrations. At the end of the trial period, each animal was subjected to a complete necropsy. Organs were weighed, then examined macroscopically, and processed for comprehensive histopathology analysis.

Results and Conclusions: Repeated oral exposure of dogs to the highest PMT dose level of 60 mg/kg BW/day induced multiple treatment-related organ-system toxic responses. These toxic responses were (1) de-pigmented tapetum lucidum of the eyes, (2) increased heart rate and QT and QTc intervals, (3) reduction of PQ interval, (4) reduced food consumption, body weight, and body weight gain, (5) clinical chemistry parameters such as alanine aminotransferase (ALAT), glutamate

dehydrogenase (GLDH), total protein, and albumin concentration were increased,(6) increased organ weights (liver, pituitary gland, thyroid gland, and gallbladder), and (7) histopathology findings (vacuolation in multiple organs and tissues, accumulation of hyaline droplets in the kidneys, increased severity of hematopoietic activity in the spleen, and increased incidence of colloid content in the thyroid gland). At 20 mg/kg BW/day, vacuolation of the artery in the tongue and of the smooth muscle in the esophagus was observed. PMT administration at 6 or 20 mg/kg BW/day induced restlessness, recumbency, tremors, and whimpering. A NOEL for PMT could not be established because these clinical observations were seen at the lowest dose level (6 mg/kg BW/day) tested.

d. **55-Week Oral (Capsule) Toxicity Study in Beagle Dogs**

Study Title: "20, 23-Di-piperidinyl-mycaminosyl-tylonolide (PMT): 55-Week Oral (Capsule) Toxicity Study in the Beagle Dog."

Study No: B00066; Intervet Reference Number: V-0045-0068

Report Date: November 24, 2008

Study Director: Dr. L. Braun

Study Location (in-life): Harlan Laboratories Ltd., Itingen/Switzerland

Purpose: This study was conducted to address the long-term potential toxic effects of PMT.

Experimental Design: Purebred beagle dogs (4/sex/group) were fed PMT repeatedly by oral gelatin capsules at dose levels of 4, 10, and 50 mg/kg BW/day for 55 consecutive weeks. Dosing was designed such that animals were fed 2 mg/kg BW/day from day 1 to 21, and fed 4 mg/kg BW/day from day 22 until the end of the study. The control group received empty gelatin capsules only. Throughout the entire exposure period, all animals were examined for any clinical signs related to PMT. Clinical signs, food consumption, body weights, and ophthalmoscopic examination were evaluated. Blood sample was taken for hematology and clinical chemistry analysis. In addition, urine samples were obtained from overnight fasting dogs for urinalysis. At the end of the in-life phase, the dogs were euthanized, detailed necropsy was performed, and various organs were weighed. A standardized set of tissues were collected, examined grossly, processed, and later examined histopathologically.

Results and Conclusions: At high dose levels (50 mg/kg BW/day), many treatment-related toxic responses were reported, such as: (1) reduced mean food consumption and body weight gain in males and females; (2) increased ALAT activity in both sexes, whereas GLDH activity increased in females only; (3) in females, total protein, globulin, and albumin concentrations increased while the albumin/globulin ratio decreased; (4) the weights of the pituitary gland, thyroid glands, and gallbladder were increased in both sexes, whereas in females, liver and

spleen weight increased as well; (5) enlarged thyroid glands in females; and (6) cellular vacuolation, involving many organ/systems, tissues, and cell types, occurred frequently in both sexes. Some of the affected organ systems were cardiovascular, nervous, skin, endocrine, reticulo-endothelial, reproductive, and respiratory. Tissues such as blood vessels, heart, liver, spleen, lungs, uterus, thyroid gland, and brain were all affected following exposure to 50 mg/kg BW/day. Cell types such as brain neurons, smooth muscle cells, cardiomyocytes, and epithelial cells were all affected. In addition, neuromorphologic changes occurred mainly in the spinal cord, brain, dorsal root ganglia, retina, sciatic nerve, and pituitary gland. Other changes that were noted at the high PMT dose level include degenerative changes in the smooth muscle fibers of many organs and tissues. No significant treatment related changes were reported at 4 or 10 mg/kg BW/day PMT. Therefore, a NOEL for PMT was established at 10 mg/kg BW/day from this study.

e. **Oral (Gavage) Developmental Toxicity Study in Rodents**

Study Title: "20, 23-Di-piperidinyl-mycaminosyl-tylonolide (PMT): Prenatal Developmental Toxicity Study in Han Wistar Rats."

Study Number: A43323; Intervet Reference Number: V-0045-0057

Report Date: June 12, 2007

Study Director: Dr. S. Whitlow

Study Location (in-life): RCC Ltd, Füllinsdorf/Switzerland, Itingen/Switzerland

Purpose: The study was performed to evaluate the cumulative toxic potential of PMT on pregnant rats and developing fetuses following prenatal exposure.

Experimental Design: 10 week-old Han Wistar rats (22 females per treatment group) were exposed to PMT *via* daily oral gavage at 0 (vehicle only), 30, 120, and 480 mg/kg BW/day at a dose volume of 10 mL/kg BW from gestational day 5 through 20. Clinical signs and mortality were observed. Food consumption and body weight were measured.

On day 21 post-coitus, the uteri from all females with live fetuses were weighed. In addition, all internal organs were examined grossly, with emphasis on the uterus, uterine contents, position of fetuses in the uterus, and number of corpora lutea. Fetuses were removed from the uterus, sexed, weighed individually, examined for gross external and visceral abnormalities, and then sacrificed. At least one half of the fetuses from each litter were examined by histology and micro-dissection. The remaining fetuses were examined for skeletal abnormalities and variation.

Results and Conclusions: Administration of 480 mg/kg BW/day PMT to pregnant rats produced statistically significant toxic responses to both

dams and fetuses. Food consumption of dams was reduced whereas head pushing through bedding was increased. In addition, there was a statistically significant reduction in body weight and body weight gain. In fetuses, this high dose increased the following endpoints: incidence of pelvic girdle caudal displacement, cervical vertebral body non-ossification on both a litter and fetus basis, and the number of non-ossified taluses on a fetus basis. There was a reduction in fetus body weight.

At the mid-dose level (120 mg/kg BW/day PMT) dams displayed a statistically significant reduction in food consumption. Therefore, the maternal toxicity NOEL of 30 mg/kg BW/day is established from this study, based on significant reduction in food consumption of dams. A fetal toxicity NOEL of 120 mg/kg BW/day PMT was established from this study, based on increased incidence of pelvic girdle caudal displacement, cervical vertebral body non-ossification on both a litter and fetus basis, and the number of non-ossified taluses on a fetus basis. No teratogenic effects were seen in this study.

f. **Oral (Gavage) Developmental Toxicity Study in Non-Rodents**

Study Title: "20, 23-Di-piperidinyl-mycaminosyl-tylonolide PMT): Prenatal Developmental Toxicity Study in the Himalayan Rabbit."

Study Number: A43356; Intervet Reference Number: V-0045-0058

Report Date: June 15, 2007

Study Director: Dr. S. Whitlow

Study Location (in-life): RCC Ltd, Füllinsdorf/Switzerland, Itingen/Switzerland

Purpose: This study was performed to evaluate the cumulative toxic potential of PMT on pregnant rabbits and developing fetuses following prenatal exposure.

Experimental Design: 18-23 week old Himalayan rabbits (20 females per treatment group) were exposed to PMT *via* daily oral gavage at 0 (vehicle only), 10, 30, and 90 mg/kg BW/day at a dose volume of 4 mL/kg BW from gestational day 6 through 27. Clinical signs and mortality were observed. Food consumption and body weight were measured. On day 28 post-coitus, the internal organs of all females were examined grossly, with emphasis on the uterus, uterine contents, position of fetuses in the uterus, and the number of corpora lutea. The uteri of all females with live fetuses were weighed at necropsy. Fetuses were removed from the uterus, weighed individually, and examined for gross external abnormalities. Following dissection, fetal organs were examined, and the sex of each fetus recorded. The cranium was examined for ossification. Heads from half of the fetuses per litter were examined histologically for soft tissue alterations.

Results and Conclusions: Administration of 90 mg/kg BW/day of PMT to pregnant rabbits produced statistically significant toxic responses to both dams and fetuses. Food consumption of dams was reduced, and the incidence of food spillage increased, thus causing unaccountable food consumption. In addition, there was a transient reduction in body weight gain. In fetuses, this same dosage increased the following endpoints: incidence of pelvic girdle displacement, the number of supernumerary ribs, the incidence of incompletely ossified phalanges on digit 5, and the number of supernumerary and rudimentary costal cartilage. At the mid-dosage (30 mg/kg BW/day PMT) dams displayed transient reduction in food consumption, whereas in fetuses both the incidence of pelvic girdle displacement and the incidence of incompletely ossified phalanges at digit 5 increased. A maternal toxicity NOEL of 10 mg/kg BW/day PMT was established from this study, based on transient reduction in food consumption of dams. A fetal toxicity NOEL of 10 mg/kg BW/day PMT was established from this study, based on increased incidence of incompletely ossified phalanges on digit 5 and increased incidence of pelvic girdle displacement seen in fetuses at the next highest dose level tested.

g. **Two-Generation Oral (Gavage) Reproductive Toxicity Study in Rats**

Study Title: "20, 23-Di-piperidinyl-mycaminosyl-tylonolide (PMT): Two-generation reproduction toxicology study in the Han Wistar rat."

Study No: A43301; Intervet Reference Number: V-0045-0062

Report Date: March 31, 2008

Study Director: Dr. S. Whitlow

Study Location (in-life): RCC Ltd, Füllinsdorf/Switzerland, Itingen, Switzerland

Purpose: This study was performed to evaluate the cumulative toxic potential of PMT on reproductive function, growth, and development. In addition, the study assessed the growth, development, morbidity, mortality, and behavior of the progeny (F_1 and F_2).

Experimental Design: PMT was administered daily by oral gavage at 0 (vehicle), 20, 80, and 320 mg/kg BW/day to Han Wistar rats (24/sex/group) in parental (P_0) and first filial (F_1) generation. P_0 rats were exposed to PMT starting from 6 weeks of age until sacrifice. Further, treatment of P_0 and F_1 generation with PMT continued for 70 days prior to pairing/mating and through cohabitation (2 weeks). Females continued to receive PMT during gestation and parturition and to the end of lactation, which ended at weaning of F_2 offspring. On day 4 post-partum, each litter was culled to 4 males and 4 females. At weaning on day 21 post-partum, weaners were randomly chosen to be F_1 breeding pairs (to produce F_2). All remaining P_0 animals and some of the remaining F_1 offspring were sacrificed and used for histopathology.

In all generations, mortality, clinical observations, body weight and food consumption were recorded. P_0 and F_1 breeders were assessed for reproductive performance, including pre-pairing estrous cyclicity, mating performance, gestation length, number of implantations, post-implantation loss, corpora lutea, litter size, and number of live births. At necropsy, all organs were subjected to a gross pathological examination, specific organ weights were recorded, and a quantity of sperm was obtained and evaluated for motility, morphology, and sperm head counted. A thorough histopathology examination was performed on a variety of reproductive and non-reproductive organs and tissues from P_0 and F_1 animals. Physical development and sexual maturation were recorded for F_1 pups. In addition, behavioral tests were conducted for F_1 animals at 6 weeks of age. At necropsy, animals were examined macroscopically, and the absolute weights of the brain, spleen, and thymus were obtained. Full histopathology examination was performed on organs and tissues from one male and one female pup of each litter selected for organ weight recording.

Results and Conclusions: Administration of PMT at 320 mg/kg BW/day to Wistar rats produced many adverse responses in P_0 and F_1 generations. These responses included reductions in food consumption, body weights, number of implantations, litter size, and normal sperm, and an increase in the number of normal sperm with complete heads but with detached tail. In P_0 males, there was an increase in the incidence of thickened thyroid gland that was correlated with thyroid gland vacuolation and hypertrophy of follicular cells. High-dosed males of P_0 generation showed increased absolute weights of the pituitary gland, coagulating gland, thyroid gland, kidneys, seminal vesicles, and epididymis. In addition, vacuolation occurred in the kidneys. In P_0 female rats, vacuolation occurred in multiple tissues including the adrenal glands, thyroid glands, and pituitary glands. In F_1 females, whereas the weight of the adrenal gland increased, the weight of the ovaries decreased. In addition, both the number of primordial and growing primordial follicles was reduced.

In F_1 (offspring) exposed to PMT at 320 mg/kg BW/day, incisor eruption, preputial separation, and landing foot splay were delayed. Brain/body weight ratio in both sexes was increased. In F_2 females exposed to the highest PMT dosage, the absolute spleen weights and spleen/brain weight ratio were decreased. The mean body weights in both F_1 and F_2 pups were reduced.

PMT treatment at 80 mg/kg BW/day was associated with some general toxic responses in the P_0 and F_1 generation. In P_0 males, PMT was associated with an increased absolute weight of the thyroid gland, an increased incidence of thickened thyroid gland that was correlated with vacuolation of follicular cells. Vacuolation occurred in the epididymis (P_0 and F_1) and oviducts (P_0).

PMT treatment at 20 mg/kg BW/day was not associated with any toxic reproductive responses on either parents or offspring. A NOEL of

20 mg/kg BW/day PMT was established for general toxicology effects and 80 mg/kg BW/day for reproductive effects.

h. **Cardiovascular Toxicity Study in Beagle Dogs**

Study Title: "20, 23-Di-Piperidinyl-Mycaminosyl-Tylonolide (PMT) Evaluation of Blood Pressure, Heart Rate and the ECG (Lead II) in Telemetric Conscious Beagle Dogs (intramuscular administration)."

Study No: ITV0002/062368

Report Date: July 21, 2006

Study Director: Stuart Purbrick, B.Sc.

Study Location (in-life): Huntingdon Life Sciences Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, England

Purpose: The objective of this study was to assess cardiovascular toxicity in beagle dogs following intramuscular (IM) administration of PMT over a period of 7 days.

Experimental Design: Purebred beagle dogs (2/sex/group) received IM injection of the vehicle or PMT at 5, 10, and 20 mg/kg BW/day at a dose volume of 0.2 mL/kg BW on each of four dosing sessions at dose intervals of 7 days using a randomized cross-over design. On the fifth dosing session, all dogs except one female received MICOTIL 300 (tilmicosin) at 5 mg/kg BW at a dose volume of 0.166 mL/kg BW [positive control] as a single IM injection. Clinical observations and body weight were recorded. Telemetry recordings were made at multiple time points prior to and after dosing, with blood pressure, heart rate, and Lead II Electrocardiogram (ECG) examined.

Result and Conclusions: There were no PMT-related effects on heart rate; systolic, diastolic, and mean arterial blood pressure; ECG Lead II intervals; and ECG waveform or morphology. However, following IM PMT administration at 20 mg/kg BW, a statistically significant reduction (~13% relative to the vehicle control) in pulse pressure was noted at 0.5 to 1 hr, but not at later time points. The mean T_{max} was 0.75 to 2.0 hours after a single oral dose of PMT (6 or 20 mg/kg BW), therefore, the reduction in pulse pressure at 0.5 to 1 hr was not considered of biological or physiological relevance. In contrast, in the positive control (tilmicosin) animals, a significant decrease in pulse pressure was reached during the 2 hr period after dosing. In addition, the positive control dose of 5 mg tilmicosin/kg BW induced increased heart rate, increased diastolic blood pressure, decreased pulse pressure, and shortened ECG Lead II PR and QT intervals. Tilmicosin at 5 mg/kg BW/day did not affect ECG Lead II waveform or morphology, with the exception of an isolated P wave seen in one dog. It was concluded that a single dose of PMT up to 20 mg/kg BW/day did not induce adverse cardiovascular effects in dogs.

i. **Bacterial Reverse Mutation Assay using *Salmonella typhimurium/Escherichia coli.* Plate Incorporation Test with and without metabolic activation (Ames Test)**

Study Title: "20, 23-Di-piperidinyl-mycamisonyl-tylonolide (PMT)-Macrolide. Bacterial Reverse Mutation Test."

Study Number: V-0045-0005

Report Date: September 20, 2004

Report Author: H. M. Kauffmann

Study Location: Aventis Pharma, Germany

Purpose: To assess the potential toxicity of PMT to induce reverse mutations in the presence or absence of mammalian microsomal enzymes.

Experimental Design: Studies were conducted with the plate incorporation procedure and pre-incubation assays. Four different strains of *Salmonella typhimurium* (TA 98, TA 1535, TA 1537, and TA 100) and one strain of *Escherichia coli* (WP2uvrA pKM101) were used. A standard plate incorporation assay was conducted in the presence (+S9) and absence (-S9) of rat liver metabolic activation mix (S9) at six different PMT dose levels ranging from 0.256 to 80.0 µg/plate.

Results and Conclusions: PMT did not precipitate at any concentration. In the plate incorporation test, toxicity was observed ±S9 from concentrations of 25.6 µg/plate and higher with tester strains TA100, TA1535, and TA98, and at 80 µg/plate with strains TA1537 and WP2uvrA. In the pre-incubation assay, toxicity was observed ±S9 from concentrations of 25.6 µg/plate and higher with tester strains TA100 and TA1535, and at 80 µg/plate with strains TA1537 and TA98. Toxicity was observed with WP2uvrA at 80 µg/plate in the absence of S9 in the first pre-incubation test. In the repeat pre-incubation test, PMT was toxic to WP2uvrA at 80 µg/plate, ±S9 activation. PMT did not cause any significant increase in the number of revertant colonies at any concentration with any of the tester strains in the presence and absence of S9, in the plate incorporation, or in the pre-incubation tests. It was concluded that PMT was not mutagenic under the conditions of the assay.

j. **In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$) Mouse Lymphoma Assay**

Study Title: "PMT. Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells."

Study No.: V-0045-0051

Report Date: January 11, 2007

Report Author: Hans-Eric Wollny

Study Location: Cytotest Cell Research, GmbH (RCC-CCR), Germany

Purpose: PMT was evaluated for mutagenic potential based on quantification of forward mutations at the thymidine kinase (TK) locus of L5178Y mouse lymphoma cells by the microtiter method.

Experimental Design: A preliminary trial was conducted to establish the optimal concentration of PMT for use in the mutagenesis assay in the absence or presence of rat liver mix (S9). L5178Y cells were exposed to water (solvent) alone or nine different concentrations of PMT ranging from 39.1 to 5000 µg/mL with S9 (+S9) or without S9 (-S9) mix using 4 or 24 hours exposure. Separately, cell population density was determined 24 and 48 hours post-exposure to PMT. L5178Y cell toxicity was measured as suspension growth of the treated cultures relative to the growth of the solvent control cultures. From the cytotoxicity assay, six PMT concentrations were chosen for the mutagenesis test. In the first set of cultures without S9, PMT concentrations ranged from 35.9 to 572 µg/mL, and in cultures with S9, PMT concentrations ranged from 69.2 to 1110 µg/mL, with both cultures exposed for 4 hours. In the second set of cultures, the concentrations ranged from 92.3 to 554 µg/mL for the -S9 cultures and 462 to 923 µg/mL for the +S9 cultures with a 4-hour exposure. In the third set, PMT concentrations ranged from 92.3 to 1850 µg/mL without S9 mix.

Results and Conclusions: PMT exposure at 462 µg/mL for 4 hours and 1110 µg/mL for 24 hours significantly increased L5178Y mutant colonies. However, these concentrations were considered very high and, therefore, extremely toxic. There were no other significant treatment-associated increases in mutational frequency in any of the trials conducted in the absence or presence of S9. Under the experimental conditions reported, PMT was negative in the absence and presence of metabolic activation.

k. **In Vitro Mammalian Chromosomal Aberration Test in Human Lymphocytes**

Study Title: "PMT. Chromosomal Aberration Test in Human Lymphocytes *in vitro*."

Study No.: V-0045-0052

Report Date: January 11, 2007

Report Author: Susanne Kunz

Study Location: Cytotest Cell Research GmbH (RCC-CCR), Germany

Purpose: This *in vitro* assay evaluates the potential of PMT to induce structural chromosomal aberrations in cultured whole blood human

lymphocytes in the presence and absence of an exogenous metabolic activation system (liver S9 mix from Phenobarbital/β-naphthoflavone treated male rats).

Experimental Design: Lymphocytes from human peripheral blood of healthy donors were cultured in Dulbecco's modified Eagle's medium/HamF12, with growth factors, antibiotics, anticoagulant, HEPES, L-glutamine, and 3 µg/mL phytohemagglutinin (mitogen). In addition, the livers of male Wistar Hanlbm rats treated consecutively for 3 days with phenobarbital (intraperitoneal) and 80 mg/kg BW/day β-naphthoflavone (orally) were used to prepare hepatic S9 mix. Ethylmethane sulfonate without S9 and cyclophosphamide with S9 were used as positive controls.

Chromosomal damage in human lymphocytes was evaluated in the following independent experiments:

1) Experiment A:

Experimental Design: Duplicate cultures were tested at PMT concentrations ranging from 174.1 to 533.1 µg/mL. For direct test without S9, cultures were treated with PMT or control, continuously for 46 hours. In the direct test containing S9 metabolic activation, cells were exposed for 4 hours to PMT or control article in serum-free medium followed by a 42-hour recovery in fresh complete culture medium. Approximately 3 hours before the end of the treatment and recovery period, Colcemid (final concentration 0.2 µg/mL), was added to each culture. Cells were harvested at the end of a 42–hour treatment (-S9) or recovery (+S9) period, stained according to fluorescent plus Giemsa staining, and scored for the presence of metaphase figures. The mitotic index was determined for the control and treated cultures by systematically scoring 1000 consecutive nuclei for the presence of metaphase figures.

Results and Conclusions: Following 46 hours of continuous PMT exposure without metabolic activation, mean mitotic indices were reduced dose-dependently (13, 11.1, and 8.0 at 174.1, 304.6, and 533.1 µg/mL, respectively). Further, there was no statistically significant increase in the frequency of polyploidy cells in PMT-treated cultures compared to control. Consistently, after 4 hours of continuous PMT exposure, in the presence of S9 mix and 42 hours of recovery, mean mitotic indices were reduced dose-dependently (12.7, 11.1, and 9.5 at 533.1, 932.9, and 1632.7 µg/mL). However, under each experimental condition these differences were not statistically different when compared to negative control (HEPES 1 mM). Alternately, in the presence of S9, after 4 hours of treatment and 42 hours of recovery, the number of aberrant cells increased dose-dependently (0.5%, 1.0%, and 3.5% at 533.1, 932.9, and 1632.7 µg/mL, respectively) when compared to control. However, increases in the number of aberrant cells were not statistically significant and

were within the performing laboratories historical control value of 0 to 3.5%.

2) Experiment B:

Experimental Design: Experiment A was repeated using a smaller concentration range to confirm if PMT increased the number of aberrant cells in the presence of metabolic activation. Duplicate cultures were tested at each PMT concentration, in the presence of S9 for 4 hours followed by 42-hour recovery and without S9 for 42 hours followed by a 4-hour recovery period. All experiments were carried out in a serum-free medium. PMT concentrations ranged from 520 to 600 µg/mL in the absence of metabolic activation, and from 2300 to 2500 µg/mL in the presence of metabolic activation. Culture medium was used as the solvent. Approximately 3 hours prior to treatment and recovery termination, 0.2 µg/mL Colcemid (final concentration) was added to each culture. At the end of the 42-hour treatment (-S9) or recovery (+S9) period, cells were harvested and stained with fluorescent plus Giemsa and scored for the presence of metaphase figures. The mitotic index was determined for the control and treated cultures by systematically scoring 1000 consecutive nuclei for the presence of metaphase figures.

Results and Conclusions: In the absence of metabolic activation and after 46 hours of continuous treatment, mean mitotic indices (an indicator of toxicity) were 18.9 (520 µg/mL), 19.5 (560 µg/mL) and 16.1 (600 µg/mL). However, these indices were not statistically significant when compared to the negative control. In addition, there was no increase in the frequency of polyploidy cells in the PMT-treated cultures compared to the control. In the presence of S9 mix, a 4-hour treatment, and a 42-hour recovery, mean mitotic indices were 13.4 (2300 µg/mL), 9.2 (2400 µg/mL) and 14.3 µg/mL (2500 µg/mL). These results demonstrate that PMT increased slightly the number of aberrant cells at 2500 µg/mL (2%) compared to control (0%), but the increase was not statistically significant and was within the performing laboratories historical control value of 0 to 3.5%. In conclusion, under the current experimental conditions, PMT does not induce structural chromosomal aberrations.

I. **Mammalian Erythrocyte Micronucleus Test Rodent Micronucleus Assay, Oral Route**

Study Title: "20, 23-Di-piperidinyl-mycamisonyl-tylonolide (PMT). Micronucleus Assay in Bone Marrow Cells of the Mouse (after oral administration)."

Study No.: V-0045-0053

Report Date: March 21, 2007

Report Author: Naveed Honarvar

Study Location: Cytotest Cell Research GmbH (RCC-CCR), Germany

Purpose: The objective of this *in vivo* micronucleus assay was to evaluate the potential of PMT to induce micronuclei (MN) in polychromatic erythrocytes (PCE) in bone marrow of male and female NMRI mice (strain HsdWin:NMRI).

Experimental Design: A preliminary maximum tolerated dose (MTD) study was conducted by administering mice (6/sex/group) with a single dose of PMT at 1750 or 2000 mg/kg BW by oral gavage, or the vehicle only (buffered aqueous solution). In the primary study, groups of animals (6/sex/ group) were administered a single dose at 0, 437.5, 875, or 1750 mg/kg BW by oral gavage. A cyclophosphamide (40 mg/kg BW) exposure group (6/sex) served as positive control. Animals were euthanized at 24 and 48 hours (highest dose group only) post-dosing, bone marrow cells were harvested, and slides were prepared. For each preparation, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. In addition, the proportion of PCE to normochromatic erythrocytes (NCE) in 1000 erythrocytes was determined as an index of target organ toxicity. All mice were observed after treatment for any adverse clinical signs of toxicity and mortality.

Results and Conclusions: During a preliminary study, three mice (1 male and 2 female) died within 1 hour following exposure to 2000 mg/kg BW. The male mouse died during blood sampling while under general anesthesia, whereas both females died spontaneously at 10 minutes and 1 hour post-PMT exposure. There was no mortality or significant adverse clinical signs of toxicity in any mice of any sex treated at 1750 mg/kg BW. Further, no significant changes in the ratios of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCEs) occurred in any sex at any dose. In contrast, cyclophosphamide significantly increased the number of micronucleated cells (3.25%) compared to control (0.13%). In conclusion, under the current experimental conditions, PMT did not induce micronuclei damage *in vivo,* in mouse bone marrow cells.

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

The lowest NOEL of 10 mg/kg BW/day for chronic exposure is established from the 55-week chronic toxicity study in dogs (Study Number B00066; V-0045-0068).

3. Determination of Toxicological Acceptable Daily Intake (ADI)

The toxicological ADI of total tildipirosin-related residues was determined from the lowest NOEL in the most sensitive species tested in the various toxicology studies conducted. Studies considered in establishing the toxicological ADI are summarized in Table IV.2.

Table IV.2. No-Observed-Effect Levels (NOEL) in toxicology studies for PMT (tildipirosin)

Study	Study No.	NOEL (mg/kg BW/day)
Subacute Oral Toxicity Study in Rats	PT04-0147	25*
Subchronic Oral Toxicity Study in Rats	A43277	20*
Subchronic Oral Toxicity Study in Dogs	A43288	None established
Chronic Oral Toxicity Study in Dogs	B00066	10
Developmental Toxicity Study in Rats	A43323	30
Developmental Toxicity Study in Rabbits	A43356	10
Two-Generation Rat Reproduction Study	A43301	20

*NOEL not established, value for established LOEL reported

Based on these toxicology studies, the chronic oral toxicity study in dogs was determined to be the most appropriate study to determine the toxicological ADI. The toxicological ADI is calculated using the following formula based on a NOEL of 10 mg/kg BW/day from the dog study and a safety factor of 200. A safety factor of 200 was used because the NOEL was from a chronic study and there were some concerns of neurotoxicity observed in the subchronic (4-week and 13-week) oral toxicity studies with rats and dogs that were not addressed in the chronic toxicity study.

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

$$= \frac{10\ mg/kg\ bw/day}{200} = \frac{10000\ \mu g/kg\ bw/day}{200} 50\ \mu g/kg\ \ bw/day$$

(Equation 1: Toxicological Acceptable Daily Intake (ADI) equals the lowest NOEL divided by the Safety Factor, which equals 10 mg/kg BW/day divided by 200, which equals 50 µg/kg BW/day.)

The toxicological ADI for PMT (tildipirosin) is 50 µg/kg BW/day.

D. Assignment of the Final ADI

Because the toxicological ADI of 50 µg/kg BW/day calculated from the 55-week chronic toxicity study in dogs is numerically equivalent to the calculated microbiological ADI of 50 µg/kg BW/day, we assign the toxicological ADI (50 µg/kg/day) as the final ADI for total PMT (tildipirosin) residues.

E. Safe Concentration for Total Residues (edible tissues and injection sites):

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

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

(Equation 2: Safe Concentration (SC) equals Acceptable Daily Intake (ADI) times Average Human Body Weight divided by Food Consumption Value.)

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

Therefore, the safe concentrations for the edible tissues are calculated as (summarized in Table IV.3):

$$SC(muscle) = \frac{50\ \mu g/kg\ bw/day \times 60\ kg}{300\ g/day} = 10\ \mu g/g = 10\ ppm$$

(Equation 3: SC(*muscle*) equals 50 µg/kg BW/day times 60 kg divided by 300 g/day, which equals 10 µg/g or 10 ppm.)

$$SC(liver) = \frac{50\ \mu g/kg\ bw/day \times 60\ kg}{100\ g/day} = 30\ \mu g/g = 30\ ppm$$

(Equation 4: SC(*liver*) equals 50 µg/kg BW/day times 60 kg divided by 100 g/day, which equals 30 µg/g or 30 ppm.)

$$SC(kidney) = \frac{50\ \mu g/kg\ bw/day \times 60\ kg}{50\ g/day} = 60\ \mu g/g = 60\ ppm$$

(Equation 5: SC(*kidney*) equals 50 µg/kg BW/day times 60 kg divided by 50 g/day, which equals 60 µg/g or 60 ppm.)

$$SC(fat) = \frac{50\ \mu g/kg\ bw/day \times 60\ kg}{50\ g/day} = 60\ \mu g/g = 60\ ppm$$

(Equation 6: SC(*fat*) equals 50 µg/kg BW/day times 60 kg divided by 50 g/day, which equals 60 µg/g or 60 ppm.)

The safe concentration for the injection site is calculated as:

SC(*injection site*) = SC(*muscle*) x 10 = 100 ppm

(Equation 7: SC(*injection site*) equals ADI times 10, which equals 10 µg/kg BW/day times 10, which equals 100 µg/g or 100 ppm.)

Table IV.3. Safe Concentrations (SCs) for Total PMT (tildipirosin) Residues in Edible Tissues of Cattle Using the Food Consumption Factors.

Edible Tissue	Amount Consumed / Day	Safe Concentration (SC)
Muscle	300 g	10 ppm
Liver	100 g	30 ppm
Kidney	50 g	60 ppm
Fat	50 g	60 ppm
Injection site	300 g	100 ppm

F. Residue Chemistry

1. Summary of Residue Chemistry Studies

a. Total Residue and Metabolism Study

The following study was conducted to permit the assignments of a target tissue, a marker residue, and a tolerance for PMT (tildipirosin) residues in cattle.

i. Study Title: "A Target Animal Metabolism Study Following a Single Subcutaneous (SC) Administration of 4 mg/kg Body Weight of [^{14}C]-PMT (20, 23-di-piperidinyl-mycaminosyl-tylonolide) to Beef Cattle." Intervet-Doc. No. V-0045-0082 (November 2006 to February 2008).

ii. Study Director: Graeme McLellan, BSc, Charles River Laboratories, United Kingdom, and Intervet Innovation GmbH, Germany.

iii. Objective: To determine PMT (tildipirosin) total residue depletion and metabolism in cattle after receiving a single subcutaneous administration of [^{14}C]-PMT at the labeled dose.

The study was conducted in acceptable compliance with the Good Laboratory Practice (GLP) regulations (21 CFR 58).

iv. Test Substance: [^{14}C]-PMT

v. Test Animals: 28 (14 male, 14 female) crossbred beef cattle, weighing 175.5 to 246.0 kg.

vi. Treatment Groups: 7 groups of 4 animals each, with 2 male and 2 female animals in each group.

vii. Dose: A single subcutaneous injection of [^{14}C]-PMT at a dosage of 4 mg PMT/kg BW.

viii. Sample Analysis: Total radioactivity was quantitated by liquid scintillation analysis. Metabolite profiling was determined by radio-HPLC analysis and mass spectrometry.

ix. Results:

Table IV.4. Mean and Standard Deviation of Total Radioactive Residues (ppb) in Edible Tissues Following Subcutaneous Administration of [^{14}C]-PMT.

Slaughter Time Point (days)	Liver	Kidney	Injection Site	Fat (Renal)	Muscle
3	23706 (2379)	21613 (1272)	42030 (15506)	789 (186)	1176 (154)
7	28852 (2796)	19753 (3092)	23842 (16497)	577 (38)	654 (47)
21	18215 (1393)	7689 (711)	5426 (4644)	435 (139)	276 (34)
35	10660 (2134)	3549 (693)	3877 (2676)	269 (193)	169 (13)
49	7749 (2292)	2127 (211)	3463 (2123)	144 (47)	96 (12)
63	4552 (1716)	1443 (255)	2388 (1105)	124 (98)	76 (14)

Table IV.5. Mean Parent PMT (Marker Residue) Concentration (ppb) Following Subcutaneous Administration of [^{14}C]-PMT.

Slaughter Time Point (Days)	Liver	Kidney	Injection Site	Fat (Renal)	Muscle
3	12323	16193	30780	815	737
7	9321	13969	14418	572	324
21	3834	4774	3218	409	131
35	2805	1989	2873	228	<LOQ
49	1228	1083	2952	106	<LOQ
63	971	586	1614	99	<LOQ

Limit of quantitation (LOQ): 50 ppb

The mean of the total residues (Table IV.4) was compared to the mean of parent PMT (Table IV.5) to determine the mean ratio of parent PMT to total residues. The results are summarized in Table IV.6.

Table IV.6. Mean Ratio of Parent PMT to Total Radioactive Residues Following Dosing with [^{14}C]-PMT.

Slaughter Time Point (Days)	Liver	Kidney	Injection Site	Fat (Renal)	Muscle
3	0.520	0.749	0.732	1.034	0.627
7	0.323	0.707	0.605	0.991	0.496
21	0.210	0.621	0.593	0.941	0.473

Slaughter Time Point (Days)	Liver	Kidney	Injection Site	Fat (Renal)	Muscle
35	0.263	0.560	0.741	0.846	NA
49	0.158	0.509	0.852	0.740	NA
63	0.213	0.406	0.676	0.800	NA

NA: not applicable

Parent PMT was the major component of the total residues in cattle tissue and excreta samples. The metabolites identified in cattle are summarized in Table IV.7.

Table IV.7. Summary of PMT Metabolites Identified in Cattle Following Subcutaneous Administration of [^{14}C]-PMT.

Cattle Liver	Cattle Kidney	Injection Site Muscle	Urine
Desmethyl-PMT	Dihydro-PMT-SO_3H	Desmethyl-PMT	Hydrolyzed-dihydro-PMT-SO_3H
S-Cysteine-PMT	Hydroxy-keto-PMT	S-Cysteine-PMT	
PMT-SO_3H	PMT-SO_3H		
Hydrolyzed-S-Cysteine-PMT	Dihydroxy-PMT		
S-Glutathione-PMT			

b. Comparative Metabolism Studies

i. PMT metabolism study in rats

a) Study Title: "A Chromatographic Investigation into the Absorption, Distribution, Metabolism and Excretion of [^{14}C]-PMT (20, 23-di-piperidinyl-mycaminosyl-tylonolide) following Multiple Oral Administration at a Dose Level of 25 µg and 25 mg PMT/kg Body Weight to Rats" – Intervet-Doc No. V-0045-0047 (February 2006 to December 2006).

b) Study Director: Chris Lowrie, BSc, Charles River Laboratories, United Kingdom.

c) Objective: To determine PMT (tildipirosin) metabolism in rats after receiving oral administration of [^{14}C]-PMT.

The study was conducted in acceptable compliance with the Good Laboratory Practice (GLP) regulations (21 CFR 58).

d) Test Substance: [^{14}C]-PMT

e) Test Animals: 14 (7 males and 7 females) Han Wistar rats, approximately 7 weeks old on arrival.

f) Treatment Groups: One low dose group and one high dose group of 3 male and 3 female animals in each group, and a control group of one male and one female animal.

g) Dose: [^{14}C]-PMT was orally administered once daily for 7 days to the low dose group at a dosage of 25 µg/kg body weight and to the high dose group at a dosage of 25 mg/kg body weight. Animals in the control group were dosed with placebo formulation once daily for 7 days.

h) Results: The highest radioactivity was detected in colon. Excretion of radioactivity was mainly through feces. Unchanged PMT accounted for a minor portion of the total residues. See Table IV.8 for metabolites identified in rats following oral administration of [^{14}C]-PMT (tildipirosin).

Table IV.8. Summary of PMT Metabolites Identified in Rats Following Oral Administration of [^{14}C]-PMT.

Liver	Colon	Urine	Feces
S-Cysteine-PMT	Hydrolyzed-dihydro-PMT-SO_3H	Hydrolyzed-dihydro-PMT-SO_3H	
Dihydro-PMT-SO_3H	Hydrolyzed-hydroxy-PMT-SO_3H		Dihydro-PMT-SO_3H

ii. PMT metabolism study in dogs

a) Study Title: "A Chromatographic Investigation into the Absorption, Distribution, Metabolism and Excretion of [^{14}C]-PMT (20, 23-di-Piperidinyl-Mycaminosyl-Tylonolide) in the Dogs Following Multiple Oral Administration at a Target Dose Level of 25 mg /kg." Intervet-Doc No. V-0045-0086 (September 2006 to May 2007).

b) Study Director: Chris Lowrie, Charles River Laboratories, United Kingdom.

c) Objective: To determine PMT (tildipirosin) metabolism in dogs after receiving oral administration of [^{14}C]-PMT (tildipirosin). The study was conducted in accordance with Good Laboratory Practice regulations.

d) Test Substance: [^{14}C]-PMT

e) Test Animals: Four (2 male and 2 female) beagle dogs, 11-13 months at dosing.

f) Dose: [^{14}C]-PMT (tildipirosin) was orally administered at 25 mg/kg BW once daily for 7 days.

g) Results: The highest radioactivity was detected in colon. Excretion of radioactivity was mainly through feces. Unchanged

PMT accounted for a minor portion of the total residues. See Table IV.9 for metabolites identified in dogs following oral administration of [^{14}C]-PMT (tildipirosin).

Table IV.9. Summary of PMT Metabolites Identified in Dogs Following Oral Administration of [^{14}C]-PMT.

Liver	Kidney	Urine	Feces
Dihydroxy-PMT	Desmethyl-PMT	Hydrolyzed-dihydro-PMT-SO_3H	
S-Cysteine-PMT		Dihydro-PMT-SO_3H	Dihydro-PMT-SO_3H
S-Glutathione-PMT			

c. Residue Depletion Study

i. Study Title: "Determination of the marker residue 20, 23-di-piperidinyl-mycaminosyl-tylonolide (PMT) in liver, kidney and muscle tissue and injection site of cattle after a single subcutaneous administration of 18% w/v PMT solution for injection at a dose of 4 mg PMT per kilogram body weight." Intervet-Doc. No. 2052-021-01 (September 2009 to April 2010).

ii. Study Director: Dr Patrick Lockwood

In-Life Phase - Intervet Inc., Terre Haute, IN

Analytical Phase - Ricerca Biosciences, LLC., Concord, OH

iii. Objective: To measure PMT (tildipirosin) marker residue concentrations in edible tissues of cattle after receiving a single subcutaneous administration of 18% w/v PMT at the labeled dose of 4 mg PMT/kg BW.

The study was conducted in acceptable compliance with the Good Laboratory Practice (GLP) regulations (21 CFR 58).

iv. Test Substance: 18% w/v PMT (tildipirosin) in the commercial formulation

v. Test Animals: 38 (19 castrated male and 19 female) crossbred beef commercial cattle, 6 to 12 months old, weighing 228 to 350 kg.

vi. Treatment Groups: Six treated groups of 3 castrated males and 3 females in each group and a single untreated control group of 1 male and 1 female.

vii. Dose: Animals in the treated groups received a single dose of 18% w/v PMT (tildipirosin) administered subcutaneously at 4 mg/kg BW. The injection site volume was limited to 10 mL per injection site.

viii. Sample Analysis: The concentrations of parent PMT were determined using a validated LC-MS/MS method.

ix. Results:

Table IV.10. Mean and Standard Deviation of Parent PMT Concentration (ppm) Following Subcutaneous Injection of 18% w/v PMT at 4 mg/kg Body Weight.

Slaughter Time Point (Days)	Liver	Kidney	Muscle
3	14.6 (3.2)	20.5 (1.8)	0.9 (0.2)
8	9.5 (1.1)	14.0 (3.4)	0.4 (0.1)
13	6.4 (1.4)	7.9 (1.4)	0.3 (0.1)
18	5.4 (1.2)	6.5 (0.3)	0.3 (0.0)
23	4.7 (1.5)	4.8 (0.9)	0.2 (0.0)
28	3.3 (0.9)	NA (NA)	0.2 (0.0)

NA: not applicable

2. Target Tissue and Marker Residue

The target tissue for residue monitoring is liver and the marker residue is parent PMT (tildipirosin).

3. Tolerance

The tolerance for parent PMT (tildipirosin) in liver is 10 ppm. The tolerance represents 32% of the PMT (tildipirosin) residue safe concentration for liver.

4. Withdrawal Period

The withdrawal period for the use of ZUPREVO 18% injectable solution in cattle is 21 days, based on the results of the residue depletion study (Intervet-Doc. No. 2052-021-01) and our statistical tolerance algorithm for the 99th percentile with 95% confidence. The withdrawal period is consistent with depletion of PMT (tildipirosin) residues at the injection site, when the injection site residue safe concentration of 100 ppm (10X the muscle safe concentration) is referenced in the residue chemistry evaluation.

G. Analytical Method for Residues

1. Determinative Procedure

After adding internal standard (10D-PMT) to ground, frozen bovine liver the sample is treated with 0.1% acetic acid and acetonitrile. After vortex and centrifuge, a portion of the extract is evaporated to dryness, the residue reconstituted in water, and then subjected to solid phase cartridge cleanup. The analytes are eluted with 0.05% formic acid in acetonitrile:water (7:3, v/v) mixture. The eluate is evaporated to dryness and reconstituted into aqueous ammonium acetate (0.05M). The resulting solution is

analyzed using liquid chromatography with mass spectrometric detection (HPLC-MS/MS)

2. Confirmatory Procedure

The sample extraction and preparation for the confirmatory procedures are identical to the ones for the determinative procedures. PMT is detected using a tandem mass analyzer (MS/MS). Three PMT-specific ion transitions are monitored to obtain ion ratios, signal to noise ratios, and retention times that meet the required acceptability criteria.

3. Availability of the Method

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

V. USER SAFETY:

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

For use in animals only. Not for human use. Keep out of reach of children. To avoid accidental injection, do not use in automatically powered syringes which have no additional protection system. In case of human injection, seek medical advice immediately and show the package insert or label to the physician.

Avoid direct contact with skin and eyes. If accidental eye exposure occurs, rinse eyes with clean water. If accidental skin exposure occurs, wash the skin immediately with soap and water. Tildipirosin may cause sensitization by skin contact.

For technical assistance or to report a suspected adverse reaction, call: 1-800-219-9286. For customer service or to request a Material Safety Data Sheet (MSDS), call: 1-800-211-3573. For additional ZUPREVO 18% information go to www.zuprevo.com. For a complete listing of adverse reactions for ZUPREVO 18% reported to CVM see: http://www.fda.gov/AnimalVeterinary/SafetyHealth.

VI. AGENCY CONCLUSIONS:

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

A. Marketing Status:

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). The decision to restrict this drug to use by or on the order of a licensed veterinarian was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product to treat bovine respiratory disease, or to use this product for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD, (b) adequate instructions cannot be written for the lay person in the safe use of the product, including the treatment of any adverse reactions, and (c) restricting this drug to use by or on the order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues.

B. Exclusivity:

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 been previously approved.

C. Patent Information:

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