Date of Approval: December 30, 2009

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 140-929

MICOTIL 300

Tilmicosin
Injectable Solution
Beef and non-lactating dairy cattle, and Sheep

To add *Pasteurella multocida* and *Histophilus somni* to the indication "For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni"* in beef and non-lactating dairy cattle.

To establish a dose range of 10 to 20 mg tilmicosin/kg body weight in beef and non-lactating dairy cattle for the treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* and for the control of respiratory disease in cattle at high risk of developing BRD associated with *Mannheimia haemolytica*.

Sponsored by:

Elanco Animal Health

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I. GENERAL INFORMATION:

A. File Number:	NADA 140-929
B. Sponsor:	Elanco Animal Health A Division of Eli Lilly & Co. Lilly Corporate Center Indianapolis, IN 46285 Drug Labeler Code: 000986
C. Proprietary Name(s):	MICOTIL 300
D. Established Name(s):	Tilmicosin
E. Pharmacological Category:	Antimicrobial
F. Dosage Form(s):	Sterile injectable solution
G. Amount of Active Ingredient(s):	300 mg tilmicosin/mL
H. How Supplied:	50 mL, 100 mL, and 250 mL glass vials
I. How Dispensed:	Rx
J. Dosage(s):	Cattle: 10 to 20 mg tilmicosin/kg body weight Sheep: 10 mg tilmicosin/kg body weight
K. Route(s) of Administration:	Subcutaneous injection
L. Species/Class(es):	Cattle/beef and non-lactating dairy Sheep
M. Indication(s):	For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida*, and *Histophilus somni*. For the treatment of ovine respiratory disease (ORD) associated with *Mannheimia haemolytica*. For the control of respiratory disease in cattle at high risk of developing BRD associated with *Mannheimia haemolytica*.

N. Effect(s) of Supplement:

To add *Pasteurella multocida* and *Histophilus somni* to the indication "For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni"* in beef and non-lactating dairy cattle.

To establish a dose range of 10 to 20 mg tilmicosin/kg body weight in beef and non-lactating dairy cattle for the treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* and for the control of respiratory disease in cattle at high risk of developing BRD associated with *Mannheimia haemolytica*.

II. EFFECTIVENESS:

A. Dosage Characterization:

The FOI Summary for the original approval of NADA 140-929 dated March 3, 1992, contains dosage characterization information for beef and non-lactating dairy cattle administered tilmicosin subcutaneously at a dose of 10 mg/kg body weight (BW). This minimum dose was selected to conduct studies to add *Pasteurella multocida* and *Histophilus somni* to the previously approved indication for the treatment of bovine respiratory disease (BRD). CVM did not require additional effectiveness studies for the supplemental approval of the 10 to 20 mg tilmicosin/kg BW dose range.

B. Substantial Evidence:

Multi-Location Clinical Field Study

a. Study Title: "Clinical Study: Clinical Field Efficacy and Safety of Tilmicosin (MICOTIL) for Use in the Treatment of Bovine Respiratory Disease". Study numbers T5C060303, T5C160304, T5C310305, and T5CCA0306. July 2004 to March 2005.

b. Investigators:

Terry TerHune, DVM, PhD, HMS Veterinary Development, Inc., Tulare, CA
Ed Johnson, DVM, Johnson Research, Parma, ID
Kelly Lechtenberg, DVM, PhD, Midwest Veterinary Services, Oakland, NE
Tim Guichon, DVM, Feedlot Health Management Services, Okotoks, Alberta, Canada

c. Study Design:

Objective: To evaluate the effectiveness of tilmicosin injectable for the treatment of naturally occurring BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* when administered as a single, subcutaneous injection of 10 mg/kg BW.

Animals: A total of 1,508 commercial beef cattle (males and females), approximately five to eleven months of age, weighing approximately 300 to 750 pounds, were enrolled in this study.

Experimental Design: The study was conducted at four sites. At each site, animals were randomly allocated to treatment and pen. Pen size and number of pens varied across the study but were consistent at a site. Treatment and control animals were commingled in pens.

An animal was enrolled into the study when it had a rectal temperature ≥ 104˚F and a depression score ≥ 2 or a respiratory score ≥ 2.

Dyspnea was assessed using the following clinical scoring scale:

0 = Normal: No abnormal respiratory symptoms. 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: Mucus 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, with one or more of the following: open mouth breathing, abdominal breathing, and/or extended head.

Depression was assessed using the following clinical scoring scale:

0 = Normal: Bright, alert, and responsive.
1 = Mild Depression: May stand isolated with its head down or ears drooping, but will quickly respond to minimal stimulation.
2 = Moderate Depression: May stand isolated with its head down and may show signs of muscle weakness (standing cross-legged or knuckling when walking). Shows a delayed response to minimal stimulation or requires greater stimulation before showing a response.
3 = Severe Depression: May be recumbent and reluctant to rise, or if standing isolated, may be reluctant to move. Ataxia, knuckling, or swaying may be evident when moving. Head carried low with eyes dull and ears drooping. Possible excess salivation and/or lacrimation.

Test Article Administration: Tilmicosin was administered as a single subcutaneous injection at a dose of 10 mg/kg BW. Saline, injected at an equivalent volume, was used as the negative control article.

Measurements and Observations: The primary variable was the treatment success rate on Day 13. Treatment success was defined as an animal with a depression score of ≤ 1, and a respiratory score of ≤ 1, and a rectal temperature < 104.0˚ F. Cattle not meeting the criteria for success were classified as treatment failures.

Cultures were performed on nasal swabs and lung samples. Other supportive variables recorded were individual scores for respiration and depression, rectal temperature, and body weight.

Statistical Analysis: Each study site was initially evaluated individually and data were combined for analysis. The analysis of Day 13 treatment success was performed using a generalized linear mixed-effects model. Mortality data

was combined across sites and statistically evaluated by the Fisher's Exact Test.

d. Results: Refer to Table 1 below. The success rate was statistically significantly higher for the tilmicosin-treated group (63.1%) compared to the control group (29.2%) at P = 0.004. The Day 13 mortality rate was statistically significantly lower for the tilmicosin-treated group (1.3%) compared to the control group (6.2%).

Table 1: Treatment Success on Day 13

Site	Saline	MICOTIL 300
T5C060303 CA	38/188 (20.2%)	82/188 (43.6%)
T5C160304 ID	53/190 (27.9%)	133/191 (69.6%)
T5C310305 NE	43/199 (21.6%)	114/199 (57.3%)
T5CCA0306 Can	85/172 (49.4%)	144/172 (83.7%)
Combined Sites	219/749 (**29.2**%)	473/750 (**63.1**%)

Eight hundred ninety *Mannheimia haemolytica* isolates, 375 *Pasteurella multocida* isolates, and 101 *Histophilus somni* isolates were isolated from nasopharyngeal swabs and lung tissue.

e. Adverse Events: Adverse events seen during the study were ocular discharge, diarrhea, and lameness. Some non BRD-related deaths occurred during the study but there were more of these deaths in the control groups than in the treated groups. None of the adverse events were considered to be drug related.

f. Conclusions: These results demonstrate that tilmicosin, when administered to beef and non-lactating dairy cattle as a single, subcutaneous injection of 10 mg/kg BW was effective for the treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*.

C. Pharmacology

1. Pharmacokinetic Study

a. Study Title: "Bovine Tonsil and Intracellular Alveolar Macrophage Tilmicosin Concentrations following Administration of Tilmicosin at 10 mg/kg BW." Study# T5C660004

b. Investigator:

Shawn Fossler, BS, Elanco Animal Health,
Greenfield, IN

c. Study Design:

Objective: To determine tilmicosin concentrations in the alveolar macrophages of cattle following a single, subcutaneous injection of 10 mg/kg BW and comparing the tilmicosin concentration with the minimum inhibitory concentration data for *Mannheimia haemolytica*.

Animals: Twenty-six healthy, crossbred cattle (14 males and 12 females), weighing between 400 and 500 kg, were used in this study.

Experimental Design: The study consisted of an untreated control group (1 male and 1 female) and six tilmicosin treatment groups (2 males and 2 females per group).

Test Article Administration: A single, subcutaneous injection of tilmicosin was administered at a dose of 10 mg/kg BW. A single treatment group was euthanized at 96, 120, 144, 168, 240, or 336 hours after treatment.

Measurements and Observations: Following euthanasia, the lungs and trachea were removed from each animal and a bronchial alveolar lavage was performed. The total intracellular alveolar macrophage volume was calculated and the alveolar macrophages were processed, assayed, and the total tilmicosin content determined. A validated high pressure liquid chromatography (HPLC) was used to assay the samples for tilmicosin concentration.

d. Results:

Table 2: Mean Tilmicosin Concentration (ppm) in Alveolar Macrophages From Healthy Cattle Treated With a Single 10 mg/kg BW Subcutaneous Injection of MICOTIL 300

Withdrawal Time (hr)	**Tilmicosin Concentration (ppm)**
96	8.96
120	13.93
144	16.10
168	5.88
240	15.11
336	6.00

e. Conclusions: These results demonstrate that the tilmicosin concentration in the alveolar macrophages is above 4 µg/mL for 14 days after a single injection of tilmicosin at 10 mg/kg BW.

2. Pharmacokinetic Study

a. Study Title: "Distribution of Tilmicosin into Bovine Peripheral neutrophils following MICOTIL treatment." Study #T5C760002

b. Investigator:

Shawn Fossler, BS, Elanco Animal Health,
Greenfield, IN

c. Study Design:

Objective: To determine the time course of tilmicosin distribution into peripheral neutrophils of cattle following a single, subcutaneous dose of tilmicosin at 10 mg/kg BW.

Animals: Six healthy, crossbred cattle (3 males and 3 females), weighing approximately 300 kg, were used in this study.

Experimental Design: The study consisted a single treatment group (3 males and 3 females) treated with tilmicosin.

Test Article Administration: Tilmicosin (300 mg/mL) was administered as a single, subcutaneous injection of 10 mg/kg BW.

Measurements and Observations: At 8 hours and 1, 2, 3, 4, 5, 6, 7, 9, 10, and 14 days after treatment, 60 mL of whole blood was obtained from each animal. Peripheral neutrophils were isolated from each blood sample and

sample purity, viability, and total cell count was determined. Tilmicosin was extracted and assayed (HPLC) and intracellular tilmicosin concentrations were calculated.

d. Results:

Table 3: Mean Tilmicosin Intracellular Concentration (µg/mL) in Peripheral Neutrophils From Healthy Cattle Treated With a Single, 10 mg/kg BW, Subcutaneous Injection of Tilmicosin

Withdrawal Time (days)	Tilmicosin Concentration (ppm)
0.33	7.03
1	13.96
2	11.00
3	6.50
4	4.19
5	6.62
6	5.57
7	4.90
9	2.53
10	3.59
14	0.00

e. Conclusions: These results demonstrate that intracellular tilmicosin concentrations in the peripheral neutrophils are above 4 µg/mL for 7 days after a single injection of tilmicosin at 10 mg/kg BW.

III. TARGET ANIMAL SAFETY

A. Toxicity Study:

1. Study Title: "Non-clinical Laboratory Study: Evaluation of the Margin of Safety of Injectable Tilmicosin in Cattle" (Study No.: MCL-LRL-0538)

2. Study Dates: (Live Phase) June 2006 to July 2006

3. Investigator: Teresa Schieber, DVM, Midwest Veterinary Services, Inc., Oakland, NE

4. General Study Design:

Objective: To evaluate the margin of safety of injectable tilmicosin solution in beef cattle administered subcutaneous doses of 0 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, or 60 mg/kg of BW at three day intervals (Study Days 0, 3 and 6).

Test Animals: 30 crossbred beef cattle (15 males, 15 females) approximately 8 to 10 months of age with an average weight of 180 kg for bulls and 174 kg for heifers

Test and Control Articles: The test article was commercially available injectable tilmicosin solution, delivered as a subcutaneous injection. The control article was commercially available 0.9% sterile saline solution.

Treatment Administration: The administration of the test and control articles to the study's treatment groups is outlined in Table 4 below.

Table 4: Treatment Groups

Treatment Group	Treatment Description	Days of Treatment Administration	Number of Animals
TG01	Sterile saline injected subcutaneously (SC) at a volume equivalent to the 60 mg tilmicosin/kg BW dose of the test article (TG05)	Study Days 0, 3, and 6	6 (3 males and 3 females)
TG02	Single SC injection of 20 mg tilmicosin/kg BW	Study Days 0, 3, and 6	6 (3 males and 3 females)
TG03	Single SC injection of 30 mg tilmicosin/kg BW	Study Days 0, 3, and 6	6 (3 males and 3 females)
TG04	Single SC injection of 40 mg tilmicosin/kg BW	Study Days 0, 3, and 6	6 (3 males and 3 females)
TG05	Single SC injection of 60 mg tilmicosin/kg BW	Study Days 0, 3, and 6	6 (3 males and 3 females)

Measurements and Observations: The following measurements and observations were made on Study Days -1, 1, and 7: physical examination, general health observations, feed and water consumption, body weights, hematology, serum chemistry, urinalysis, and fecal analysis. Animals were necropsied on Day 7 and organ weights and tissue samples for histopathology were obtained.

Statistical Methods: The body weight (in kg), and organ weight (in kg) data expressed as percent of body weight for each organ were analyzed by analysis of variance with the model containing treatment, sex, and sex by treatment interaction terms in the model. The hematology and urinalysis variable values were analyzed by a repeated measure model with treatment, sex, day, treatment by sex, sex by day, treatment by day, and treatment by sex by day terms in the

model. Day -1 values were used as a covariate. Treatment effect was evaluated at the 10% significance level.

5. Results:

General Health Observations: No abnormalities were observed.

Physical Examinations: No health abnormalities, except injection site lesions, were observed in any of the study animals.

Feed Consumption: Decreased feed consumption was seen in the treated groups when compared to the control group.

Water Consumption: Decreased water consumption was seen in the treated groups when compared to the control group.

Body Weights: No statistically significant test article-related effects were observed.

Hematology: Test article administration resulted in statistically significant, dose independent, slight increases in mean neutrophil and eosinophil counts in all tilmicosin-treated groups as compared to controls. Although some values fell outside the normal reference ranges, the variations from the normal reference ranges were minor and considered secondary to inflammation and/or edema at the injection sites and therefore, not a clinically relevant test article-related effect.

All treatment groups had slightly elevated (above the reference range) red blood cell counts. The red blood cell elevations were considered to be related to decreased water consumption and edema at the injection sites.

Serum Chemistry: Minimal to slight decreases in mean total protein due to decreases in mean albumin concentrations occurred in all tilmicosin-treated groups as compared to the control group. Although some values fell outside the normal reference ranges, the variations from the normal reference ranges were minor and considered secondary to inflammation and/or edema at the injection sites and therefore, not a clinically relevant test article-related effect. Minimal decreases in mean serum calcium concentrations were likely secondary to the decreases in albumin concentrations. Means for calcium in all treatment groups were within normal ranges.

Urinalysis: No statistically significant test article-related effects were observed.

Fecal Analysis: No statistically significant test article-related effects were observed.

Gross Pathology: Lesions related to test article administration were limited to injection sites of animals in the treated groups. Lesions were described as being

generally more severe and occurred at higher frequency rates in the animals treated with higher doses of tilmicosin.

Organ Weights: Statistical analysis of organ weights (as a percent of body weight) revealed no dose-related findings or test article-related observations.

Histopathology Results: Lesions related to test article administration were limited to injection site reactions of all animals in each of the treated groups (TG02, TG03, TG04, and TG05). Injection sites were generally characterized by interstitial edema in the subcutaneous tissues. The edema was multi-focal in distribution and occurred in areas between muscle layers and between the skin and underlying tissue. Acute inflammation with occasional hemorrhage scattered throughout the subcutaneous tissues was observed.

6. Conclusions

This study demonstrated that tilmicosin is safe when administered at 20 mg/kg BW as a single subcutaneous injection to beef and non-lactating dairy cattle.

IV. HUMAN FOOD SAFETY:

A. Toxicology:

1. Summary of Toxicology Studies

A summary of toxicology studies performed with tilmicosin are included in the original approval for this NADA (see the FOI Summary for the original NADA 140-929, approved March 3, 1992).

An assessment was prepared to determine if a microbiological Acceptable Daily Intake (ADI) was needed for tilmicosin residues present in edible tissues of cattle. The following studies were performed to provide data needed to answer the questions asked in a stepwise approach to establishing a microbiological ADI.

a. Activity of tilmicosin against bacterial strains representing the normal human intestinal microbiota: determination of minimum inhibitory concentration (MIC).

Study No: 035/05
Report date: February 26, 2006
Starting date: September, 2005
Termination date: February, 2006
Study Director: Andrew Pridmore, BSc, PhD.
Whitley Scientific Ltd.
United Kingdom

Minimum inhibitory concentrations (MICs) for tilmicosin were determined against 10 bacterial isolates from each of 10 bacterial groups representative of human intestinal flora obtained from feces of healthy human volunteers. An additional 20 strains of *Bifidobacterium* spp. were also tested (study DWS 030/06) to confirm the susceptibility of this species. The test system was standardized agar dilution MIC methodology as recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines. The study was performed in compliance with GLP (21 CFR Part 58) regulations of the European Union, United States, and Japan. Table 5 below summarizes the results of the MIC tests, including the results of 30 species of *Bifidobacterium*:

Table 5: Results of MIC Testing With Tilmicosin Against Representatives of the Human Intestinal Flora

Bacterial group	**MIC range**	**MIC_{50}**	**MIC_{90}**
Bacteroides fragilis	1 - >128	16	128
Bacteroides (other species)	0.5 - >128	8	64
Bifidobacterium	0.016 - 16	0.125	8
Clostridium	0.062 - >128	16	>128
Enterococcus	8 - 32	8	16
E. coli	64 - >128	128	>128
Eubacterium	All 32	32	32
Fusobacterium	2 - 128	32	32
Lactobacillus	1 - >128	16	>128
Peptostreptococcus	All 32	32	32
All strains (n=100)	0.016 - >128	32	>128

The results demonstrated that tilmicosin activity varied considerably between bacterial groups and also within bacteria of the same group. The lowest tilmicosin activity was shown to be against *Escherichia coli* strains. Tilmicosin was most active against *Bifidobacterium* spp.

b. Effect of fecal binding on the antibacterial activity of tilmicosin.

Study No: 036/05
Report date: February 24, 2006
Starting date: November 22, 2005
Termination date: December 16, 2005
Study Director: Andrew Pridmore, BSc, PhD.
Whitley Scientific Ltd.
United Kingdom

The objective of the study was to assess the effect of fecal binding on the antibiotic activity of tilmicosin.

Tilmicosin concentrations of 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 µg/mL were mixed with fecal concentrations of 0, 10%, 25%, and 50% weight per volume (w/v) of samples from three different donors. The mixtures were incubated for 30 minutes, 1, 2, 6, 8, and 12 hours and samples were taken at each time point. After removal of fecal solids by centrifugation, supernatants were inoculated with a susceptible strain of *Staphylococcus aureus* (ATCC strain) and incubated for 24 hours to assess antibacterial activity. Differences in activity before and after interaction with feces were used to calculate the percentage of tilmicosin bound to feces.

Results were consistent among samples of the three fecal donors and showed correlation between fecal concentration and the percentage of binding. In two cases, tilmicosin bound to 10% feces at a level of 87.5% within 30 minutes. In 25% feces, it was bound between 75% and 94% before incubation and the binding increased to >96% during incubation. In 50% feces, tilmicosin was bound between 87% and 94% before incubation and >96% after incubation. The incubation time to achieve maximum binding was between 1 and 6 hours. Binding showed greater time dependency in the presence of higher fecal concentrations (25% and 50%) and reached a higher level than in 10% feces.

Based on the results of this study, it is concluded that binding of tilmicosin to feces is reliably estimated to be greater than 96% for fecal concentrations above 25% tilmicosin. For practical reasons, the 50% w/v fecal concentration used in the study is assumed to be most similar to the *in vivo* situation in relation to binding of tilmicosin residues to the intestinal contents.

c. Microbiological end-point determination for two antibiotics.

Study No: 3.1164 and 1164/2
Report date: April 16, 1993
Starting date: Study 1: September 11, 1992 (arrival of the animals to the laboratory and dosing with human feces)
Study 2: November 6, 1992 (arrival and dosing of animals with human feces)
Termination date: Study 1: October, 1992; Study 2: December, 1992
Study Director: C.J. Rumney, BSc, PhD
BIBRA Toxicology International
United Kingdom

The objective of the study was to use human flora-associated rats to determine the effect of low doses of tilmicosin and spiramycin on colonization barrier disruption and development of resistant *Enterobacteriaceae* on human intestinal flora.

Two studies were conducted in which germ-free rats were initially associated with a suspension of pooled human feces from healthy volunteers (two in

Study 1 and three in Study 2). Two male and two female rats were randomly allocated to the treatment groups one week after dosing with the fecal suspension (time necessary for the dosed material to reach equilibrium in the rat). The treatment groups included a negative control (dosed with deionized water), two groups dosed with tilmicosin at 400 and 120 µg/kg BW/day, respectively, and a positive control group dosed with spiramycin at 500µg/kg BW/day. The animals were maintained in isolators under controlled environmental conditions during the study.

Rat fecal samples from each rat were studied on the following days: two days prior to treatment (pre-treatment period), five days during antibiotic treatment, and two days after antibiotic treatment (post-treatment period). The following bacterial counts were performed on each sample: total anaerobes, total *Enterobacteriaceae*, total *Enterobacteriaceae* resistant to tilmicosin, and total *Enterobacteriaceae* resistant to spiramycin. Bacterial counts were performed in appropriate selective media. The proportion of total *Enterobacteriaceae* and *Enterobacteriaceae* resistant to tilmicosin and spiramycin were reported as the difference between the log counts.

The no observed adverse effect level (NOAEL) for this study is 400 µg/kg BW/day. Results showed that this dose produced an increase in the anaerobe population and a significant but transient increase in the counts of *Enterobacteriaceae* and tilmicosin-resistant *Enterobacteriaceae*. However, the flora recovered rapidly. The high tilmicosin dose could be considered to be very close to the low observed adverse effect level (LOAEL); however, this dose was set as the NOAEL because of the wide variability among the control animals in both studies and the inconsistency of many of the results. This was observed in the bacterial counts of sensitive anaerobes and *Enterobacteriaceae* as well as in the results for tilmicosin-resistant *Enterobacteriaceae*.

2. No Observed Adverse Effect Level (NOAEL)

The NOAEL for establishing the toxicological ADI for tilmicosin based on the basic package of toxicology studies was 4 mg/kg BW/day. This NOAEL was obtained from Study D07187 entitled "*A One-Year Chronic Toxicity Study in Beagle Dogs Given Oral Doses of Tilmicosin"* (details are in the FOI Summary for NADA 140-929, dated March 3, 1992).

3. Acceptable Daily Intake (ADI)

Toxicological ADI

The toxicological ADI for tilmicosin residues is calculated as follows:

Acceptable Daily Intake (ADI) = Lowest NOAEL/Safety Factor

A safety factor (SF) of 100 was used because the ADI is based on chronic data.

The lowest NOAEL was 4 mg/kg
Toxicological ADI = 4 mg/kg/100
= 0.04 mg/kg or 40 µg/kg BW/day

Microbiological ADI

Based on data obtained from *in vitro* and *in vivo* studies and the assessment presented by the sponsor, it is concluded that there is a need to determine a microbiological ADI for tilmicosin residues. Of the two endpoints of concern (i.e., disruption of colonization barrier and increase of the population(s) of resistant bacteria), a microbiological ADI is determined for disruption of the colonization barrier. The assessment on the effects of tilmicosin residues present in edible tissues of cattle on the human intestinal flora concluded that the amount of active tilmicosin residues reaching the human colon following a 28-day withdrawal period could affect the colonization resistance property of the human intestinal colon. The increase in the population of resistant bacteria is not relevant at this time because 1) residue levels in edible tissues other than injection site are relatively low compared with MICs of the majority of the genera tested; and 2) resistant populations already exist in the human intestinal flora.

The microbiological ADI for tilmicosin residues is determined from *in vitro* susceptibility data and following an accepted step-by-step approach. Although data from *in vivo* studies are usually considered more relevant to assess effects in humans, in this case the ADI is determined from the more recent *in vitro* data because the *in vivo* study was poorly designed, and the effects observed were transient and inconsistent.

The step-by-step approach followed to calculate the microbiological ADI for disruption of the colonization barrier of the human colon included:

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

Yes. Results of Study 035/05 "Activity of tilmicosin against bacterial strains representing the normal human intestinal microbiota: determination of Minimum Inhibitory Concentration (MIC)" showed that tilmicosin was most active against *Bifidobacterium* spp. and had the lowest activity against *E. coli*.

Step 2. Do residues enter the human colon?

Yes. Tilmicosin residues present at the injection site reach the human colon. Injection site residues will be used for determining the microbiological ADI because they represent the worst case scenario (they are at the highest concentration compared to residues in remote muscle). According to residue

depletion data obtained from animals dosed with 20 mg/kg BW, the amount of residues at the injection site that could reach the human colon would be 0.56 - 2.18 mg of active tilmicosin. This value is determined based on the following:

- A residue concentration at the injection site after a 28-day withdrawal time of 8.3 ± 4.9 mg/kg or 1.02 – 3.96 mg/300 grams of injection site considering the consumption value for muscle.
- Metabolism data from cattle, swine, and rats showing that approximately 55% of an original oral dose is excreted in animal feces. This translates into 0.56 – 2.18 mg of tilmicosin reaching the colon with a concentration of 2.5 – 10 µg/mL (considering 220 grams of colon content).
- Data obtained from an *in vitro* fecal binding study performed with human fecal slurries (Study 036/05) showing that 96% of tilmicosin residue binds to fecal solids and approximately 4% is free for interaction with bacteria. The 4% of 2.5 – 10 µg/mL would be 0.1 to 0.4 µg/mL. For the purpose of the assessment, it will be considered that 0.4 µg/mL of active tilmicosin remains in the colon for interaction with bacteria.

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

Yes. Residues entering the colon are microbiologically active tilmicosin residues, according to results of *in vitro* and *in vivo* studies performed with tilmicosin.

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

Yes. The increase in the population of resistant bacteria is not a relevant endpoint at this time because 1) residue levels in edible tissues other than injection site are relatively low compared with MICs of the majority of the genera tested; and 2) resistant populations already exist in the human intestinal flora.

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

The microbiological ADI for disruption of the colonization barrier of the human colon is determined based on *in vitro* MIC data and applying the following formula as recommended in GFI #159.

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

MIC_{calc}: calculated based on the MIC_{50} of the following bacterial genera: *Bacteroides* other than *B. fragilis, Enterococcus*, and *Bifidobacterium*. These

genera had MIC_{50} values $\leq$8 µg/mL. The MIC_{calc} was determined to be 0.146 µg/mL.

Mass of colonic content = 220 grams (as stated in GFI #159).

Fraction of oral dose available to microorganisms: The fraction of oral dose available for interaction with colonic flora is 2.2% of the total tilmicosin entering the colon. This percentage reflects metabolism of the residues while passing through the gastrointestinal tract and also the percentage of binding of tilmicosin residues to human feces. The actual active tilmicosin concentration in the colon is 0.4 µg/mL (2.2% of 3.96 mg ingested in 300 grams of injection site muscle).

Consequently, the microbiological ADI for tilmicosin residues is calculated as follows:

$$\text{Microbiological ADI} = \frac{0.146\ \mu g/mL \times 220\ g}{0.022 \times 60\ kg} = \frac{32.12}{1.32} = \textbf{24.3 µg/kg BW/day or 1.46 mg/person/day}$$

Although the concentration of active tilmicosin in the colon (0.4 µg/mL) is higher than the MIC_{50} obtained for the genus *Bifidobacterium* (0.125 µg/mL), no major concerns on the safety of tilmicosin residues exist for the following reasons:

(1) A total of three bacterial groups were considered in the determination of the MIC_{calc}, including *Bacteroides* other than *B. fragilis* group (which are the major bacterial groups present in the human intestinal flora), *Bifidobacterium*, and *Enterococcus*. Two of these bacterial groups had MIC_{50} values higher than the concentration in the colon (*Bacteroides* = 8 µg/mL; *Enterococcus* = 8 µg/mL). The *Bacteroides* are not relevant for this compound because macrolides are of no real value in treating infections caused by these groups. However, they were included in the calculation because of their low MIC_{50} values.

(2) The increased sensitivity of a single genus of intestinal flora (*Bifidobacterium*) may not be predictive of disruption of the colonization barrier. Disruption of the colonization barrier is "a function of the normal intestinal flora that limits colonization of the colon by exogenous microorganisms, as well as overgrowth of indigenous, potentially pathogenic microorganisms." It is yet unknown which bacterial groups (or a combination of) are important in maintaining this function. Nevertheless, it is unknown if there is a threshold value for the population of any bacterial group (or a combination of) that is important for maintaining this function. Therefore, it is highly unlikely that a decrease in the population of only one bacterial genus could disrupt the function of the intestine. It is well documented that hundreds if not thousands of bacterial groups constitute this complex system that is the human intestinal flora. This complex system

is well balanced and maintained more or less unchanged during the life of an individual, unless disrupted by substances with antimicrobial activity.

(3) The use of MICs to assess the potential for a drug to disrupt the colonization barrier does not take into account the complexity of the human intestinal flora. Therefore, the MIC_{50} of the most relevant genus/genera for which the drug is active results in a conservative estimate of a NOAEC for disruption of the colonization barrier. The NOAEC is conservative because, among other reasons, the inoculum density is orders of magnitude lower than the bacterial population in the intestinal tract. In addition, MIC testing is performed in specific culture media that is appropriate for the different types of bacterial groups tested. Consequently, a lower bacterial population in under optimal growth conditions would be more sensitive to the action of an antimicrobial drug than the same bacterial group with a much higher bacterial density, in the presence of many more bacterial groups in the human intestinal environment.

The microbiological ADI is lower than the toxicological ADI calculated from chronic oral toxicity data (40 µg/kg BW/day); therefore, the microbiological ADI of 24.3 µg/kg BW/day is the final ADI for tilmicosin residues.

The previously established ADI for tilmicosin residues was 25 µg/kg BW/day. This ADI was assigned in 1996 based on the default upper acceptable limit for microbiological residues, according to a 1996 guideline that no longer reflects the Agency's current thinking. The current microbiological ADI of 24.3 µg/kg BW/day was calculated based on the data and approach presented in this summary. The new microbiological ADI is very similar to the previously published ADI of 25 µg/kg BW/day; therefore, the ADI for tilmicosin residues is maintained as published in 21 CFR 556.735.

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

$$\text{Safe Concentration (SC)} = \frac{\text{Acceptable Daily Intake (ADI) x Human Weight}}{\text{Grams of Tissue Consumed/Day}}$$

The average human weight is approximated as 60 kg. The daily consumption values of tissues are approximated as 300 g for muscle, 50 g for fat or kidney, and 100 g for liver[1].

[1] SECTION IV. Guideline for Establishing a Safe Concentration, In: *General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals*, revised July 1994

SC (muscle) = $\frac{24.3\ \mu g/kg\ BW/day \times 60\ kg}{300\ g/day}$ = 4.86 µg/g = 4.86 ppm

SC (fat or kidney) = $\frac{24.3\ \mu g/kg\ BW/day \times 60\ kg}{50\ g/day}$ = 29.16 µg/g = 29.16 ppm

SC (liver) = $\frac{24.3\ \mu g/kg\ BW/day \times 60\ kg}{100\ g/day}$ = 14.58 µg/g = 14.58 ppm

B. Residue Chemistry:

1. Summary of Residue Chemistry Studies

a. Total residue depletion studies

The total residue depletion and metabolism in the target species and comparative metabolism in the toxicological species for MICOTIL 300 (tilmicosin) injectable solution are summarized in the FOI Summary for NADA 140-929 (approval date March 3, 1992).

b. Residue depletion studies

The following pivotal study was conducted to permit decisions on tolerances and the withdrawal time.

Study 007-00993--"Non-clinical Laboratory Study (GLP): Marker Residues of Tilmicosin in Liver and Muscle following Fourteen Days of Oral Administration with or without Concurrent Use of MICOTIL in Feedlot Cattle"

Study Director: Erin I. Weich, D.V.M., Southwest Bio-Labs, Inc., Las Cruces, NM 88005

Test Animals: Thirty-six (18 males and 18 females) cross-bred cattle, weighing 369 to 399 pounds at the start of the study

Treatment Design: In the study, groups of animals received tilmicosin in the feed at 12.5 mg/kg BW for 14 days. One group of the animals was slaughtered at 28 days post last dose in feed and tissue samples were analyzed for tilmicosin concentrations. Another group of the animals received concomitant tilmicosin injectable at a dose of 20 mg/kg BW on Day 14, after being fed with tilmicosin for 14 days. These animals were subjected to either a 28-day or a 35-day withdrawal period post injection prior to slaughter. Tissue samples were analyzed for tilmicosin concentrations.

Marker Residue Depletion Data: The liver and injection site residue data from cattle that received feed and concomitant injectable treatment are summarized in Table 6, below.

At 28 days withdrawal, the mean concentration of the injection site residues was 1.07 ppm. Although the individual and mean residue concentrations were below the research tolerance limit of 5 ppm for injection site, the 99% tolerance limit with 95% confidence exceeded 5 ppm.

At 35 days withdrawal, the individual, mean and 99% tolerance limit with 95% confidence were well below 5 ppm.

Table 6: Liver and Injection Site Parent Tilmicosin Residue Concentrations From Cattle That Received Tilmicosin in Feed and Up to 13.5 mL Tilmicosin Injectable at the Injection Site Analyzed*

Days	**Animal No.**	**Liver (ppm)**	**Inj. Site (ppm)**	**Days**	**Animal No.**	**Liver (ppm)**	**Inj. Site (ppm)**
-	-	-	-	35	4803	0.338	1.02
28	4804	0.394	0.242	35	4810	0.423	1.51
28	4805	2.06	0.693	35	4814	0.408	0.172
28	4811	0.256	2.27	35	4817	0.628	0.0315
28	4813	0.261	2.98	35	4819	0.980	0.841
28	4821	0.500	0.391	35	4824	0.665	1.30
28	4822	0.220	0.197	35	4826	0.672	0.416
28	4835	0.789	0.717	35	4831	0.479	0.433
	Mean	0.64	1.07		Mean	0.57	0.72
	S.D.	0.66	1.10		S.D.	0.21	0.54
99% Statistical Tolerance		3.7 ppm	6.2 ppm	99% Statistical Tolerance		1.5 ppm	3.1 ppm

* The injection site residue data shown in Table 6 were from animals that received 11 to 13.5 mL tilmicosin injectable at the injection site analyzed, except for one animal that was excluded from the table because its injection site residue concentration was below the limit of quantitation. The maximum single injection site volume allowable in the product label is 10 mL. The residue data in Table 6 were obtained from a study in which the treated animals received tilmicosin in feed and a concomitant tilmicosin injectable treatment. The use condition represents a worse-case scenario than tilmicosin injectable treatment alone. Therefore, the data support making conservative Residue Chemistry decisions.

The parent tilmicosin residue concentrations in individual muscle samples of all the treated animals in the study were below 0.05 ppm at 28-day withdrawal and onward.

2. Target Tissue and Marker Residue Assignment

The target tissue for residue monitoring is liver and the marker residue is parent tilmicosin.

3. Tolerance Assignments

Based on the results in Table 6, above, the codified tolerance of 1.2 ppm (21 CFR 556.735) for parent tilmicosin in liver has been maintained to take into account the injection site residues resulting from the treatment with tilmicosin injectable at the dose range of 10 to 20 mg/kg BW and the assigned withdrawal period.

In addition, the codified tolerance of 0.1 ppm for parent tilmicosin in muscle has been maintained.

A research tolerance of 5 ppm for parent tilmicosin at injection site has been established for making decisions regarding the safety of the injection site residues. The injection site safe concentration is the same as the remote muscle safe concentration because the microbiological endpoint used to set the final ADI was an acute effect and, consequently, a 10 x factor nominally applied to calculating injection site safe concentration from remote muscle safe concentration does not apply. Because tilmicosin is considered the only microbiologically active residue of concern, the injection site safe concentration and the injection site research tolerance are of the same value of 5 ppm.

4. Withdrawal Time(s)

A 42-day withdrawal period has been assigned.

C. Microbial Food Safety:

Elanco Animal Health submitted a microbial food safety *hazard characterization,* correctly addressing the microbial food safety hazard as human illness caused by macrolide-resistant *Campylobacter* spp., attributable to consumption of contaminated beef, and treated with an antibiotic from the macrolide class. The hazard characterization addressed the expansion of the current dose from 10 mg/kg BW (1 mL/30 kg or 1.5 mL/100 lb of body weight) to 10 to 20 mg/kg body weight (1 to 2 mL/30 kg or 1.5 to 3.0 mL/100 lb of body weight). The hazard characterization included information on tilmicosin, specifically its spectrum of antibacterial activity, mechanisms of macrolide resistance in *Campylobacter*, and current prevalence of macrolide-resistant *Campylobacter* in retail beef. In addition, data from studies assessing the potential of tilmicosin phosphate to select for

macrolide resistant *Campylobacter* spp. in cattle when administered at the high end of the dose range (20 mg/kg body weight), were provided.

The Agency determined that the magnitude of the hazard associated with the proposed new use of tilmicosin phosphate in cattle (a single injection of 10 to 20 mg/kg BW for 1) the treatment of bovine respiratory disease (BRD) in cattle associated with *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni*; and 2) for the control of respiratory disease in cattle at high risk of developing of BRD associated with *Mannheimia haemolytica* was adequately addressed by the information provided in the *hazard characterization*, and no further microbial food safety assessment was warranted. The proposed conditions of use correlate with risk management/mitigation steps for an antimicrobial drug belonging to a class of antimicrobial drugs used in food-producing animals and considered critically important to human medicine, and include prescription (Rx) only marketing status, use in individual, diseased animals, and continued monitoring by the National Antimicrobial Resistance Monitoring System (NARMS).

D. Analytical Method for Residues:

Information regarding regulatory analytical method has been provided (see the FOI Summary for the approval of NADA 140-929, approval dated March 3, 1992).

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to MICOTIL 300:

Not for human use. Injection of this drug in humans has been associated with fatalities. Keep out of reach of children. Do not use in automatically powered syringes. Exercise extreme caution to avoid accidental self-injection. In case of human injection, consult a physician immediately and apply ice or cold pack to injection site while avoiding direct contact with the skin. Emergency medical telephone numbers are 1-800-722-0987 or 1-800-428-4441. Avoid contact with eyes.

Read Note to the Physician, Spanish translation, and other important information fully before use.

The product labeling includes a client information sheet titled Important Client Information. It contains the following information.

Safe Handling Practices When Using MICOTIL 300 Tilmicosin Injection, USP

Please read this information before you start using MICOTIL. This information is a summary and is not intended to take the place of discussions with your veterinarian. MICOTIL can only be prescribed by a licensed veterinarian who has information specific to your operation. You should discuss with your veterinarian how to use MICOTIL, human warnings associated with the product and recommended safe

handling and use practices. For emergency medical information call 1-800-722-0987 or 1-800-428-4441. If you have any questions about MICOTIL, talk with your veterinarian or call Elanco at 1-800-428-4441. To report an adverse drug event contact Elanco at 1-800-428-4441.

1. WHAT ARE THE POSSIBLE EFFECTS OF ACCIDENTAL HUMAN INJECTION?

   Human injections of MICOTIL have been associated with fatalities. Clinical signs from human exposure include off taste in the mouth, nausea, headache, dizziness, rapid heart rate, chest pain, anxiety or lightheadedness. Local reactions such as injection site pain, bleeding, swelling or inflammation have been reported.

2. WHAT SHOULD I DO IN THE CASE OF ACCIDENTAL HUMAN INJECTION?

   - Immediately seek medical attention.
   - Apply ice or cold pack to injection site, while avoiding direct contact with the skin, and transport immediately to a hospital.
   - Call 1-800-722-0987 or 1-800-428-4441 for further emergency information.

3. WHAT SHOULD MY PHYSICIAN KNOW IN THE CASE OF ACCIDENTAL HUMAN INJECTION?

   - The cardiovascular system is the target of toxicity and should be monitored closely.
   - Cardiovascular toxicity may be due to calcium channel blockade.
   - Intravenous calcium administration reversed the cardiovascular effects of MICOTIL in dogs and may provide benefit in patients exhibiting low blood pressure (hypotension) or rapid heart rate (tachycardia).
   - Dobutamine improved some of the cardiac function in dogs given MICOTIL.
   - Epinephrine increased the toxicity of MICOTIL in pigs, resulting in death.
   - Propanolol (a beta-adrenergic antagonist), further decrease cardiac function in dogs given MICOTIL.
   - The active ingredient in MICOTIL is tilmicosin phosphate and persists in tissue for several days.
   - Call 1-800-722-0987 or 1-800-428-4441 for further emergency information.

4. WHAT ARE THE PROPER WAYS TO HANDLE AND STORE MICOTIL?

   - Store at or below 86°F (30°C), out of direct sunlight, in a safe location, not easily accessible to the general public.
   - Read, understand and follow all label use directions.
   - Keep the needle capped until ready to use.
   - Never carry a loaded syringe with an attached needle in pocket or clothing.
   - Wash hands thoroughly with soap and water after handling.

5. WHAT ARE THE PROPER METHODS FOR ADMINISTERING MICOTIL?

- Properly restrain animals prior to administration.
- Work in a team, or if alone, advise someone of your location and how long you plan to be there.
- For subcutaneous use. Do not use in automatically powered syringes.
- Use a 1/2-inch to 5/8-inch, 18- to 16-gauge needle.
- With a single hand on the syringe, insert the needle subcutaneously, at a top-down angle, while avoiding penetration of underlying muscle.
- For cattle and sheep, injection under the skin in the neck is suggested. If not accessible, inject under the skin behind the shoulders and over the ribs.
- Administer a single subcutaneous dose of 1.5 to 3.0 mL of MICOTIL per 100 lbs of body weight, in either of the two areas noted in the adjacent drawing.
- For beef cattle, Beef Quality Assurance recommends injection site 1, unless this site is inaccessible or places the operator in a potentially dangerous situation.
- Ensure proper disposal of sharp needles and syringes.
- Wash hands thoroughly with soap and water after administration.
- Do not administer intravenously (IV) as IV administration will be fatal.
- Intramuscular injection will cause a local reaction, which may result in trim loss.
- Do not inject more than 10 mL per injection site.
- Do not use in lambs less than 15 kg body weight.

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 MICOTIL 300, 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* and for the control of respiratory disease in cattle at high risk of developing BRD associated with *Mannheimia haemolytica*. Additionally, data demonstrate that residues in food products derived from cattle treated with MICOTIL 300 will not represent a public health concern when the product is used according to the label.

A. Marketing Status:

Labeling continues to restrict this drug to use by or on order of a licensed veterinarian. This decision was based on the following factors: (a) adequate directions cannot be written to enable laypersons to appropriately diagnose and subsequently use this product to treat BRD and ORD, (b) administration by other than approved routes and dosages, or uses in species other than cattle and sheep can cause signs of toxicity, including death, and (c) there is a potential danger to the person administering the product if it is accidentally self-injected or to other persons if it is accidentally injected. Because of these effects, extensive warning and caution

statements are provided in the labeling which are deemed to be adequate to protect users from accidental injection and to discourage extra label use.

B. Exclusivity:

Under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the date of the approval. The three years of marketing exclusivity applies only to the new indication and dose range for which this supplement is approved.

C. Supplemental Applications

In accordance with the Center's supplemental approval policy 21 CFR 514.106(b)(2), this is a Category II change which did not require a re-evaluation of safety and effectiveness data in the parent application.

D. Patent Information:

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