I. GENERAL INFORMATION

A. File Number

NADA 140-929

B. Sponsor

Elanco Animal Health
A Division of Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285

C. Proprietary Name

MICOTIL® 300

D. Established Name

Tilmicosin Phosphate

E. Dosage Form

Tilmicosin (MICOTIL 300) will be marketed as an injectable antibiotic in 100 mL and 250 mL multidose amber glass bottles.

F. Dosage Regimen

The drug is to be administered subcutaneously to cattle only as a single injection of 10 mg tilmicosin per kg of body weight (1 mL MICOTIL 300 per 30 kg or 1 1/2 mL per 100 lbs of body weight).

G. Route of Administration

Injection

H. Indication

For the treatment of respiratory disease (BRD) associated with Pasteurella haemolytica in cattle.

II. EFFECTIVENESS

The following studies were considered pivotal in determining the effectiveness of tilmicosin in bovine respiratory disease:

A. PIVOTAL STUDIES

1. *In Vitro* Activity:

   Tilmicosin has an *in vitro* antibacterial spectrum that is predominantly gram-positive with activity against certain gram-negative microorganisms. Activity against several mycoplasma species has also been detected. The minimal inhibitory concentrations (MICs) of some of the organisms tested are shown as follows:

Microorganisms	**MIC (µg/mL)**
Pasteurella haemolytica	3.12
P. multocida	6.25
Haemophilus somnus	6.25
Mycoplasma dispar	0.097
M. bovirhinis	0.024
M. bovoculi	0.048

Ninety-five percent of the *Pasteurella haemolytica* isolates were inhibited by 3.12 µg tilmicosin/mL or less. Many of these isolates were resistant to one or more different antibiotics including tetracycline.

2. Pharmacokinetic Study:

Investigators:

T. D. Thomson and J. S. Peloso
Lilly Research Laboratories
Greenfield, Indiana 46140

Serum and lung tissue concentrations of tilmicosin were determined in cattle following a single subcutaneous injection of either a 5 mg or 10 mg/kg dose. The steers weighed an average 216 kg for the 5 mg dose study (T5C768903) and 240 kg for the 10 mg dose study (T5C768902). The experimental procedures were similar in both studies, which were conducted as follows: Three steers were assigned to each of 5 withdrawal periods of 8, 24, 48, 72 and 96 hours after dosing. All animals received a single injection of either 5 mg or 10 mg/kg dose subcutaneously in the dorsolateral chest. At each withdrawal period, a two dimensional measurement was made of the injection site and a blood sample was obtained from each animal in the group. The animals were then euthanized and the lungs removed, placed into an uniquely identified container and stored frozen at -20 C until bioassayed. Blood samples were allowed to clot at room temperature for three hours, centrifuged, the serums placed into containers and frozen at -20 C until bioassayed. Serum samples were analyzed for tilmicosin activity with a validated agar well bioassay using *Micrococcus lutea*(ATCC 9341) as the test organism, and lung tissue tilmicosin concentrations were determined with a validated HPLC method.

Results:

The results are shown in the following Table.

Dose - 5 mg/kg (Hrs)	**Dose - 10 mg/kg Serum Conc.**	**Dose - 5 mg/kg (mcg/ml)**	**Dose - 10 mg/kg Lung Conc.**	**Sample Time (mcg/ml)**
8	0.31	0.35	4.29	5.50
24	0.16	0.29	4.34	7.17
48	0.08	0.15	2.69	5.02
72	0.02	0.07	1.60	3.63
96	0.01	0.04	1.22	2.55

As shown above, the serum tilmicosin activities following a single 5 or 10 mg/kg subcutaneous injection were much lower than the concentrations obtained in lung tissues at various time periods. Tilmicosin is typical of many of the other macrolide antibiotics, which have very large volumes of distribution and low serum levels. Thus, even with the 10mg dose, peak serum levels rarely exceeded 1 mcg/ml in the cattle. However, a higher concentration of tilmicosin was obtained in lung tissues of steers given the 10 mg dose compared to the 5 mg dose and the concentration remained above the tilmicosin MIC 95% of 3.12 mcg/ml for *P. haemolytica*for at least three days.

3. Feedlot Trials:

A series of six trials numbered T5C168701, T5C488702, T5C398703, T5C488704, T5CCA8703, and T5CCA8705 utilizing a total of 228 mixed breed feedlot calves in feedlot environments were conducted. Tilmicosin was administered subcutaneously at levels of 0, 5, 10 or 20 mg/kg of body weight in order to conduct dosage evaluation studies of tilmicosin as a single injection for the treatment of naturally occurring bacterial pneumonia in cattle.

Trial No. T5C168701 - A Clinical Field Study

Investigator:

E. G. Johnson, D.V.M.
Johnson Research
Route 1, Box 1142
Parma, Idaho 83660

Monitor:

Jack W. McAskill
Lilly Research Laboratories
3131 South Vaughn Way
Aurora, Colorado 80014

Forty-eight mixed breed feedlot calves of both sexes, weighing on an average 179 kg and exhibiting signs of bacterial pneumonia with a temperature of 105 F, were randomly assigned to four treatments (0, 5, 10 and 20 mg/kg) of

12 each. Nasal swabs were taken from each animal prior to the treatment for the isolation of organisms. Following a single subcutaneous injection with the respective treatments, clinical evaluation and temperature of each animal were recorded daily for days 0-10 and day 28.

Results:

Treatment with tilmicosin at levels in 10 and 20 mg/kg was effective in reducing body temperature and mortality and in improving general condition scores. Two animals died from each control group and the 5 mg/kg treated group. The 5 mg dose did not reduce the incidence of mortality but did reduce the temperature and improve general condition to a point intermediate to the controls and the 10 and 20 mg/kg levels. Of the 48 nasal swabs collected for culture isolation, only 7 samples were positive for both *P. haemolytica*and *P. multocida. P. haemolytica*was also isolated from 3 of the 4 lung tissue samples collected from the animals that died during the trial. The average weight gains for the 28 days were 13.50, 76.00, 72.08 and 76.25 lbs per head for the 0, 5, 10 and 20 mg/kg treated animals. No unexpected reactions were observed at the injection sites.

Trial No. T5C488702 - A Clinical Field Study

Investigator:

David T. Bechtol, D.V.M.
Rt. 1, Box 37
Canyon, TX 79016

Monitor:

L. H. Carroll, D.V.M.
Lilly Research Laboratories
P. O. Box 819019
Dallas, TX 75381

Thirty-two mixed breed feedlot steers, weighing on an average 193 kg and exhibiting signs of bacterial pneumonia with temperature of at least 105 F, were randomly assigned to the four treatment groups (0, 5, 10 and 20 mg/kg) of 8 each. Nasal swabs were collected from each animal for culture isolation prior to treatment. Following single subcutaneous injection with the respective treatments, clinical evaluation and temperature of each animal were recorded daily for days 1-10 and day 28.

Results:

All treatments were effective in reducing the body temperature within 24 hours. Mean temperature of the 20 mg/kg treated group appeared to be better than the 5 mg or 10 mg dosed animals from day 6 through 10. Four animals (1 from control, 1 from 10 mg and 2 from 20 mg groups) died during the 28 day trial period. Lung lesion scores for all dead animals were in the severe category (>20% involved). *P. haemolytica*was isolated from the lungs of the dead animals and *P. multocida*was isolated from only one of the animals.

*P. haemolytica*was isolated from 22 of the 32 nasal swabs taken on day 0. *P. multocida*was also isolated from one of the 22 swabs.

The average gains for the 28 days were 14.96, 16.88, 38.29 and 18.00 lbs per head for the 0, 5, 10 and 20 mg/kg treated groups. No unexpected reactions were observed at the injection sites.

Trial No. T5C398703 - A Clinical Field Study

Investigator:

Dr. Gary W. Davis
Greenbriar Veterinary Services, Inc.
6040 Dublin Road
Delaware, OH 43015

Monitor:

Dr. Lee E. Watkins
7277 Lithopolis Road
Groveport, OH 43125

Forty-four mixed breed feedlot heifer calves, weighing on an average 178 kg and exhibiting clinical signs of bacterial pneumonia with a temperature of at least 105 F, were randomly assigned to four treatments (0, 5, 10 and 20 mg/kg) of 11 each. Nasal swabs were taken from each animal for culture isolation. All animals were given their respective subcutaneous treatments once and clinical evaluation and temperature of each animal were recorded daily for days 0-10 and on day 28.

Results:

All doses of tilmicosin produced a rapid drop in body temperature from 105 F to 103 F within 24 hrs. However, after two days, the temperature of the 5 and 10 mg/kg treated animals tended to move within the same range as the controls. The temperature in the 20 mg treated animals remained constantly below the control animals through 10 days.

One heifer died in the control group and another heifer aborted a calf in the 10 mg group. A diagnosis of multifocal pneumonia was made in the dead heifer without any positive isolation of *P. haemolytica*. *P. haemolytica*was isolated from 32 of the 44 nasal swabs. *P. multocida*was also isolated from one of these 32 swabs.

The average gains for the 28 days were 12.97, 15.97, 21.95 and 25.81 kg per head for the 0, 5, 10 and 20 mg/kg treated groups. No unexpected reactions were observed at the injection sites.

Trial No. T5C488704 - A Clinical Field Study

Investigator:

David Hutcheson, Ph.D.
Texas A&M University
Agricultural Research and Extension Center
6500 Amarilo Boulevard
West Amarilo, TX 79016

Thirty-two mixed breed feedlot steers, weighing on an average 197 kg and exhibiting typical signs of bacterial pneumonia with temperature of at least 105 F, were randomly assigned to the four treatment groups (0, 5, 10 and 20 mg/kg) of 8 each. Nasal swabs were taken from each animal for culture isolation. All animals were given their respective subcutaneous treatments once and clinical evaluation and temperature of each animal were recorded daily for day 1 through day 10 and day 28.

Results:

All dosages of tilmicosin produced a reduction in body temperature within 24 hours and maintained this reduced temperature for 3 days. On day 4, the average temperature of all treated and the control groups was very similar and remained at 103 F or less for up to 28 days. One control animal died during the trial period and the lung tissue yielded positive cultures of *P. haemolytica. P. haemolytica*was also isolated from 8 of the 32 nasal swabs taken on day 0. *P. multocida*was also isolated from one of the 32 swabs.

The average gains for the 28 days were 12.83, 37.53, 34.70 and 28.35 kg per head for the 0, 5, 10 and 20 mg/kg treated groups. No adverse reactions were observed at the injection sites.

Trial No T5CCA8703 - A Clinical Field Study

Investigator:

Tim Guichon, D.V.M.
Feedlot Health
Management Services
Box 247
Okotoks, Alberta T0L 1T0

Monitor:

John K. Merrill, Ph.D.
Elanco Products Company
B13, 6020 - 2nd Street S.E.
Calgary, Alberta T2H 2LB

Thirty-six mixed breed calves, weighing on an average 288 kg and exhibiting typical signs of bacterial pneumonia with a temperature of at least 105 F, were randomly assigned to the four treatment groups (0, 5, 10 and 20 mg/kg) of 9

each. Nasal swabs were collected from each animal for culture isolation on day 0. All animals were given a single subcutaneous injection of the respective treatments and clinical evaluation and temperature of each animal were recorded daily for day 1 through day 10 and day 28.

Results:

All dosages of tilmicosin produced a 2-degree reduction in body temperature within 24 hours and this lowered temperature was maintained for about 8 days after which the temperature in both control and treated groups remained at 102-103 F during the trial period.

Three control animals died between day 5 and day 10 and each showed >20% lung involvement with positive isolation of *P. haemolytica culture. P. haemolytica*was also isolated from 10 of the 36 nasal swabs collected from each animal on day 0.

The average gains for the 28 days were 26.50, 54.11, 43.56 and 41.44 kg per head for the 0, 5, 10 and 20 mg/kg treated groups.

The 20 mg/kg dose of tilmicosin produced a significant injection site swelling reactions which disappeared by day 28.

Trial No T5CCA8705 - A Clinical Field Study

Investigators:

Ken Bateman, D.V.M.
Ontario Veterinary College
Guelph, Ontario
P. A. Kotzeff, D.V.M.
Chesley Vet. Clinic
Chesley, Ontario

Monitor:

Tom Wheat, D.V.M.
Elanco Products Company
305 Consortium Court
London, Ontario N6E 2S8

Thirty-six mixed breed feedlot steers, weighing on an average 267.4 kg and with a temperature of at least 105 F, were randomly assigned to four treatment groups (0, 5, 10 and 20 mg/kg) of 9 each. Nasal swabs were collected from each animal on day 0 for culture isolation. All animals were given a single subcutaneous injection of the assigned treatments and clinical evaluation and temperature of each animal were recorded daily for days 1-10 and day 28.

Results:

All dosages of tilmicosin were effective in reducing the body temperature within 24 hours and the reduced temperature was maintained at the same level in all three dosages throughout the study period.

Two control animals died during the 28 day trial and each animal had >20% lung involvement. *Haemophilus somnus*and *P. multocida*were isolated from the lungs of the dead animals. *P. haemolytica*was isolated from 12 and *P. multocida*was isolated from 10 of the 30 nasal swabs cultured.

The average gains for the 28 days were 27.47, 43.34, 44.40 and 44.75 kg per head for the 0, 5, 10 and 20 mg/kg treated groups.

Moderate to severe swelling was noted at the injection site in the 10 and 20 mg/kg groups. The mobility of one animal was affected. However, the injection site swelling subsided over the 28 day trial period with no evidence of infection or abscessation.

B. Corroborative Studies:

Trial No T5CCA8601 - A Clinical Field Study

Investigator:

Kee Jim, D.V.M.
Feedlot Health
Management Services
Box 247
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Monitor:

John K. Merrill, Ph.D.
Elanco Products Company
B13, 6020 - 2nd Street S.E.
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Thirty-six mixed breed calves, weighing on an average 282 kg and all with body temperature of 105 F, were randomly assigned to four treatments (0, 5, 10 and 20 mg/kg) of 9 each. Upon arrival, all animals were given an IBR-P13- *H. somnus*vaccine, 8-way clostridial bacterin, ivermectin, anabolic implant and 30 ml of a long-acting oxytetracycline injection. After 4 days, tilmicosin treatment was initiated.

Nasal swabs were collected from each animal on day 0 for culture isolation but the samples were lost. All animals were then given a single subcutaneous injection of the assigned treatments and clinical evaluation and temperature of each animal were recorded daily for days 1-10 and day 28.

Results:

The mean temperatures of calves treated with 5, 10 and 20 mg/kg dosages of tilmicosin for days 1-10 were lower than the control group of animals. Mean temperatures of calves treated with 10 and 20 mg doses were similar but that of calves treated with the 5 mg dose were intermediate between these and the controls.

Five animals died during the trial, four from the control and one from the 5 mg treated group. Each of the 5 dead animals had severe lung lesion score (20% involved). Three additional animals became chronic during the trial and were euthanized on day 28. One of these (a control, #2746) had bovine viral diarrhea, another (a 5 mg treated animal, #2687) had pneumonia, and the other (a 10 mg treated animal, #2730) had pneumonia and myocarditis. The average gain for the 30 days were 6.75, 29.86, 35.00 and 38.78 kg per head for the 0, 5, 10 and 20 mg/kg treated groups. The average gains for the control was determined from the four animals that were alive when the final weight was taken.

There was a significant injection site reaction particularly to high doses. However, the injection site swelling had virtually disappeared by day 7.

The lungs of the necropsied animals that died had acute fibrinous pneumonia lesions which yielded positive isolation of *P. haemolytica*.

Trial No T5CCA8602 - A Clinical Field Study

Investigator:

Eugene G. Janzen, D.V.M.
Large Animal Clinic
Western College Vet. Medicine
University of Saskatchewan
Saskatoon, Saskatchewan S7N OWO

Monitor:

John K. Merrill, Ph.D.
Elanco Products Company
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Calgary, Alberta T2H 2L8

Thirty-six mixed breed calves, weighing on an average 291 kg and with a temperature of at least 105 F, were randomly assigned to four treatment groups (0, 5, 10 and 20 mg/kg) of 9 each. Upon arrival, animals were given an IBR-PI3-*H. somnus*vaccine, 8-way clostridial bacterin, ivermectin, anabolic implant and 30 ml of a long-acting oxytetracycline injection. After 6 days, tilmicosin treatment was initiated.

Nasal swabs were collected from each animal on day 0 for culture isolation but the samples were lost. All animals were then given a single subcutaneous injection of tilmicosin and clinical evaluation and temperature were recorded daily for days 1-10 and day 28.

Results:

All treatments were effective in lowering the body temperature within 24 hours and preventing mortality from *P. haemolytica*infection. Mean temperatures of all three treatments were very similar.

Three animals died in the control group with fibrinous pneumonia while none died in the 5 and 20 mg/kg treated groups. One animal in the 10 mg group died from peritonitis. The three dead animals had severe lung lesions (20% involved) with positive isolation of *P. haemolytica* cultures.

The average gains for the 28 days were 24.83, 28.89, 41.63 and 40.89 kg per head for the 0, 5, 10 and 20 mg/kg treated groups. Higher doses of tilmicosin produced injection site swelling which disappeared by day 5 or 6.

Conclusions:

Of the three dosages of tilmicosin evaluated as a single subcutaneous injection in eight clinical field trials, the 10 and the 20 mg/kg dosages were equally effective and somewhat more efficacious than the 5 mg/kg dose in naturally occurring pneumonia caused by *P. haemolytica*in the cattle. The higher levels reduced the body temperature more than the lower dose during the 10 day post-treatment period and also prevented mortality and improved weight gain during the entire 28 days of the trial. The results indicate that in cases where the infection would be more severe than it was in these trials, cattle treated with the lower dose (5 mg/kg) would have a higher incidence of mortality and reduced weight gain than those treated with either of the higher doses (10 or 20 mg/kg). That the 5 mg dose was suboptimal in these field trials is further supported by the results of a tissue residue depletion study in steer in which the 5 mg dose given as a single subcutaneous injection produced tilmicosin concentration in lung tissues slightly greater than the minimal inhibitory concentration (MIC) of 3.12 mcg/ml for *P. haemolytica*and maintained it for about 36 hours. The 10 mg dose, also given as a single subcutaneous injection, produced a lung tilmicosin concentration more than double the MIC of *P. haemolytica*within 24 hours and the concentration gradually declined but remained above the MIC for 72 hours. Since lung is the target organ for *Pasteurella*infection in cattle, the use of a single 5 mg/kg dose subcutaneously to control the infection can not be clinically supported. Therefore, based on the MICs of *Pasteurella*spp, the concentration of tilmicosin in lung tissues and the efficacy data of tilmicosin in steers, a single subcutaneous injection of 10 mg tilmicosin/kg body weight is the optimum effective dose for the treatment of pneumonia in cattle caused by *P. haemolytica*.

III. TARGET ANIMAL SAFETY

Three target animal (cattle) safety studies were conducted to address the safety of and tolerance to tilmicosin. Based on the results of these studies, tilmicosin is safe when administered subcutaneously at doses up to 50 mg (5X the recommended dose) tilmicosin per kg body weight once every third day for three injections. Local swelling reaction at the site of injection is deemed transient and not detrimental to the safety of a sick animal.

PIVOTAL STUDIES

A. The Target Animal Safety of Tilmicosin Administered Subcutaneously to Cattle.

Investigator:

William H. Jordan, D.V.M., Ph.D.
Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

Purpose: A safety evaluation of tilmicosin injected subcutaneously for the treatment of bovine respiratory disease in the most frequently affected population of cattle (feedlot cattle).

Study Number: VX8702

Species and Strain: Cattle, Hereford

Weight: Steers: 147 to 197 kg, Heifers: 159 to 223 kg

Number per group: 4/sex/group

Initial age: Approximately 10 months old

Dosage form: Injection

Dosages used: 0, 10, 30, and 50 mg/kg/injection, (0, 1x, 3x, and 5x the approved dose level)

Frequency: Once every third day for three injections

Route of administration: Subcutaneous

Start Date: October 21, 1987

Termination Date: November 4, 1987

Test Duration: 2 weeks

Parameters Studied and Results:

- Survival: One control heifer died on test day 2 due to pneumonia. All other cattle survived to termination of the study.

- Clinical Observations: Twice daily observation of the cattle during the study period revealed no clinical signs of toxicity. Observation of the injection sites revealed swelling which was dose-related in degree and duration.

- Body Weight: During the treatment period all treated groups of cattle had weight gains equal to or greater than the control cattle.

- Feed Consumption: No treatment-related differences in feed consumption between control and treated groups of cattle were detected.

- Feed/Gain: Results for the feed gain ratio indicated no adverse treatment effects.

- Hematology: Samples for hematology were collected on pretreatment days 14 and 7, immediately prior to the first treatment, at 6 and 24 hours post first injection and on test days 6 and 14. No treatment-related changes occurred in erythrocyte count, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, erythrocyte morphology, total and differential leukocyte counts or thrombocyte count.

- Clinical Chemistry: Serum samples for clinical chemistry were collected at the same time as blood samples for hematology. Mild increases in serum creatinine phosphokinase activity in individual cattle of each group and mild decreases in mean serum protein concentration, including albumin, in high-dose cattle were attributed to tissue damage and edema at the injection site. Neither change was considered to be of toxicological importance. No treatment-related changes occurred in glucose, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, sorbitol dehydrogenase, lactate dehydrogenase, calcium, sodium potassium, inorganic phosphorus, cholesterol, globulin or chloride.

- Organ Weights: Both the absolute organ weights and the organ weights relative to body weight were normal for all organs weighed including kidney, liver, heart, adrenal, and thyroid (with parathyroids).

- Pathology: Subcutaneous edema occurred at the injection sites in all treated cattle. The only gross or histopathologic evidence of toxicity in tilmicosin-treated cattle was focal myocardial necrosis of the left papillary muscle in two of eight high-dose (50 mg/kg) cattle.

- Conclusion: Clinically, no evidence of toxicity was observed. There were no compound- related adverse effects on body weight, feed utilization efficiency, hematology, clinical chemistry or organ weight parameters studied.

The most prominent treatment related changes consisted of dose-related slight to marked swelling at the injection sites characterized histologically as noninflammatory subcutaneous edema and sometimes accompanied by necrosis of small areas of adjacent skeletal muscle. In the high dose group (50 mg tilmicosin/kg body weight), two of the eight animals histologically demonstrated small foci of myocardial necrosis in the papillary muscle as a sign of toxicity. Otherwise, except for the injection site edema, no other gross toxicity was observed in animals given three injections of 50 mg/kg dose (5X the recommended dose).

B. The Drug Tolerance Study of Tilmicosin Administered Subcutaneously to Beef Cattle

Investigator:

William H. Jordan, D.V.M., Ph.D.
Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

Purpose: The acute effects of large doses of tilmicosin in feeder cattle were demonstrated in this two week study.

Study Number: VX8701

Species and Strain: Cattle, Hereford

Weight: Heifers: 197 to 227 kg, Steers: 204 to 208 kb

Number per group: Placebo group (one male and one female)

Treatment group (two heifers and two steers)

Initial age: Approximately 10 months

Dosage form: Injection

Dosages used: Placebo control and 150 mg/kg, tilmicosin (0 and 15x the approved dose)

Frequency: Once every other day for four treatments

Route of administration: Subcutaneous

Start date: April 22, 1987

Termination date: May 6, 1987

Test Duration: 2 weeks

Parameters Studied and Results:

- Survival: One heifer on the 150 mg/kg dose died approximately one hour following the third injection. The second heifer on the 150 mg/kg dose died one hour after the fourth injection. Both treated steers survived to termination of the study.

- Clinical Observations: Clinical observations in treated cattle included swelling at the injection site, reluctance to move, slight to deep depression, and mortality.

- Electrocardiograms and Heart Rates: Subcutaneously implanted electrodes connected to battery-powered transmitters were used for radio-telemetric collection of electrocardiograms. Recordings were made pretest, immediately prior to dosing, at 1, 4, and 24 hours post-injection and at additional times as indicated by clinical signs or recordings at scheduled recording times. Transient tachycardia and increased amplitude of the Q and T waves were detected after each of the four injections. After the second and subsequent doses, the above changes developed into mild resting bradycardia and decreased wave form amplitudes.

- Body Weight: Control cattle continued to gain weight during the study. Cattle receiving tilmicosin lost weight due to reduced feed consumption.

- Feed Consumption: Cattle in the control group continued to consume feed at a level equivalent to or greater than the pretest periods. Feed consumption for the cattle receiving tilmicosin was greatly reduced following the first injection.

- Hematology: All cattle were bled at pretreatment days 14 and 7, just prior to the initial treatment, at 6 and 24 hours post first injection, and on test days 7 and 14 to provide blood samples for the determination of erythrocyte counts, hemoglobin, packed cell volume, mean corpuscular hemoglobin concentration, erythrocyte morphology, total and differential leukocyte count, and thrombocyte count. Hematologic changes included mild neutrophilia and mild hemoconcentration. All other values were within normal ranges.

- Clinical Chemistry: Serum samples for clinical chemistry were collected at the same times as blood samples for hematology. Minimal to slight increases in activities of the enzymes lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) occurred as early as six hours after the first injection. LDH values remained slightly elevated while CPK values returned to normal at the end of the two-week study. The isoenzyme patterns suggested that increased serum activities of LDH and CPK were a result of skeletal muscle damage at the injection site. Serum alkaline phosphatase activity and serum concentrations of total protein, albumin, globulin, calcium, inorganic phosphorus, sodium, and cholesterol were mildly decreased. These changes were compatible with decreased feed intake, combined with massive loss of serum components into the subcutaneous tissue at the injection sites. Values for glucose, urea nitrogen, total bilirubin, creatinine, potassium, and sorbitol dehydrogenase were normal.

- Organ Weights: Kidney, liver heart, adrenal, and thyroid weights were determined and evaluated for differences in absolute and organ weight relative to body weight. The adrenal weights of the treated steers were moderately increased both on an absolute and on a relative body weight basis. The weights for the kidney, liver, heart, and thyroids were similar to those of the two control animals.

- Pathology: Treatment-related gross and histopathologic changes in cattle given multiple 150 mg/kg subcutaneous injections of tilmicosin were

prominent subcutaneous edema at the injection sites and minimal to moderate focal myocardial necrosis of the left ventricular papillary muscle.

Conclusion:

The acute manifestations of multiple injections of large (150 mg/kg) subcutaneous doses of tilmicosin included small foci of myocardial necrosis, prominent noninflammatory injection site edema and some mortality. The noninflammatory nature of the injection site edema and the absence of prominent morphologic changes in the heart suggest that cattle that survive large doses of tilmicosin should be expected to become clinically normal with no important residual lesions.

C. AN EIGHT-DAY STUDY IN CATTLE TO DETERMINE THE ACUTE TOXICITY OF A SINGLE INTRAVENOUS INJECTION OF A BOVINE SUBCUTANEOUS INJECTABLE TILMICOSIN FORMULATION

Study No.: VX8803

Starting date: June 15, 1988

Termination date: June 22, 1988

Study Director: Dr. W. H. Jordan

Location of the Study: The Elanco Products Company, Greenfield, Indiana

Identification of Substance and Dosage Form: Tilmicosin Phosphate, Injectable

Species and Strain: Bovine, Hereford

Weight: Steers 223.5 kg and Heifers 210.8 kg

Drug Levels Tested and Duration of Dosing: 0, 2.5, 5.0 mg/kg administered once as a single injection.

Number of Animals Per Sex Per Treatment Group: 0 and 2.5 mg/kg groups: 1/sex, 5 mg/kg group: 2/sex

Route of Administration: Intravenous

Parameters Studied and Results:

- Survival and Clinical Signs: No mortality or clinical signs were observed in the control or low-dose groups. Both heifers injected with the high-dose died within 10 minutes. Increased and shallow respirations were noted in both high-dose steers, and one steer was recumbent from 2 to 12 minutes following dosing. Both steers returned to normal in approximately one hour.

Conclusion:

Tilmicosin, formulated for subcutaneous injection, was tested for toxicity when given by the intravenous route. Rapid intravenous injection of 5 mg tilmicosin/kg body weight caused death in two of two heifers and was acutely toxic, but nonlethal, to two of two steers. No clinical signs were observed in cattle that received an intravenous injection of 2.5 mg of tilmicosin/kg of body weight.

D. CORROBORATIVE STUDY - Adverse Reactions at the Injection Site

Investigators:

T. D. Thomson and G. E. Weinantz
Lilly Research Laboratories
Greenfield, Indiana 46140

A separate study (No. T5C769812) was conducted to generate photographic records of the areas in the immediate vicinity of the subcutaneous injection sites. Four Angus cross steers, weighing 425-511 kg, each received 4 subcutaneous injections of 10 mg/kg dose of tilmicosin at different locations at weekly intervals. The injection sites were located caudal to the shoulder and over the last ribs on both sides in areas designated as right front, right rear, left front and left rear. The injection schedule and locations were assigned such that at necropsy all 4 injection locations were represented for each post-injection period. Two weeks after the last tilmicosin injection, the steers were sacrificed. The carcasses were hung by the hind legs and the injection sites were exposed. Gross evaluations and photographs were made of the internal surface of the skin over the injection site, the subcutaneous injection site surface, and the incised subcutaneous site surface for all 16 treatment sites.

The results show that subcutaneous injection of tilmicosin at 10 mg/kg dose in feedlot cattle does not result in grossly apparent damage to either the skin or the muscle just below the injection area by 14 days post-treatment, and that no trimming of these injection areas would be indicated by 28 days post-treatment.

IV. HUMAN FOOD SAFETY

A. TOXICITY STUDIES

CARCINOGENIC POTENTIAL

Carcinogenicity studies in laboratory animals were not required to support the use of this injectable antibiotic in domestic livestock. The absence of a carcinogenicity study in the tilmicosin data package is consistent with a low level of concern for carcinogenicity as defined by FDA guidelines.[1]

Specific factors indicating that a carcinogenicity study was not needed for this macrolide antibiotic included the lack of chemical structural relationship to known carcinogens, the lack of important pathological changes in toxicity studies, the absence of genotoxicity and anticipated low human exposure levels. Literature searches failed to reveal any evidence of carcinogenicity due to other macrolide antibiotics. Repeated dose toxicity studies did not produce any signs or symptoms

to suggest a potential for neoplasia. Toxicology studies were conducted in which rats and dogs were given daily oral doses for up to 3 months and 1 year, respectively. Although a specific target organ was not defined in the rats, studies in dogs clearly demonstrated the heart to be the most important target organ of toxicity for tilmicosin. The heart was subsequently confirmed as the target organ of toxicity in cattle, pigs, and monkeys. The cardiac effects are transient and would not be expected to lead to neoplasia. Furthermore, no proliferative lesions attributable to the direct effects of tilmicosin were observed in any species tested. The absence of mutagenic or clastogenic effects in a series of well accepted tests indicated that tilmicosin was not genotoxic and was unlikely to induce genetic effects that might lead to neoplasia.

[1]U.S. FDA (1982). Toxicological principles for the safety assessment of direct food additives and color additives used in food. U.S. Food and Drug Administration, Bureau of Foods, Washington, DC.

1. THE TOXICITY OF EL-870 GIVEN ORALLY TO BEAGLE DOGS FOR THREE MONTHS

   Study No.: D08286

   Starting date: September 11, 1986

   Termination date: December 16, 1989

   Study Director:

   Dr. W. H. Jordan
   Toxicology Division
   Lilly Research Laboratories
   Greenfield, Indiana

   Identification of Substance and Dosage Form: Tilmicosin oral capsule

   Species and Strain: Dog, Beagle

   Number of Animals Per Sex Per Treatment Group: Four groups of four dogs of each sex

   Drug Levels Tested and Duration of Dosing: 0, 6, 20, and 70 mg/kg/day

   Route of Administration: oral

   Parameters Studied and Results:

   - Survival: Four high-dose dogs (two/sex) died during the first month of the study. All remaining animals survived to study termination.

   - Clinical Observation: The only treatment-related clinical observations were pale mucous membranes in a male and a female from the high-dose group prior to death. The high-dose female was also ataxic on one occasion.

- Ophthalmic Examination: All dogs were normal pretest. All surviving dogs were examined approximately one week prior to study termination and again one day prior to termination. At both examinations, one male and one female high-dose dog had bilateral multifocal areas of subretinal fluid, primarily in the tapetal region. Although the condition was seen in each dog, there was a much greater effect in the male than in the female. Each area was less than 1/2 disk diameter in size. The male also had some areas of focal retinal degeneration. Miosis was seen in the female, but with normal pupillary light responses and a normal response to the mydriatic. There was virtually no change in either dog's condition during the re-examination near study termination. The remaining dogs had no treatment-related changes.

- Physical Examination: No treatment-related effects were observed during the physical examination conducted at study termination.

- Electrocardiograms: Daily doses of 6 mg/kg showed no significant electrocardiographic effects, doses of 20 mg/kg produced moderate to severe tachycardia; and daily doses of 70 mg/kg caused very severe and possibly lethal tachycardia and a significant sag (depression) in the ST segment.

- Body Weight: No treatment-related changes were observed during the study.

- Food Consumption: Treatment with tilmicosin did not affect the quantity of food consumed.

- Hematology: Hematology samples were collected during weeks 1, 2, 4, 8, and at termination. No treatment-related effects were detected for leukocyte count, erythrocyte count, hemoglobin, packed cell volume, erythrocyte indices, thrombocyte count, leukocyte differential, erythrocyte morphophology, or activated partial thromboplastin time.

- Bone Marrow: The bone marrow evaluation including estimated M:E ratios of bone marrow smears was normal for all dogs at termination.

- Clinical Chemistry: Serum samples were collected during weeks 1, 2, 4, 8, and at termination. Values for glucose, urea nitrogen, creatinine, total bilirubin, and alkaline phosphatase were normal. Serum alanine transaminase for high-dose dogs of both sexes was increased by day 12 and continued to increase to termination of the study.

- Urinalysis: Parameters evaluated for urine collected from each dog during the study and at termination included color, clarity, pH, specific gravity, protein, glucose, occult blood, ketones, bilirubin, and urobilinogen. Urinalysis data from treated animals did not differ from those of control dogs. A significant increase in mean activity of p-nitroanisole O-demethylase was observed in the high-dose group females. There was no effect on enzyme induction in males.

- Serum Concentration of Tilmicosin: The serum content of tilmicosin during the study indicated that absorption of the test compound was dose-related. Comparisons of the mean 3- and 24-hour serum levels indicated that the compound was slowly eliminated from the serum compartment. Apparent increases in the 24-hour mean serum levels were evident for the middle- and high-dose groups through week 9; no significant increases were evident thereafter.

- Organ Weights: Treatment-related increases in liver weights occurred in high-dose females and in heart weights in high-dose dogs of both sexes. The significance of slightly increased relative kidney weights in high-dose females was not apparent. The treatment did not affect the weights of the adrenal glands, thyroids, ovaries, testes, or brain.

- Pathologic Findings: There were neither gross nor microscopic lesions to explain the cause of death in the four high-dose dogs that died during the study. Upon gross examination, the heart was found to be slightly enlarged in one terminally killed high-dose male. Histologically, mild diffuse mucosal edema occurred in the gall bladders of one high-dose dog of each sex. No additional treatment-related changes were observed.

Conclusion:

Daily oral administration of tilmicosin at a dose of 70 mg/kg for three months resulted in the deaths of four of eight dogs. Dogs that survived doses of 20 to 70 mg/kg sustained moderate to severe tachycardia without clinical pathologic or morphologic evidence of permanent tissue damage. The no-observed-effect level for daily oral administration of tilmicosin was 6 mg/kg (3 mg/kg BID).

2. THE TOXICITY OF TILMICOSIN GIVEN ORALLY TO Crl:CD(SD) RATS FOR THREE MONTHS

Study No.: R09886

Starting date: June 16, 1986

Termination date: September 18, 1986

Study Director:

Dr. W. H. Jordan,
Toxicology Division
Lilly Research Laboratories
Greenfield, Indiana

Identification of Substance and Dosage Form: Tilmicosin, an aqueous oral solution

Species and Stratin: Rat, Crl: CD(SD)

Number of Animals Per Sex Per Treatment Group: Four groups (00, 01, 02, and 03) with 20/sex/group (except group 01 which contained 21 males and 19 females)

Drug Levels Tested and Duration of dosing: 0, 50, 250, and 1000 mg/kg administered daily

Route of Administration: oral (gavage)

Parameters Studied and Results:

- Survival: Survival at the end of the study was 100%, 100%, 100%, and 45% for males and 100%, 94.7%, 75%, and 35% for females in the control, low-, middle-, and high-dose groups, respectively.

- Antemortem Observations: Antemortem observations noted in some high-dose animals shortly before death included thinness, ventral soiling, chromorhinorrhea, alopecia, chromodacryorrhea, and poor grooming.

- Ophthalmic Examinations: There were no compound-related ocular effects.

- Body Weight: Mean body weights and weight gains in high-dose males were significantly lower than controls throughout the study. Middle-dose females had body weights significantly lower than controls at 8, 12, and 13 weeks.

- Food Consumption: High-dose males consumed significantly less food than controls throughout the study.

- Efficiency of Food Utilization: EFU was significantly decreased in high-dose males throughout the study and in high-dose females during the last two weeks of the study.

- Hematology: No important treatment-related effects were detected for erythrocyte count, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, erythrocyte morphology, total and differential leukocyte counts, thrombocyte count, or activated partial thromboplastin time.

- Clinical Chemistry: No treatment-related effects were detected for serum concentrations of glucose, urea nitrogen, creatinine, total bilirubin or activities of the enzymes alkaline phosphatase and alanine transaminase.

- Urinalysis: The pH of the urine from high-dose females was slightly lower than that of controls and a slight increase in occult blood was noted for high-dose rats of both sexes. Parameters considered normal included color, clarity, protein, glucose, specific gravity, ketones, bilirubin, and urobilinogen.

- Enzyme Induction: No significant increases in mean activity of p-nitroanisole O-demethylase were observed at any of the dose levels tested in this study.

- Organ Weights: Moderate increases in adrenal weights were noted for middle-dose males and high-dose rats of both sexes. Absolute or relative weights were also increased for hearts, kidneys, and livers of middle- and high-dose rats. Weights for the thyroid (with parathyroid), prostate, testis, ovary, uterus, spleen, and brain were considered either to be within normal limits or changed due to the poor body condition of high-dose males.

- Pathologic Findings: Grossly, high-dose and a few middle-dose rats appeared thin and had other nonspecific changes such as dehydration, nasal discharge and decreased size of the spleen. Histologically, tilmicosin at a dose of 1000 mg/kg was associated with minimal to moderate hypertrophy of the zona fasciculata of the adrenal and a low incidence of myocardial degeneration and skeletal muscle necrosis. Despite some deaths at a dose of 250 mg/kg, no morphologic evidence of toxicity was found at this dose. No evidence of toxicity was found in low (50 mg/kg) dose rats.

Conclusion:

Daily oral gavage of tilmicosin doses of 250 and 1000 mg/kg were toxic to Crl:CD(SD) rats as demonstrated by increased mortality and organ weight and histopathologic effects on several tissues. The no-observed-effect level for daily administration of tilmicosin by gavage to Crl:CD(SD) rats was 50 mg/kg.

3. REPRODUCTIVE EFFECTS OF EL-870 ADMINISTERED ORALLY VIA GAVAGE TO Crl:COBS CD (SD)BR RATS FOR TWO GENERATIONS, WITH TWO LITTERS PER GENERATION

Study No.: 112-001

Starting date: February 3, 1987

Termination date: February 22, 1989

Study Director: Alan M. Hoberman, Ph.D.

Location of the Study:

Argus Research Laboratories, Inc.
935 Horsham Road
Horsham, Pennsylvania 19044

Identification of Substance and Dosage Form: Tilmicosin, an aqueous oral solution

Species and Strain: Rats, Crl:COBS CD (SD)BR

Number of Animals Per Sex Per Treatment Group: 30 rats per sex

Drug Levels Tested and Duration of dosing: 0, 10, 45, and 200 mg/kg/day. The males were dosed for 70 days and the females were dosed 14 days prior to breeding.

Route of Administration: oral

Parameters Studied and Results:

- Parental Mortality and Necropsy Findings: There were 2, 1, 8, and 8 deaths of the F0 males, F0 females, F1 males, and F1 females, respectively. The death of the one F0 female of the 200 mg/kg/day group was treatment-related. The other deaths were attributed to intubation accidents or unknown causes. There were no treatment-related effects or lesions found at necropsy.

- Parental Clinical Observations: The incidence of excess salivation was statistically significant in the 200 mg/kg/day group in the F0 males and females and the F1 males. Sporadic incidences of excess salivation were observed in the F1 females of the 200 mg/kg/day group. No other clinical observations were treatment-related.

- Body Weights: Statistically significant, treatment-related depression in body weight gain occurred in the F0 females of the 45 and 200 mg/kg/day groups during the first 14 days of treatment. Statistically significant, treatment-related depression in body weight gain also occurred in the F0 females of the 200 mg/kg/day group on days 0 to 6 of the first (F1a) gestation period. There were no statistically significant, treatment-related effects on body weight or body weight gain in the F0 and F1 males or the F1 females.

- Food Consumption: Statistically significant, treatment-related depression in food consumption occurred in the F0 females of the 45 and 200 mg/kg/day groups during the first 14 days of treatment. In addition, statistically significant, treatment-related depression in food consumption occurred in the F0 females of the 200 mg/kg/day group on days 0 to 6 of the first (F1a) gestation period. There were no treatment-related effects on food consumption in the F0 and F1 males or the F1 females.

- Mating Performance and Fertility: There were no treatment-related differences in mating performance or fertility.

- Reproductive Parameters: There were no treatment-related differences in litter size.

- Progeny Survival: Pup mortality was slightly increased during the first four days pospartum in both the F1a and F1b litters of the 200 mg/kg/day group. This pup mortality was statistically significant in the

F1b litters of the 200 mg/kg/day group. There were no treatment-related effects on mortality of the F2a or F2b litters.

- Progeny Body Weights and Sex Distribution: There were no treatment-related differences in progeny body weights or sex ratios.

- Progeny Clinical Observations and Necropsy Findings: There were no treatment-related differences observed clinically or at necropsy of progeny.

Conclusion:

Parental toxicity was indicated by depressed body weight gain and depressed food consumption in the F0 female rats in the 45 and 200 mg/kg/day groups. No comparable parental toxicity was observed in the F0 male rats or the F1 rats. Developmental toxicity was indicated by a small increase in pup mortality during the first 4 days postpartum in the F1a and F1b litters of the 200 mg/kg/day group. This increase in mortality did not recur in the F2a and the F2b litters. No other adverse effects on reproductive performance were observed.

4. A TERATOLOGY STUDY OF TILMICOSIN (EL-870, COMPOUND) ADMINISTERED ORALLY TO CD RATS

Study No.: R13387

Starting date: August 3, 1987

Termination date: August 27, 1987

Study Director: Dr. W. H. Jordan

Location of the Study: The Elanco Products Company, Greenfield, Indiana

Identification of Substance and Dosage Form: Tilmicosin, oral aqueous solution

Species and Strain: Rat, Crl:CD(SD)

Weight: 213.6 grams

Number of Animals Per Sex Per Treatment Group: 25 females per group

Drug Levels Tested and Duration of Dosing: 0, 10, 70, and 500 mg/kg/day for 10 days

Route of Administration: oral

Parameters Studied and Results:

- Maternal Survival and Signs of Toxicity: All the animals from each dose group completed the study. There was increased salivation in rats given 70 mg/kg/day and increased salivation and alopecia in rats given 500 mg/kg/day.

- Maternal Body Weight and Food Consumption: Body weight gain was depressed during the first half of the treatment period in rats given 70 mg/kg/day and body weight gain and food consumption were depressed during the first half of the treatment period in rats given 500 mg/kg/day. During the post-treatment period, food consumption was elevated for the middle and high-dose group animals and their total body weight gain for the entire gestation period was comparable to control values.

- Maternal Reproduction Parameters: The numbers of pregnant rats for evaluation on gestation day 20 were 21, 18, 19, and 21, from the control, low-, middle-, and high-dose groups, respectively. The numbers of corpora lutea and implantations and the proportions of live fetuses and resorptions were not affected by treatment with tilmicosin. There were no dead fetuses in this study.

- Fetal Parameters: Fetal sex ratios, fetal body weights, the proportions of fetal runts, and the proportions of fetuses with developmental variations, deviations, or malformations were not affected by treatment. Male or female fetal body weights were significantly elevated in litters of dams given 500 mg/kg/day; however, the elevation was interpreted to be unrelated to tilmicosin treatment because it was slight and exaggerated by the atypically low weights of control fetuses in comparison to recent historical control data. The proportion of male fetuses with developmental deviations was significantly elevated in litters of dams given 10 mg/kg/day. However, these deviations were interpreted to be unrelated to tilmicosin treatment because there was no dose-response relationship, and the incidence of each type of deviation was in close agreement with recent historical control data.

Conclusion:

Based on a depression of body weight gain during the first half of the treatment period, the no-observed-effect level for tilmicosin maternal toxicity in the rat was 10 mg/kg/day. There was no evidence of embryo/fetal toxicity or teratogenicity related to tilmicosin treatment up to 500 mg/kg/day, the highest dose tested.

5. A ONE YEAR CHRONIC TOXICITY STUDY IN BEAGLE DOGS GIVEN ORAL DOSES OF TILMICOSIN

Study No. - D07187

Starting date: September 29, 1987

Termination date: March 1, 1989

Study Director: Dr. W. H. Jordan

Location of the Study: The Elanco Products Company, Greenfield, Indiana

Identification of Substance and Dosage Form: Tilmicosin, oral powder in capsules

Species and Strain: Dog, Beagle

Number of Animals Per Sex Per Treatment Group: Four/sex/group

Drug Levels Tested and Duration of Dosing: 0, 4, 12, and 36 mg/kg/day total dose divided for twice a day administration for one year

Route of Administration: Oral

Parameters Studied and Results:

- Dose Administration: The compound was administered orally twice-daily (b.i.d.) as equally divided doses separated by a minimum of five hours. Each dog was offered 120 g of food approximately one hour prior to each of the two daily dose administrations. A visual estimation of the amount of food consumed was made after the dogs had access to the food for one hour. Only the dogs that had consumed at least one-half of the food were administered a dose. The same dosing/feeding regimen was repeated a minimum of five hours later for the second daily dose. There were sporadic occasions in each treatment group in which the first and/or second daily doses were not administered because of insufficient food consumption. The number of dogs affected and the total number of these occasions were similar between the control, low-, and middle-dose groups. Instances of dogs not being dosed due to insufficient food consumption were slightly lower in the high- dose group.

- Survival: All animals survived to the scheduled termination of the study.

- Clinical Observations: No toxicologically important clinical observations were detected in tilmicosin-treated dogs. Peripheral redness, first observed at five-months, consisted of redness of the ventral aspect of the pinna and occasionally the muzzle and/or abdomen. Peripheral redness was observed sporadically, in all tilmicosin-treated groups, during the last seven months of the study, but was always mild and was considered to be of no toxicological importance.

- Ophthalmic Examinations: No treatment-related ophthalmic changes were observed during the study.

- Physical Examinations: No treatment-related effects were detected.

- Electrocardiograms: An increased incidence of moderate to marked heart rate increases was detected in six of eight high-dose dogs. In the latter half of the study, the positive chronotropic effect in three of the dogs was accompanied by sporadic instances of ST segment depression. There was no evidence of tilmicosin-induced disturbances in cardiac rhythm or conduction in high-dose dogs and no evidence of tilmicosin-induced cardiotoxicity in mid- and low-dose dogs.

- Body Weight: Although all dogs gained weight at approximately the same rate for the first three to four months of the study, the body weight gains for the middle- and high-dose dogs were slightly to moderately less than those of control and low-dose animals during the last eight months of the 12-month study. These differences were slightly greater and occurred earlier for females than for males.

- Food Consumption: No treatment-related effects on food consumption were observed during the study. There were sporadic occasions when doses were not administered because of insufficient food consumption; however, the total ration was consumed during each 24 hour period.

- Hematology: No toxicologically important changes occurred in erythrocyte count, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, erythrocyte morphology, total and differential leukocyte counts, thrombocyte count, or activated partial thromboplastin time. Bone marrow cytology evaluation, including an estimated M:E ratio, was also normal.

- Clinical Chemistry: No treatment-related changes occurred in serum glucose, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, alanine transaminase, aspartate transaminase, lactate dehydrogenase, creatinine phosphokinase, calcium, inorganic phosphorus, sodium, potassium, chloride, cholesterol, triglycerides, total protein, albumin, globulin, or albumin/globulin ratio.

- Urinalysis: No treatment-related changes occurred in urine color, clarity, specific gravity, pH, protein, glucose, occult blood, ketones, bilirubin, or urobilinogen.

- Organ Weights: Organs weighed at necropsy included: kidney, liver, heart, lungs, spleen, thymus, salivary glands, ovaries, uterus, testes, prostate, adrenals, thyroids with parathyroids, pituitary, and brain. The absolute and relative heart weights of high-dose males and relative heart weights of high-dose females were increased relative to controls. No additional treatment-related effects on organ weights were detected.

- Pathology: Upon gross examination, the hearts of four males and one female from the high-dose group were found to be slightly dilated, but were histologically normal. Mild dermatitis in treated dogs was judged to be of no toxicologic importance.

Conclusion:

Oral tilmicosin doses of 12 or 36 mg/kg/day given as twice-daily divided doses for one year caused slightly depressed body weight gains and, at the 36 mg/kg dose, an increased incidence of tachycardia and mild ST segment depression in electrocardiograms. The no-observed-effect level was 4 mg/kg/day.

6. THE EFFECT OF COMPOUND 177370 (EL-870) ON THE INDUCTION OF DNA REPAIR SYNTHESIS IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES

Study No.'s: 851008UDS2449 and 851015UDS2449. Each of these two studies was conducted as an independent UDS assay.

Starting dates: October 8, 1985 and October 15, 1985, respectively

Termination dates: October 9, 1985 and October 16, 1985, respectively

Study Director: G. S. Probst, Ph.D.

Location of the Study:

Toxicology Division
Lilly Research Laboratories
Greenfield, Indiana

Identification of Substance and Dosage Form: Tilmicosin, solution

Species and Strain: Rats, Fischer 344

Number of Animals Per Sex Per Treatment Group: Hepatocytes harvested from one male rat per study

Drug Levels Tested and Duration of Dosing: 1000, 500, 100, 50, 10, 5, 1, and 0.5 µg/mL

Route of Administration: Cell culture perfusion

Parameters Studied and Results:

Unscheduled DNA synthesis (UDS) in hepatocytes was quantified. The number of silver grains over the cell nucleus was counted. The net nuclear grain count represents the difference between the gross nuclear grain count and the mean cytoplasmic background grain count. Nuclei of 20 morphologically unaltered cells, judged to be representative of the UDS responsiveness of the cell population and containing at least four grains, were counted for each treatment. Autoradiographic grain counts were conducted for the highest compound concentration that did not produce pronounced cytotoxicity and for all lower concentrations of the test compound.

Conclusion:

Cytoxicity resulted from treatment with tilmicosin at concentrations of 1000, 500, 100, and 50 µg/mL. No induction of UDS was observed at concentrations of 10, 5, 1, and 0.5 µg/mL. These observations were noted in both studies.

The cultured adult rat hepatocytes were sensitive to the induction of UDS both by the positive controls MNNG and 2AAF, and tilmicosin was inactive for the induction of UDS.

7. THE EFFECT OF COMPOUND 177370 (EL-870) ON THE INDUCTION OF FORWARD MUTATION AT THE THYMIDINE KINASE LOCUS OF L5178Y MOUSE LYMPHOMA CELLS

Study No.'s: 851106MLA2449 and 851113MLA2449

Starting dates: November 6, 1985 and November 13, 1985, respectively

Termination dates: November 8, 1985 and November 26, 1985, respectively

Study Director: G. S. Probst, Ph.D.

Location of the Study:

Toxicology Division
Lilly Research Laboratories
Greenfield, Indiana

Identification of Substance and Dosage Form: Tilmicosin, solution

Species and Strain: Mouse cell line, TK +/- cells, (TK3.7.2C)

Number of Animals Per Sex Per Treatment Group: not applicable

Drug Levels Tested and Duration of Dosing: Nonactivated: 900, 800, 700, 600, 500, 400, 200 and 100 µg/ml. Activated: 1000, 900, 800, 700, 600, 500, 400, and 200 µg/ml.

Route of Administration: cell culture perfusion

Parameters Studied and Results:

In this *in vitro*forward mutation assay, mutagenic agents induce the heritable loss of Thymidine kinase (TK) activity in formerly TK competent L5178Y cells (TK +/-). The resulting L5178Y TK -/- mutants lack the salvage enzyme thymidine kinase and are therefore detected by their resistance to the lethal thymidine analogs 5-bromodeoxyuridine or trifluorothymidine. Rat liver microsomal enzymes (S9) have been incorporated into this test for the activation of mutagenicity.

L5178Y (TK +/-) mouse lymphoma cells were treated *in vitro*with tilmicosin to evaluate mammalian cell point-mutation. The test was conducted with and without metabolic activation by an S9 liver microsomal fraction obtained from Aroclor 1254-induced rats. To confirm the responsiveness of the test, ethylmethanesulfonate (EMS) and 3- methylcholanthrene (3MC) were also tested and served as positive controls.

Conclusion:

In the nonactivated test, concentrations of 900 µg/ml and 800 µg/ml were not evaluated for mutagenicity due to cytotoxicity. All concentrations in the activated test were evaluated. In the nonactivated test, the percent survival ranged from 19% to 99% and the mutation index ranged from 0.4 to 0.9. In the activated test the percent survival ranged from 61% to 99% and the mutation index ranged from 0.5 to 1.2. No mutagenicity was observed at any dose with or without activation. This system was sensitive to the positive controls as evidenced by the positive responses observed with EMS and 3MC, and tilmicosin was not mutagenic to L5178Y TK+/- cells, with or without metabolic activation.

8. THE EFFECT OF TILMICOSIN ON THE INDUCTION OF FORWARD MUTATION AT THE HGPRT + LOCUS OF CHINESE HAMSTER OVARY CELLS

Study No.'s: 881130CHT2449 and 890111CHO2449

Starting dates: December 1, 1988 and January 5, 1989, respectively

Termination dates: April 21, 1989 and April 21, 1989, respectively

Study Director: K. K. Richardson, Ph.D.

Location of the Study:

Toxicology Division
Lilly Research Laboratories
Greenfield, Indiana

Identification of Substance and Dosage Form: Tilmicosin, solution

Species and Strain: Chinese hamster ovary HGPRT+ cell line CHO-K1-BH4

Number of Animals Per Sex Per Treatment Group: Not applicable

Drug Levels Tested and Duration of Dosing: Nonactivated: 250, 200, 175, 150, 125, 100, 50, and 24 µg/ml. Activated: 300, 275, 250, 225, 200, 150, 100, and 50 µg/ml.

Route of Administration: infusion into cell culture

Parameters Studied and Results:

Tilmicosin was tested *in vitro*for the induction of mammalian cell mutation in the HGPRT+ Chinese hamster ovary cell assay with and without liver microsomal activation. The assay used is a modified method. Cells are maintained in suspension for exposure of the compound and then plated in soft-agar for selection of mutants. EMS and 3MC served as positive controls for the nonactivated and activated assays, respectively.

Conclusion:

In the nonactivated test, the highest concentration of tilmicosin (250 µg/ml) was not evaluated for mutagenicity due to cytotoxicity. In the activated test, concentrations of 275 µg/ml and 300 µg/ml were not evaluated due to cytotoxicity. In the nonactivated test, the percent survival ranged from 15% to 89% and the mutation index ranged from 0.0 to 1.7. In the activated test, the percent survival ranged from 7% to 98% and the mutation index ranged from 0.2 to 1.1. No treatment, whether nonactivated or activated, showed any evidence of induced mutation. This system was sensitive to both EMS and 3MC as evidenced by the induction of mutants. Tilmicosin was not mutagenic in HGPRT+ Chinese hamster ovary cells, with or without metabolic activation.

9. MUTAGENICITY TEST ON TILMICOSIN IN THE RAT BONE MARROW CYTOGENETIC ASSAY

Study No.: 10646-0-452

Starting date: January 11, 1989

Termination date: April 26, 1989

Study Director: Dr. J. L. Ivett

Location of the Study:

Hazleton Laboratories America, Inc.
5516 Nicholson Lane
Suite 400
Kensington, Maryland, 20895

Identification of Substance and Dosage Form: Tilmicosin, solution

Species and Stratin: Rats, Sprague-Dawley

Number of Animals Per Sex Treatment Group: 5 males and 5 females were used per treatment group

Drug Levels Tested and Duration of Dosing: 180, 600, and 1800 mg/kg (acute dosage) and subchronically for 5 consecutive days at 100, 333, and 1000 mg/kg, respectively.

Route of Administration: oral gavage

Parameters Studied and Results:

Tilmicosin was tested to evaluate whether it or its metabolites could interact with the chromosomes from rat bone marrow cells to produce gross lesions (the acute dosage) and whether these changes were of a type which could survive more than one mitotic cycle of the cell (the subchronic dosage). All aberration figures detected by these assays would have resulted from breaks in the chromatin, which either failed to repair or were repaired in atypical combination. The cell transit time for bone marrow is normally 10-14 hours. The assay designs were such that bone marrow samples were taken at 6, 18, and 30 hrs after a single dose of tilmicosin in the acute study and 6 hrs after the last 5 daily doses of tilmicosin in the subchronic study. The rationale for harvesting bone marrow at 30 hrs in the acute study was for detection of chromosome aberrations in cells that may have been delayed in their progression through the mitotic cycle.

Conclusion:

The test article, tilmicosin, was considered negative for inducing chromosomal aberrations in bone marrow cells of both male and female rats under the acute and subchronic dosing conditions of this assay. The acute dose of 1800 mg/kg approximated 80% of the median lethal dose in nonfasted rats.

10. THE EFFECT OF TILMICOSIN ON THE *IN VIVO* INDUCTION OF SISTER CHROMATID EXCHANGE IN BONE MARROW OF CHINESE HAMSTERS

Study No.: 881114SCE2449

Starting date: November 14, 1988

Termination date: January 23, 1989

Study Director: M. L. Garriott, Ph.D.

Location of the Study:

Toxicology Division
Lilly Research Laboratories
Greenfield, Indiana

Identification of Substance and Dosage Form: Tilmicosin, solution

Species and Strain: Chinese hamsters, Cricetulus friseus

Number of Animals Per Sex Per Treatment Group: 3 females per treatment group

Drug Levels Tested and Duration of Dosing: 450, 900, or 1800 mg tilmicosin/kg as a single dose

Route of Administration: oral gavage

Parameters Studied and Results:

Tilmicosin was tested in vivo for the induction of sister chromatid exchange (SCE) in bone marrow of Chinese hamsters. Cyclophosphamide was administered as the positive control for this study. Ten percent aqueous acacia, the vehicle used for tilmicosin suspensions, was used as the negative control.

In order to achieve the sister chromatid differentiation necessary to score SCE, a bromodeoxyuridine tablet was implanted in each hamster. Five hours later tilmicosin and the positive and negative controls were administered. Approximately 19 hr later, animals were killed, bone marrow was harvested from the femurs, and slides were prepared, stained, and scored for SCE.

Conclusion:

The *in vivo* assay for SCE formation was sensitive to the positive control, cyclophosphamide, and tilmicosin was inactive for the induction of SCE in Chinese hamster bone marrow at a dose that approximated 50% of the median lethal dose in that species.

11. THE EFFECT OF COMPOUNDS 177370 (EL-870) ON THE INDUCTION OF REVERSE MUTATIONS IN SALMONELLA TYPHIMURIUM USING THE AMES TEST

Study No.'s: 850930AMS2449, 851028AMS2449, and 851111AMS2449. Each of these three studies was conducted as an independent Ames test.

Start dates: September 20, 1985, October 28, 1985, and November 11, 1985, respectively

Termination dates: October 4, 1985, November 1, 1985, and November 15, 1985, respectively

Study Director: Dr. G. S. Probst

Location of the Study:

Toxicology Division
Lilly Research Laboratories
Greenfield, Indiana

Identification of Substance and Dosage Form: Tilmicosin solution, agar/bacterial cell culture administration

Species and Strain: Salmonella typhimurium LT-2, strains TA1535, TA1537, TA1538, TA98, and TA100

Number of Animals Per Sex Per Treatment: Not applicable

Drug Levels Tested and Duration of Dosing: Activated and Nonactivated 100, 50, 10, 5 and 1 µg/plate

Route of Administration: Agar/Bacteria plate inoculation

Parameters Studied and Results:

Tilmicosin was tested in the Ames assay for the induction of bacterial mutation using five histidine auxotrophs of *Salmonella typhimurium*. Concentrations of 100, 50, 10, 5 and 1 µg/plate of tilmicosin were tested with and without metabolic activation using an S9 fraction prepared from the livers of Aroclor 1254-induced rats. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 2-nitrofluorene (2NF), and 9-aminoacridine (9AmAc) served as the positive controls for the nonactivated test, while 2-aminoanthracene (2AA) served as the positive control for the activated test. Dimethyl sulfoxide, the solvent for tilmicosin, served as the negative control, with and without metabolic activation.

Conclusion:

Treatment with MNNG, 2NF, 9AmAc, and 2AA resulted in a dose-related induction of reverse mutations in the appropriate Salmonella tester strains in the nonactivated and activated tests. Treatment with tilmicosin resulted in concentration-related decreases in counts of reverent colonies in all tester strains, both with and without metabolic activation. This was considered a manifestation of the antibacterial properties of the compound. Concentrations of <=10 µg/plate yielded survival values >=30 percent in all tester strains under both activation conditions and informative reverent frequency data were confined to concentrations of 10, 5 and 1 µg/plate. Over this concentration range, treatment with tilmicosin did not result in the induction of Salmonella reverent with or without activation. Tilmicosin was not mutagenic in the Ames Salmonella/mammalian microsome test for bacterial mutation.

B. CALCULATIONS OF TILMICOSIN ACCEPTABLE DAILY INTAKE

1. Studies Considered in Establishing a Tolerance

Study	**No-Observed-Effect-Level NOEL**
A One Year Chronic Toxicity Study in Beagle Dogs Given Oral Doses of Tilmicosin, Study D07187	4 mg/kg/day
The Toxicity of EL-870 Given to Beagle Dogs for 3 Months, Study D08286	6 mg/kg/day
The Toxicity of Tilmicosin Given Orally to Crl:CD(SD) Rats for 3 Months, Study R09886	50 mg/kg/day
Reproductive Effects of EL-870 Administered Orally Via Gavage to Crl:COBS CD(SD) BR Rats for Two Generations, with Two Litters per Generation, Study 112-001	200 mg/kg/day (Reproductive Effects) 10 mg/kg/day (Maternal Effects)
A Teratology Study of Tilmicosin EL-870, Compound 177370) Administered Orally to CD Rats, Study R13387	500 mg/kg/day (Fetal Effects) 10 mg/kg/day (Maternal Effects)

2. Safe Concentrations for Tilmicosin Residues

   The no-observed-effect-level (NOEL) for establishing the safe concentration of the total residues of tilmicosin is 4 mg/kg/day.

   Safe Concentration calculations are as follows:

   Liver was selected as the target tissue based upon data described in Section D.

   ADI = NOEL/Safety Factor (SF) SF = 100 because a one year chronic dog study was used therefore:

   ADI = 4 mg/kg/100 = 0.04 mg/kg or 40 µg/kg

   Safe Concentration = Acceptable Daily Intake x Human Weight / Food Factor x 500 g/day
   where:

   Human Wt. = 60 kg
   Daily Meat Consumption = 500 g
   Food Factor for Bovine Muscle = 1
   Food Factor for Bovine Liver = 0.5
   Food Factor for Bovine Kidney = 0.333
   Food Factor for Bovine Fat = 0.25

   Therefore for Bovine MUSCLE:

   Safe Concentration
   = 40 µg/kg x 60 kg / 1 x 500 g/day
   = 4.8 µg/g/day
   = 4.8 mg/kg/day or 4.8 ppm

   For Bovine LIVER:

   Safe Concentration
   = 40 µg/kg x 60 kg / 0.5 x 500 g/day
   = 9.6 µg/g/day
   = 9.6 mg/kg/day or 9.6 ppm

   For Bovine KIDNEY:

   Safe Concentration
   = 40 ug x 60 kg / 0.333 x 500 g/day
   = 14.4 µg/g/day
   = 14.4 mg/kg/day or 14.4 ppm

   For Bovine FAT:

Safe Concentration
= 40 µg/kg x 60 kg / 0.25 x 500 g/day
= 19.2 µg/g/day
= 19.2 mg/kg/day or 19.2 ppm

C. Total Residue and Metabolism The levels of total drug-related residues of tilmicosin in the tissues of cattle treated with 14C tilmicosin were determined in a tissue residue study conducted by Elanco.

Dose: 10 mg/kg subcutaneous injection

Test Animals: 12 Hereford cattle (7 male, 5 male); ~200 kg

Withdrawal Schedule: 3, 14, 28, 42, and 56 days post dosing

Tissue samples of muscle, liver, kidney, injection site and fat from each of the animals were radioassayed for total drug related residues. The results from the study are shown in the table that follows:

Total Radioactivity (ppm) in the Edible Tissues of
Cattle Receiving 10 mg/kg Dose of 14C-Tilmicosin
Withdrawal Day

Tissue	**3**	**14**	**28**	**42**	**56**
Liver	19.441 (±1.886)	11.626 (±3.485)	5.743 (±1.293)	3.518 (±0.571)	2.721 (±0.227)
Muscle	0.405 (±0.085)	0.090 (±0.010)	<0.048	NDR[1]	
Kidney	18.094 (±0.075)	2.509 (±0.340)	0.588 (±0.139)	0.267 (±0.039)	
Fat	0.244 (±0.076)	0.053 (±0.03)	<0.039		
Injection Site	73.528 (±19.308)	13.815 (±9.321)	5.072 (±0.899)	0.939 (±0.540)	0.332 (±0.136)

[1]NDR = No detectable residue

Livers from cattle in the residue study described above were subjected to wet chemistry procedures to establish the profile of 14C-tilmicosin metabolites present. The approach involved solvent extractions followed by thin layer chromatography (TLC), autoradiography, and high performance liquid chromatography (HPLC). At three days withdrawal, approximately 86% of the 14C-tilmicosin residues present in cattle liver was freely extractable into methanol/water (80/20). By 56 days post dosing, 57% of the residues were extractable.

The extraction and fractionation of the radiolabeled residues in liver tissue demonstrated that there were three primary components of the liver tissue. At three days withdrawal parent tilmicosin and compounds designated as T-1 and T-2

represented 33.7%, 16.0% and 8.7% of the liver radioactivity respectively. At 28 days, that distribution changed to 7%, 5%, and 21%. Metabolites T-1 and T-2 were isolated and characterized through mass spectrometry (MS) and nuclear magnetic resonance analysis (NMR). Those data showed a molecular weight of 854 for metabolite T-1 and indicated that it is N-desmethyl tilmicosin with the change in structure on the mycaminose sugar.

Metabolite T-2 was found by comparative HPLC to be identical with an impurity known to be present in the tilmicosin bulk active ingredient. This impurity had a molecular weight of 1609 and consisted of components of two macrolide and one piperidine ring. Compound T-2 is considered to be a dimer of tilmicosin and not a likely metabolite. Its presence as a residue in liver tissue is probably due to its presence in the injected drug rather than due to metabolism of tilmicosin.

One minor metabolite (T-3) was isolated from cattle feces. This metabolite was closely related to tilmicosin and appeared to result from replacement of the dimethylamino group on the mycaminose sugar with a hydroxyl group. Metabolic profiles of tilmicosin residues in the urine, feces and livers of orally dosed rats were also determined using similar extraction procedures. The resulting HPLC chromatograms indicated that the rat had been exposed to the same metabolites as those found in cattle. The HPLC assays of the rat liver and urine indicated that little if any T-2 was present. Similar data had been collected for orally dosed swine.

D. Selection of Target Tissue and Marker Residue for Tilmicosin in Cattle

The data in the table residue values shown above establish that liver contains the highest levels of total drug related residues of tilmicosin and that it is the tissue in cattle from which residues are the slowest to deplete to the safe concentration. These observations suggested liver as the likely choice of target tissue for tilmicosin in cattle.

The metabolism data, summarized in Section C., confirmed liver as the target tissue and led to the selection of the parent tilmicosin as the marker substance. Those data demonstrated that the parent was present in sufficiently high concentration and had the proper depletion characteristics in liver to serve as the marker residue in that tissue.

E. Tolerance for the Marker Residue

The Rm (tolerance) for tilmicosin was set from data obtained from the analysis of liver tissue from cattle in the total residue study described in Section C. The livers from cattle in that study were assayed for parent tilmicosin by the determination assay of the regulatory method. The method involves an initial extraction of the tissues with methanol. The extracts are further partitioned with carbon tetrachloride and chloroform. The resulting residue is then dissolved in the HPLC mobile phase for HPLC analysis by UV detection at 280 nm as discussed in Section G.

The results demonstrated that at 3 days withdrawal the parent compound represented 34% (6.61 ppm) of the total residue in liver. At 14 and 28 days withdrawal, the parent compound represented 17.1% (1.991 ppm) and 6.6%

(0.377 ppm) of the total residue respectively. By plotting the linear regression of the residue depletion data for the total and marker residues, it was determined that when the total residues depleted to the safe concentration (9.6 ppm), the marker residue reached a value of 1.2 ppm. Therefore, the Rm was established as 1.2 ppm.

F. Study Establishing the Withdrawal Period

Depletion of the marker residue following the subcutaneous administration of tilmicosin was determined in a study utilizing 12 head of cattle (8 males, 4 females). Each animal received a single dose at 10 mg/kg. The cattle were euthanized in groups of three (two steers and one heifer per group) at 14, 28, 35 and 42 days post-dosing.

Liver, kidney, muscle, fat and injection site samples were collected at the time of slaughter and assayed for the marker residue using the determinative procedure. The results are presented in the table below.

Tilmicosin (ppm)

Day	Liver	Kidney	Muscle	Fat	Inj. Site
14	0.924	0.924	<0.05	<0.05	18.938
28	0.258	0.140	<0.05	<0.05	2.923
35	0.175	0.109	<0.05	- - -	0.784
42	<0.089	<0.058	- - -	- - -	0.294

A statistical analysis of the depletion data, using an upper tolerance limit containing 99 percent of the population with a 95% confidence limit, supported a withdrawal period of 28 days. As a result, a withdrawal period of 28 days has been established for the injectable form of tilmicosin used as a single subcutaneous injection at 10 mg/kg.

G. Regulatory Method

Tilmicosin Determinative Assay Procedure

The determinative assay for measuring tilmicosin residues in treated cattle consists of extraction of the parent drug from liver, and measurement of the parent drug in the extract by high performance liquid chromatography (HPLC).

Ground cattle liver is homogenized with methanol and centrifuged to separate the solids. The methanol extract is diluted with sodium chloride solution, partitioned with carbon tetrachloride (CCl4), and the CCl4 layer is discarded. The aqueous methanol phase is made basic with sodium carbonate solution, and tilmicosin is extracted into chloroform/hexane. The chloroform/hexane extract is evaporated to dryness, and the residue is reconstituted in a solution of dibutylammonium phosphate in aqueous methanol. This solution is analyzed by HPLC, using a reversed-phase phenylsilyl stationary column and a three-solvent gradient, with detection at 280 nm.

The limit of quantification (LOQ) of the method is 0.1 ppm.

Tilmicosin Confirmatory Assay Procedure

In the confirmatory procedure, a sample is processed as above, through the point at which the chloroform/hexane extract is evaporated. The residue from that evaporation is then dissolved in chloroform, applied to a silica gel cartridge, and washed with chloroform/ methanol. Tilmicosin is eluted from the column with chloroform/ methanol/ ammonium hydroxide, and the solution is evaporated to dryness. The residue is dissolved in methanol and analyzed by tandem liquid chromatography/ mass spectrometry. The mass spectrometry procedure involves selective ion monitoring for three ions characteristic of tilmicosin.

Method Validation

A method trial of the determinative and confirmatory assays was satisfactorily completed by FDA and USDA laboratories.

Method Location

The validated regulatory analytical methods for tilmicosin residues are on display in Dockets Management Branch (HFV-305), Park Building (room 1-23), 12420 Parklawn Drive, Rockville, MD 20855. They are attached to the FOI summary. They will be filed in the Animal Drug Analytical Manual.

V. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of Section 512 of the Food Drug and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that tilmicosin (Micotil® 300), a macrolide antibiotic, when administered to cattle by a single subcutaneous injection at 10 mg/kg, is safe and effective for the treatment of bovine respiratory disease (BRD) associated with Pasteurella haemolytica.

A tolerance is established for total tilmicosin residues in edible cattle tissues based on a marker residue concentration of 1.2 ppm parent (unmetabolized) tilmicosin in the target tissue (liver). A parent tilmicosin concentration of 1.2 ppm in liver corresponds to a safe concentration of total tilmicosin residues of 9.6 ppm in liver. The other respective safe concentrations are: 4.8 ppm in muscle, 14.4 ppm in kidney, and 19.2 ppm in fat. A regulatory analytical method has been developed to determine the concentration and confirm the identity of tilmicosin in liver at 1.2 ppm.

A withdrawal period of 28 days has been established for tilmicosin used as a single subcutaneous injection at 10 mg/kg dosage based on a statistical analysis of the depletion data, using an upper tolerance limit containing 99 percent of the population with a 95% confidence limit.

Labeling restricts this drug to use by or on the order of a licensed veterinarian. This decision was based on the following factors: (a) the product contains a new antimicrobial entity intended only for therapeutic purposes, (b) adequate directions can not be written to enable laypersons to appropriately diagnose and subsequently use this product to treat bovine respiratory disease associated with Pasteurella haemolytica, (c) intravenous drug administration is lethal to cattle, (d) higher doses are toxic to swine and monkeys and can cause death, with the cardiovascular system

being the target of toxicity, and (e) 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 extralabel use.

The agency has carefully considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human environment and that an environmental impact statement is not required. The agency's finding of no significant impact (FONSI) and the evidence supporting that finding are contained in an environmental assessment which may be seen in the Dockets Management Branch (HFV- 305), Park Building (Room 1-23), 12420 Parklawn Drive, Rockville, MD 20855.

Section 512(c)(2)(F)(i) of the act provides a five-year exclusivity period for a drug, no active ingredient (including any ester or salt of the active ingredient) of which has been approved in any other application under subsequent (b)(1). This NADA qualifies for such an exclusivity period. Tilmicosin is under patent number U.S. 4,820,695 expiring April 11, 2006.

VI. ATTACHMENTS

Copies of applicable labels may be obtained by writing to the:

Freedom of Information Office
Center for Veterinary Medicine, FDA
7500 Standish Place
Rockville, MD 20855

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.