Approval Date: December 17, 1996

FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION

A. File Number

NADA 141-064

B. Sponsor

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

C. Proprietary Name

Pulmotil® 90

D. Established Name

tilmicosin phosphate

E. Dosage Form

Type A medicated article containing tilmicosin phosphate at 90.9 g/lb (200 g/kg), to be diluted either in a Type B medicated article or in a finished Type C medicated feed for swine. PULMOTIL® 90 is supplied in 25-lb bags.

F. Dispensing Status

This Veterinary Feed Directive (VFD) drug shall be fed to animals only by or upon a lawful veterinary feed directive issued by a licensed veterinarian in the course of the veterinarian's professional practice.

G. Recommended Dosage

PULMOTIL® 90 should be fed at a dose rate of 181.8 g to 363.6 g tilmicosin phosphate per ton of complete feed (200 to 400 ppm). Feed continuously as the sole ration for a 21-day period, beginning approximately seven (7) days before an anticipated disease outbreak.

H. Route of Administration

Oral, in feed.

I. Indication

PULMOTIL® 90 is indicated for the control of swine respiratory disease associated with *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*.

II. EFFECTIVENESS

A. Dose Determination

1. Investigator:

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2. Experimental Disease Model: Four trials were conducted under the same protocol using a total of 400 pigs weighing 30 to 80 lb at trial initiation. For each trial, a group of pigs was inoculated with one of four *Actinobacillus pleuropneumoniae* (App) isolates from independent sources to serve as disease "seeder pigs," inducing bacterial pneumonia in non-infected penmates. "Seeder pigs" (2 to 5) were then placed in each trial pen for 2 to 10days in order to induce bacterial pneumonia in penmates. "Seeder pigs" (2 to 5) were introduced into the non-infected trial pens 7 days after the administration of medicated feeds had begun and removed 2 to 10 days later, when pneumonia characteristic of App was evident in penmates.

3. Experimental Design: Each trial consisted of 2 randomized blocks of 5 pens each (a total of 10 pens per trial). Each block contained either a light or heavy replicate. Tilmicosin phosphate dose levels were randomly assigned to pens within blocks. Trial pigs were randomly assigned to the pens within weight categories within the location blocks. There were equal numbers of each gender in each pen. Each pen started with 10 trial pigs and PEN was the experimental unit.

4. Test Article Administration: Four levels of tilmicosin phosphate plus a zero level (0, 100, 200, 300, 400 ppm) were tested. Complete feeds were formulated using a Type A medicated article containing 90.9 g tilmicosin phosphate per pound (200 g/kg). All pigs were fed a non-medicated feed for at least 3 days before being given medicated feeds. The *ad libitum* feed medication of the trial pigs began 7 days before (Day -7) the day of introduction of the "seeder pigs" (Day 0) and continued for 14 days (Days 0 to 13), for a total medication period of 21 days. A non-medicated ration was fed for the last 14 days (Days 14 to 27).

5. Decision Variables:

   MORTALITY was recorded at the time of observation. CLINICAL IMPRESSION SCORE was the attending veterinarian's assessment of disease severity (0 = none, 1 = mild, 2 = moderate, 3 = severe) based on an animal's attitude, abdominal appearance, eyes, the observed level of hydration, quality of respiration, appearance of the hair coat, and any other perceived abnormality. Clinical observations were recorded daily beginning on Day -7. BODY TEMPERATURE was recorded daily on Days -2 through 1. WEIGHT was recorded on each pig at allotment and on Days -7, 0, 14, and 28.

   PERCENT PNEUMONIC LUNG involvement was determined during necropsy of each pig, either following disease-related death or scheduled euthanasia (Days 14 and 28). Measurement was first by visual estimate, then by weight, of pneumonic tissue.

6. Results: Pivotal decision criteria are summarized in Table 4.1.

MORTALITY: There were too few deaths in the trials to determine a significant treatment effect on mortality. CLINICAL IMPRESSION SCORE: The pooled results indicated a significant reduction in clinical signs of respiratory distress and body temperatures at all non-zero doses. Pigs fed tilmicosin phosphate at 200 ppm and above had significantly improved clinical scores over those fed the 100 ppm level. A linear reduction in BODY TEMPERATURE was observed with tilmicosin phosphate fed at 100 ppm to 400 ppm.

PERCENT PNEUMONIC LUNG: The lung involvement calculated by weighing gave percentages with less random variability than the visual estimates. By trimmed weight, 14 days after the seeder pigs were introduced, pigs fed the 300 and 400 ppm levels had significantly less pneumonic tissue involvement than those fed the 200 ppm level. By Day 28 there were no significant differences in lung pneumonic involvement among the remaining tilmicosin phosphate treated animals (all control pigs were necropsied on Day 14).

Table 4.1. Mortality, clinical impression scores and percent pneumonic lung in four dose determination trials using infected "seeder pigs" to induce *Actinobacillus pleuropneumoniae* (App); 0-level group was terminated on Day 14.

Tilmicosin (pmm)	**Mortality (Days 0-14)***	**Clinical Impression Scores**	**Percent Lung Involvement (Day 14 Trimmed Weights)**
0	5/80	0.713	24.792
100	1/80	0.395	14.854
200	1/80	0.235	5.111
300	0/79	0.214	1.888
400	0/80	0.185	1.034

* Number of Pig Deaths/Number of Pigs

7. Conclusions

   Although feeding 100 ppm provided some benefits to disease-exposed pigs, feeding higher doses was sufficiently superior to warrant testing the 200 and 400 ppm dose levels in field trials to determine the lowest use dose for the control of pneumonia caused by *Actinobacillus pleuropneumoniae* in swine. The effect of tilmicosin phosphate on *Pasteurella multocida* could not be evaluated because of insufficient numbers of pigs infected with that organism. The results for pulmonic involvement and body temperature suggest that tilmicosin phosphate administered at levels greater than 200 ppm may be indicated when severe disease is anticipated.

8. Adverse Reactions: There were no drug-related adverse reactions observed by the investigator at any level of tilmicosin phosphate feeding during these trials.

B. Field Investigations:

Seven clinical field trials conducted by six different investigators in six different geographical locations were used to confirm the efficacy and safety of the dose and

dosage range of tilmicosin phosphate as a Type A medicated article in feed for the control of naturally-occurring bacterial pneumonia due *to Actinobacillus pleuropneumoniae* (App) and/or *Pasteurella multocida* (Pm). Trials were replicated in one location.

1. Investigators:

   Dr. Melissa Fleck Veenhuizen
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   Hilliard, Ohio 43026
   Trial No.'s T5C399301 and T5C399310

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   Trial No.T5C399309

2. Experimental Design, Materials and Methods: Twelve trials were conducted in nine geographic regions in commercial swine grower/finisher facilities that were representative of production systems currently in use in the swine industry. Each of the facilities contained pigs, or was repopulated with pigs, from commercial swine herds with a history of having death losses from *Actinobacillus pleuropneumoniae* (App) and/or *Pasteurella multocida* (Pm).

   Pigs ranged in weight from 30 to 165 pounds at the time of initiation of the trials. Each trial consisted of two randomized blocks of three pens each (a

total of 6 pens per trial). The blocks contained either light or heavy replicates and tilmicosin phosphate dose levels were randomly assigned to pens within the blocks. The trial pigs were randomly assigned to pens within weight categories within the location blocks. Depending upon availability of trial pigs, the pens within each trial were either normalized by sex or contained equal numbers of each sex. The numbers of pigs per pen varied between trials (15 to 30) and depended on pen size and the routine stocking density in each facility. The PEN was the experimental unit.

The dose levels of tilmicosin phosphate in the feed were 0, 200 and 400 ppm. Complete feeds containing 0, 200, and 400 ppm of tilmicosin phosphate were formulated at Lilly Research Laboratories (LRL) in Greenfield, Indiana using a Type A medicated article containing 90.9 g tilmicosin phosphate per pound (200 g/kg) of corn cob grits carrier. The feed bags were color- and letter-coded, and the trial investigator and personnel were blinded to the medication levels.

All pigs were fed a non-medicated feed for at least 3 days before being given medicated feeds. The *ad libitum* feeding of the medicated feeds was started approximately 7 days prior to the anticipated onset of clinical pneumonia due to App and/or Pm as determined by the investigator/veterinarian from each herd's clinical disease history. *Ad libitum* feeding continued for a total of 21 days at which time the trial ended. Data was summarized for the period was from Days 0 through 21.

3. Measurements:
   CLINICAL IMPRESSION SCORE was the attending veterinarian's daily assessment of disease severity (0 = none, 1 = mild, 2 = moderate, 3 = severe) based on an animal's attitude, abdominal appearance, eyes, the observed level of hydration, quality of respiration, appearance of the hair coat, and any other perceived abnormality. MORTALITY was recorded at the time of observation.

   Data summarized but not analyzed included the visual percent pneumonic lung, bacterial cultures of lung lesions, and MICs (App and/or Pm) of pigs that died or were euthanized. These data are useful in demonstrating the incidence of infection with App and/or Pm and serve as an indicator of the level of morbidity in the zero (0) level groups within each trial.

4. Trial Inclusion/Exclusion in the Pooled Analysis:

   At trial termination, five or more selected pigs on non-medicated feed were necropsied to determine the rate of pneumonia and to identify the etiological agent. Each trial was evaluated independently to assess suitability for inclusion in the pooled analysis with the primary criteria being presence of pneumonia caused by App and/or Pm. For inclusion in the pooled analysis, at least 20% of the control pigs necropsied had to have pneumonic lesions greater than 10% of the total lung parenchyma and at least 20% be culturally-positive for App and/or at least 20% be positive for Pm. Two trials were eliminated because of insufficient number of isolates. These were the same trials that failed to meet lung lesion inclusion criteria.

The averages of the CLINICAL IMPRESSION SCORES for the non-medicated pens within each pivotal trial had to be significantly different than 0 (normal) for the test herd to be considered a clinically-ill herd. Five of the original 12 trials were eliminated. An additional three trials were excluded because they did not have a sufficient number of non-zero clinical impression scores.

The data from the remaining seven trials were pooled and analyzed with regard to mortality, animal weight gain, and feed efficiency. Six trials were analyzed together because App was recovered from at least 20% of the necropsied control pigs in these trials. Similarly, three trials were analyzed together because Pm was recovered from at least 20% of the necropsied control pigs.

5. Data Analysis: The experimental unit in these trials was the PEN, therefore analysis procedures were run on the pen means. Four efficacy variables were statistically analyzed: MORTALITY, AVERAGE DAILY GAIN, FEED EFFICIENCY (feed to gain ratio), and daily CLINICAL IMPRESSION SCORES. In the categorical analysis of mortality, PEN was included as a population parameter. The SOURCE of pigs was considered an independent and random effect in the statistical models. Two trials were conducted in the same location but with different sources (herd of origin) of pigs, therefore they were considered independent and random.

6. Results: First signs of clinical pneumonia were observed in the control pigs between 1 and 16 days after the beginning of the feeding of treatment feeds. From seven trials, a total of 10 control pigs receiving no tilmicosin phosphate died. None of the pigs that were treated with either the 200 ppm or 400 ppm level of tilmicosin phosphate died. Pooled data are summarized in Table 4.2.

Table 4.2. Mortality due to naturally-occurring pneumonia (number of pig deaths/number of pigs) in pigs fed tilmicosin phosphate for 21 days

Tilmicosin(ppm)	All trials (7)	App Trials (6)	Pm Trials (3)
0	12/164*	11/143*	7/63*
200	0/256*	0/218**	0/110**
400	0/274**	0/234**	0/112**

*, ** Ratios in the same column without a common superscript are significantly different P<.001.

Analysis of CLINICAL IMPRESSION SCORES from the seven acceptable trials demonstrated that feeding of both levels of tilmicosin phosphate resulted in significant improvement in the clinical appearance of the treated pigs compared to the controls. The results were similar for the seven trial analysis, the six App trial analysis and the three Pm trial analysis (Table 4.3).

Table 4.3. Clinical impression scores for Days 0 through 14*** (least squares means and standard errors)

Tilmicosin(ppm)	All trials (7)	App Trials (6)	Pm Trials (3)
0	0.364 ± 0.0905*	0.398 ± 0.104*	0.427 ± 0.161*
200	0.054 ± .0905**	0.063 ± 0.104**	0.028 ± 0.161**
400	0.084 ± .0905**	0.098 ± 0.104**	0.054 ± 0.161**

*** Clinical impression scores for the observation period after treatment was ended were not provided.
*, ** Ratios in the same column without a common superscript are significantly different P<.05.

7. Conclusions:

   These field investigations demonstrates that tilmicosin phosphate in feed at 200 and 400 ppm is safe and effective in the control of swine respiratory disease associated with *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* when fed continuously as the sole ration for a 21-day period, beginning approximately seven (7) days before an anticipated disease outbreak. Tilmicosin phosphate at 400 ppm did not appear to be significantly more effective than 200 ppm, however these outbreaks did not incur high mortality.

8. Adverse Reactions:

   A total of 940 animals were medicated with tilmicosin phosphate at 200 or 400 ppm in these trials (470 at each dose) with no observation of adverse reactions.

C. Studies to Support Labeling

1. *In Vitro* Activity:

   Tilmicosin phosphate has an *in vitro* antibacterial spectrum that is predominantly gram-positive with activity against certain gram-negative microorganisms, and is summarized in Table 4.4. Activity against several *Mycoplasma* species has also been detected.

   Table 4.4. Minimum inhibitory concentrations (MICs) obtained for selected swine pathogens susceptibility tested *in vitro* by a standardized procedure

Microorganism	**MIC (µg/mL)**
*Actinobacillus pleuropneumoniae**	16.0
*Pasteurella multocida**	8.0
*Mycoplasma hyopneumoniae***	0.5
*Escherichia coli***	>64.0
*Salmonella choleraesuis***	>64.0
*Streptococcus suis***	>64.0

   * Activity against these organisms has been demonstrated clinically.

   ** The clinical significance of this *in vitro* data has not been demonstrated.

   Similar *in vitro* susceptibility of swine pathogens to tilmicosin has been reported in the following published articles, for which verbatim abstracts are provided.

   a. S.S. Salmon, et al. 1995. Comparison of MICs of Ceftiofur and Other Antimicrobial Agents Against Bacterial Pathogens of Swine from the

United States, Canada, and Denmark. *J. Clin. Microbiol*. 33:2435-2444.

The MICs of ceftiofur and other antimicrobial agents, tested for comparison, for 515 bacterial isolates of pigs from the United States, Canada, and Denmark with various diseases were compared. The organisms tested included *Actinobacillus pleuropneumoniae, Escherichia coli, Pasteurella multocida, Salmonella choleraesuis, Salmonella typhimurium, Streptococcus suis, Streptococcus dysgalactiae* subsp. *equisimilis, Streptococcus equi* subsp. *equi*, and *Streptococcus equi* subsp. *zooepidemicus*. In addition to ceftiofur, the following antimicrobial agents or combinations were tested: enrofloxacin, ampicillin, sulfamethazine, trimethoprim-sulfadiazine (1:19), erythromycin, lincomycin, spectinomycin, lincomycin-spectinomycin (1:8), tilmicosin, and tetracycline. Tilmicosin was only tested against the U.S. isolates. Overall, ceftiofur and enrofloxacin were the most active antimicrobial agents tested against all isolates, with MICs inhibiting 90% of isolates tested (MIC90s) of (2)2.0 and (2)1.0 µg/mL, respectively. Erythromycin, sulfamethazine, spectinomycin, and lincomycin demonstrated limited activity against all of the organisms tested, with MIC90s of (3)8.0, (3)256.0, (3)32.0, and (3)16.0 µg/mL, respectively. Trimethoprim-sulfadiazine was active against isolates of *Actinobacillus pleuropneumoniae*, *S. choleraesuis, S. typhimurium, P. multocida, S. equi,* and *S. suis* (MIC90s, (2)5.0 µg/mL) but was less active against the *E. coli* strains tested (MIC90s, (2)16.0 µg/mL). Ampicillin was active against *the P. multocida, S. suis*, and *S. equi* isolates tested (µg/mL, 0.5, 0.06, and 0.06 µg/mL, respectively) and was moderately active against *S. typhimurium* (MIC90s, 2.0 µg/mL). However, this antimicrobial agent was much less active when it was tested against A*. pleuropneumoniae, S. choleraesuis*, and *E. coli* (MIC90s, 16.0, >32.0, and >32.0 µg/mL, respectively). Against the U.S. isolates *of A. pleuropneumoniae* and *P. multocida*, tilmicosin was moderately active (MIC90s, 4.0 and 8.0 µg/mL, respectively). However, this compound was not active against the remaining U.S. isolates (MIC90s, >64.0 µg/mL). Differences in the MICs from one country to another were not detected with enrofloxacin, ceftiofur, or lincomycin for the strains tested, but variations in the MICs of the remaining antimicrobial agents were observed.

b. T. Inamoto et al. 1994. Antibiotic Susceptibility of *Mycoplasma hyopneumoniae* Isolated from Swine. *J. Vet. Med. Sci*.. 56(2):393-394.

The antibiotic susceptibility of thirty-nine strains of *Mycoplasma hyopneumoniae* isolated from swine between 1970-1981 and 1989-1990 was investigated. From the present results, it is suggested that the susceptibility to chlortetracycline has been decreasing in Japan. On the other hand, all the strains were sensitive to lincomycin, thiamphenicol and macrolides. Newly developed macrolides such as tilmicosin, acetylisovaleryl-tylosin and mirosamycin had equal or higher activity than general macrolides.

2. Pharmacology:

Generally, the duration and degree to which antibiotic serum or tissue concentrations exceed the minimum inhibitory concentration (MIC) of the suspected bacterial pathogen are used as a guide to understand the relationship between dose and clinical improvement. However, certain antibiotic compounds, such as macrolides, barely achieve detectable levels in serum but are nevertheless recognized to be highly efficacious in the treatment of infectious diseases. Evidence suggests that many macrolides undergo significant accumulation within macrophages, reaching very high ratios of intracellular to extracellular drug concentrations (Blais, *et al*., 1994; Butts, 1994; Carlier, *et al*., 1987; Gladue, *et al*., 1989). This observation has been used to explain the high degree of efficacy associated with these compounds despite the presence of negligible serum concentrations. Compounds falling into this category include clarithromycin and azithromycin. Preliminary *in vitro* data suggest that tilmicosin may similarly accumulate (approximately 50 fold) within primary swine alveolar macrophages (Blais and Chamberland, 1994) but the clinical significance of these preliminary data have not been confirmed.

Other measurements of drug accumulation have been useful for explaining the efficacy of these antibiotics. Butts (Butts, J.D. 1994. Intracellular Concentrations of Antibacterial Agents and Related Clinical Implications. *Clin. Pharmacokinet.* 27:63-84) states that "intracellular to extracellular concentration ratios may be a more reliable predictor of antibacterial effectiveness. The intracellular to extracellular concentration ratio may simply provide a somewhat better predictor for clinical outcome than serum antibacterial concentrations alone."

a. Serum and Lung Tilmicosin Concentration in Swine Following Dosing with Tilmicosin Fortified Feed. Study T5CAX9302:

Investigators:

T.D. Thomson, V.M.D., Ph.D., *et al.*
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

Daily oral intake in grower rations by 30 kg pigs of 200 or 400 ppm tilmicosin phosphate resulted in serum tilmicosin concentrations which were below (at a dose of 200 ppm) or just slightly above (at a dose of 400 ppm) the high performance liquid chromatography assay limit of quantification (0.1 µg/mL). Substantial drug accumulation was seen in the lungs of all animals, regardless of treatment level. Mean ± SD lung tilmicosin concentrations at the 2-, 4-, 7-, 10-, and 14-day sample time were 0.73 ± 0.19, 1.11 ± 0.55, 0.59 ± 0.19, 1.43 ± 1.13, and 1.20 ± 0.46 ppm, and 1.11 ± 0.24, 2.29 ± 0.27, 2.24 ± 0.88, 2.59 ± 1.01, and 1.61 ± 0.68 ppm, respectively. Thus, the degree of drug accumulation was found to be proportional to dose.

b. Intracellular Accumulation of Tilmicosin in Primary Swine Alveolar Macrophages.

Investigators:

S. Chamberland, M.Sc., Ph.D.
Department of Microbiology
Faculty of Medicine
University of Laval
Quebec, CANADA

Uptake and intracellular accumulation of (14)C-labeled tilmicosin in primary swine alveolar macrophages were compared to (14)C-labeled erythromycin. Concentrations of cell-associated drugs were derived from standard curves, and uptake was expressed as the ratio of intracellular concentration (I) to extracellular concentration (E). Tilmicosin reached concentrations as high as 1.5 mg/mL in alveolar macrophages after 24-hour incubation in culture medium containing 20 µg/mL of antibiotic. Under these conditions tilmicosin and erythromycin reached I/E ratios of 75.04 ± 0.03 and 6.20 ± 0.15, respectively. The uptake of both macrolides was abolished when cells were incubated at 4 °C, indicating the possibility of an active transport process, not simple diffusion. For both macrolides tested, the intracellular concentration of drug was proportional to the amount of antibiotic in the culture medium.

c. Evaluation of the Inhibitory Activities of Antibacterial Agents on Swine and Bovine Pathogens and the Effects of Antibiotics on the Production of Virulence Factors:

Investigators:

M. Jacques, Ph.D.
Faculty of Veterinary Medicine
University of Montreal
Quebec, Canada J2S 7C6 F. Malouin, Ph.D.
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Central Hospital of the
University of Laval
Quebec, Canada G1V 4G2

The objectives of this study were to assess the ability of tilmicosin (and other antibiotics) to alter bacterial growth, the production of virulence factors (capsule, lipopolysaccharides, toxins), and the expression of essential cell surface components (iron regulated proteins, iron uptake pathways) at drug levels below the minimum inhibitory concentration (MIC) and to measure the post-antibiotic effect (PAE) of the drug. Strains of *Actinobacillus pleuropneumoniae, Pasteurella multocida, Streptococcus suis, Bordetella bronchiseptica*, and *Pasteurella hemolytica* were evaluated in this study.

For most strains tested, addition of tilmicosin to the culture medium had a profound effect on growth, even at 1/8 of the antibiotic's MIC, while *S. suis* was not affected by high concentrations of the drug. The PAE caused by tilmicosin on *A. pleuropneumoniae, P. multocida, B. bronchiseptica*, and *P. haemolytica* was determined. Results revealed a significant delay of growth for tilmicosin treated bacteria after removal of the drug from cultures, even when using 1/2 of the MIC.

III. ANIMAL SAFETY

A. Toxicity Studies with Unformulated Drug and an Approved Tilmicosin Injectable Solution

In acute toxicity studies using dogs, tilmicosin was given by intravenous administration at 1.0 and 5.0 mg/kg, producing tachycardia, peripheral vasoconstriction, increased pulmonary pressure, increased wedge pressure, and increased pulmonary vascular resistance as well as decreased cardiac output, stroke volume, stroke work index, and femoral flow.

A tilmicosin solution (MICOTIL®), approved for treatment of bovine respiratory disease by subcutaneous injection, was tested for safety in swine by singly dosing 16 animals intramuscularly (i.m.) at either 0, 10, 20, or 30 mg/kg. There was no mortality in the 10 mg/kg group, but 3 of 4 pigs in the 20 mg/kg group died, and all pigs in the 30 mg/kg group died. All pigs in the two higher dose groups showed similar signs of toxicity: recumbency, convulsion, vomiting, increased respiration, tremors, ataxia, restlessness, labored breathing, lethargy and squealing. The gilt who survived in the 20 mg/kg group returned to normal within 4 hours. Signs of toxicity were mild in the low dose group, except for one pig that convulsed. All pigs in the low dose group recovered within an hour of injection. Necropsy findings of survivors were limited to injection site reactions.

B. Pivotal Safety Studies with PULMOTIL® 90

1. Drug Tolerance Test

   i. Investigators:

   J.M. Darby, et al.
   Lilly Research Laboratories
   Division of Eli Lilly and Company
   Greenfield, Indiana 46140

   ii. Animals and Test Article: Twenty-four 8-week-old Yorkshire-crossbred pigs, weighing 24 kg, consumed *ad libitum* rations containing 0 and 4000 ppm tilmicosin phosphate for 22 days. The 4000-ppm dose level corresponds to 10X the upper end of the approved dose range for PULMOTIL® 90. Each treatment group consisted of 6 castrate males and 6 females.

   iii. Measurements: Individual body weights and pen feed consumption were recorded at the start and end of the treatment period and pigs were observed twice daily for clinical signs.

Blood samples for hematological and clinical chemistry determinations were collected from each animal on study at Days -5 and 21. The parameters examined were: white blood cell count, red blood cell count, hemoglobin, hematocrit, cell indices, white blood cell differential, red blood cell morphology, glucose, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, alanine transaminase, aspartate transaminase, calcium, inorganic phosphorus, sodium, potassium, chloride, total protein, albumin, globulin, and A/G ratio.

Urine was collected from each pig at necropsy via bladder puncture and was analyzed for: color, clarity, specific gravity, pH, protein, glucose, occult blood, ketones, bilirubin, and urobilinogen.

During necropsy, weights were recorded for the kidneys, liver, heart, adrenals, and thyroids. Kidney, liver, and heart organ weights were analyzed as ratios of organ weight to total weight (g/kg).

iv. Results: One castrate male in the 4000 ppm treatment group died on Day 18 without prior clinical signs. There were no abnormalities found at necropsy and the cause of death was not established.

All other pigs survived the treatment period. No treatment-related clinical signs were observed during the study. The mean body weight gain, mean feed consumption, and mean gain/feed ratio were not affected by tilmicosin treatment. Treatment did not affect hematological, clinical chemistry, or urinalysis parameters and there were no treatment related gross or histological lesions.

Least square means for the heart ratio were 4.112 for control and 4.448 for 10X-treated pigs and were significantly different (p=.0179). The weights of hearts were within the normal range for pigs of this age and did not have gross or histological abnormalities.

v. Conclusions: Tilmicosin given parenterally is known to be cardiotoxic to several species, including swine. The mechanism of action for the toxicity in dogs is a disruption of the electro-magnetic currents in the heart, which is not associated with specific lesions (see Freedom of Information Summary, NADA 140-929, MICOTIL®, dated March 24, 1992). Thus, the cause of the single barrow death may be treatment-related.

2. Toxicity Test

i. Investigators:

J.M. Darby, et al.
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

ii. Animals and Test Article: Forty-eight 8-week-old Yorkshire-crossbred pigs, weighing 25 kg, consumed *ad libitum* rations containing 0, 400, 1200, or 2000 ppm tilmicosin phosphate for 43 or 44 days. These dose levels correspond to 0, 1X, 3X, and 5X the upper end of the approved dose range for PULMOTIL® 90. Treatment groups consisted of 6 castrate males and 6 females.

iii. Measurements: Individual body weights and pen feed consumption were recorded at the start (Study Day 0), Day 21, and at the end of the treatment period (Day 43 or 44), and pigs were observed twice daily for clinical signs.

Blood samples for hematological and clinical chemistry determinations were collected from each animal on Days -5, 21, and 42. Parameters examined were: white blood cell count, red blood cell count, hemoglobin, hematocrit, cell indices, white blood cell differential, red blood cell morphology, glucose, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, alanine transaminase, aspartate transaminase, calcium, inorganic phosphorus, sodium, potassium, chloride, total protein, albumin, globulin, and A/G ratio.

Urine was collected from each pig at necropsy via bladder puncture and was analyzed for: color, clarity, specific gravity, pH, protein, glucose, occult blood, ketones, bilirubin, and urobilinogen.

During necropsy, weights were recorded for the kidneys, liver, heart, adrenals, and thyroids.

iv. Results: Pigs were monitored for the toxic symptoms, such as collapse and sudden death due to heart arrhythmias, that had been observed with use of the parenteral form of the drug in swine and laboratory species. The pigs showed no adverse clinical signs during the test period and gained weight normally. The heart and other organs of all animals were carefully examined at necropsy. Although adrenal size was decreased in weight in the 2000 ppm group barrows compared to the control and lower dose groups, the histology of this organ was normal. All other organ weights and histology were unremarkable.

The statistical analysis of the blood chemistries was re-evaluated by CVM because some animals in each treatment group had hemolysis in their serum samples. We did not discount animals with slight or trace amounts of hemolysis, but we discounted those with moderate amounts of hemolysis, or those in which hemolysis was seen in more than one time point. No drug-related abnormalities were noted in the blood chemistries that we evaluated.

v. Conclusion: This pivotal safety trial demonstrated no drug-related toxicity in eight-week-old pigs given doses up to 5X (2000 ppm) the high dose of the approved range of 200 to 400 ppm. PULMOTIL® 90 in swine feed is safe when used according to label instructions.

C. Corroborative Safety Studies with PULMOTIL® 90

A total of 940 animals were medicated with tilmicosin phosphate at 200 or 400 ppm in field investigations (470 at each dose) with no observation of adverse reactions.

IV. HUMAN FOOD SAFETY

A. Toxicity Tests: Laboratory animal toxicology studies and *in vitro* mutagenicity studies for tilmicosin phosphate were addressed in the Freedom of Information (FOI) Summary for NADA 140-929 (MICOTIL® Injectable Solution).

B. Safe Concentration of Total Residues

1. No-Observed-Effect Level (NOEL)

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

2. Calculation of Acceptable Daily Intake (ADI):

   Acceptable Daily Intake (ADI) = Lowest NOEL Safety Factor

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

   The lowest NOEL is 4 mg/kg, so ADI = 4 mg/kg 100 = 0.04 mg/kg or 40 µg/kg/day

   However, the CVM guideline entitled *Microbiological Testing of Antimicrobial Drug Residues in Food*, dated January 1996, limits the maximum ADI for microbiologically-active residues to 1.5 mg/person/day (25 µg/kg body weight/day for a 60 kg person). Since this ADI is lower than the NOEL-based ADI calculated above, it is used for tilmicosin residues in swine.

3. Safe Concentration (SC) Calculations:

   Safe Concentration (SC) = Acceptable Daily Intake (ADI) x Human Weight 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 (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) = 25 µg/kg bw/day x 60 kg / 300 g/day = 5 µg/g = 5 ppm

   SC (fat or kidney) = 25 µg/kg bw/day / 50 g/day x 60 kg = 30 µg/g = 30 ppm

SC (liver) = 25 µg/kg bw/day / 100 g/day x 60 kg = 15 µg/g = 15 ppm

C. Total Residue Depletion and Metabolism Studies

1. (14)C Tilmicosin Steady-State Tissue Residue Study in Swine. Study ABC-0407:

   i. Investigators:

   A.L. Donoho, Ph.D., *et al.*
   Lilly Research Laboratories
   Division of Eli Lilly and Company
   Greenfield, Indiana 46140

   ii. Objectives: This study was conducted to: 1) determine the magnitude of residues of radioactivity in edible tissues of swine dosed orally with (14)C-tilmicosin, and slaughtered at a practical zero-hour withdrawal; 2) determine the dosing interval required to achieve steady-state residues; and 3) characterize the radioactive residues in tissues and excreta of treated swine.

   iii. Test Article: The (14)C-tilmicosin used in the study had a specific activity of 0.100 uCi/mg and a radiochemical purity of 95.8 percent. The lot consisted of equimolar quantities of tilmicosin labeled in the piperidine ring and tilmicosin labeled in the desmycosin macrolide ring.

   iv. Design: Nine crossbred swine, six castrated males and three females, weighing 22 to 26 kg were fed a ration containing (14)C-tilmicosin equivalent to 150 grams/ton (165 ppm) of feed for 4, 8, or 12 days. Two castrate males and one female were assigned to each of the three dose groups. All were slaughtered at the "zero-hour" sample time of 12 hr after the last dose.

   v. Assays: Samples of muscle, liver, kidney, back fat, skin and bile were assayed for total radioactivity by solubilization or combustion, coupled with liquid scintillation counting. Muscle, liver, and kidney tissues were assayed for parent tilmicosin by HPLC. Characterization of radioactivity in urine, feces, and selected tissues was conducted to determine the pattern of radioactive residues.

   vi. Results: Mean radioactivity concentrations in primary edible tissues are summarized in Table 6.1.

Table 6.1. Total radioactivity in tissues of swine sacrificed at 12 hours withdrawal following treatment with 165 ppm (14)C-tilmicosin in feed

Dosing Interval	**(14)C-Tilmicosin Equivalents* (ppm)**			
	Liver	**Kidney**	**Muscle**	**Fat**
4 Days	1.830	1.993	0.148	0.063
8 Days	1.895	1.953	0.139	0.056
12 Days	2.023	1.953	0.155	0.063

*Each tissue residue value represents the mean of three animals.

vii. Conclusions: The magnitude of residues from this study agree with data from a preliminary tissue residue study, ABC-0390, in which pigs were fed 150 grams per ton (165 ppm) (14)C-tilmicosin labeled in only the dimethylpiperidine ring. Tissue residues were not statistically different ($P<0.05$) in the 4-day and 12-day dose groups, indicating that steady-state residues were achieved within 4 days of dosing at the 165 ppm level.

Most of the radioactivity in the edible tissues was parent tilmicosin. HPLC analysis indicated that tilmicosin accounted for approximately 73%, 67%, and 62% of the total residue in muscle, liver, and kidney, respectively. There were no major metabolites. A minor metabolite, identified as N-desmethyl tilmicosin, was found in liver and kidney. This metabolite was described previously and was found to be present in liver and feces of (14)C-tilmicosin-fed rats. Parent tilmicosin was the primary component in the extractable portion of the residue from urine and feces.

2. Tilmicosin Metabolism Study in Tissues and Excreta of Pigs Fed 400 ppm (14)C-Tilmicosin. Study T5C759201:

i. Investigators:

A.L. Donoho, Ph.D., *et al*.
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

ii. Design: Six crossbred swine, three castrate males, and three females, weighing approximately 22 kg were fed (14)C-tilmicosin phosphate equivalent to 400 ppm tilmicosin base in the feed for 5 days. The (14)C-label was either at the 3 and 5 positions in the piperidine ring or at various positions in the macrolide ring of tilmicosin. At 12 hours after the last dose ("zero-hour" withdrawal), one male and one female were euthanized and tissues and bile were taken for assay. Similar pairs were euthanized at 7 and 14 days withdrawal. Two untreated pigs were maintained as controls.

iii. Assays: Muscle, liver, kidney, and fat were assayed for total radioactivity by solubilization and liquid scintillation counting (LSC). Bile samples were assayed by direct LSC. Selected tissues were

assayed for parent tilmicosin by HPLC. Urine and feces from two pigs were assayed to determine the rate and route of excretion of the (14)C-tilmicosin. Metabolism studies were conducted on selected tissue and excreta samples to determine the distribution of radioactivity and the metabolite pattern.

iv. Results: Liver and kidney had the highest concentrations of radioactivity, and liver was the tissue with the most slowly depleting residues. Muscle and fat residues declined to nondetectable concentrations (limit of quantitation for the assay was 0.01 ppm for muscle and 0.005 ppm for fat) by 14 days withdrawal. HPLC assays indicated that most of the radioactivity in the primary edible tissues was due to the parent tilmicosin. HPLC values are not corrected for percent recovery. In liver (target tissue) samples, parent tilmicosin (marker residue) accounts for approximately 50 percent of the total radioactivity at all three withdrawal times. Results of assay for radioactivity in tissues are summarized in Table 6.2.

Withdrawal Time (days)	**Assay**	**Mean tilmicosin concentration***			
		Liver	**Kidney**	**Muscle**	**Skin/Fat**
0	RA	4.546	4.311	0.386	0.122
	HPLC	2.33	2.34	0.24	0.13
7	RA	1.420	0.700	N.D.R.	0.020
	HPLC	0.75	0.35	<0.05	<0.05
14	RA	0.380	0.162	N.D.R.	N.D.R.
	HPLC	0.19	0.09	<0.05	N.A

* Each tissue residue value represents the mean of two animals.

RA = Total radioactivity in ppm tilmicosin

HPLC = Parent tilmicosin as measured by HPLC regulatory method

N.D.R. = No detectable residue (less than 0.01 ppm muscle or 0.005 ppm fat)

N.A. = Not assayed

The radioactive dose was excreted primarily in the feces. Total recovery of the dose was 70.4 and 69.9 percent, respectively, for the male and female in the 14-day group. Only 5.7 to 5.8 percent was found in the urine.

v. Conclusions: Fractionation of radioactivity from liver and kidney samples indicated that there were no major metabolites greater than 10 percent of the total radioactivity in these tissues. From the zero-time tissues, approximately 90% of the radioactivity was extracted. The 7- and 14-day livers revealed 13.5% and 25.8% of nonextractable radioactivity. The extractable radioactivity was primarily recovered under the peak for parent tilmicosin with a small amount of Metabolite T-1 (N-desmethyl tilmicosin). Radioactivity in muscle and fat was too low for good quantitative fractionation, but the comparative results

from radioactivity and HPLC assays indicate that parent tilmicosin is the primary component.

3. (14)C-Tilmicosin Tissue Residue Decline Study in Swine Dosed Orally at a Level of 600 ppm in the Feed. Study T5C759101:

   i. Investigators:

   A.L. Donoho, Ph.D., *et al*.
   Lilly Research Laboratories
   Division of Eli Lilly and Company
   Greenfield, Indiana 46140

   ii. Objectives: The purpose of this study was to determine: 1) the concentration of radioactive residues in tissues of swine which were dosed orally for 5 days with the equivalent of 600 ppm (14)C-tilmicosin in the feed; and 2) the rate of decline of radioactivity after withdrawal from the drug.

   iii. Test Article: The radiochemical purity of the (14)C-tilmicosin, which consisted of equimolar quantities of tilmicosin labeled in the piperidine ring and tilmicosin labeled in the desmycosin macrolide ring, was >95%.

   iv. Design: Nine crossbred swine, three castrate males and six females weighing approximately 17 kg were fed (14)C-tilmicosin equivalent to 600 ppm in the feed for 5 days. At 6 hr after the last dose ("zero-hour" withdrawal), one male and two females were euthanized and tissues and bile were taken for assay. Similar groups of three swine were euthanized at 14 and 28 days withdrawal. One untreated pig was maintained as a control.

   v. Assays: Muscle, liver, kidney, fat, skin, and lung were assayed for total radioactivity by solubilization and liquid scintillation counting (LSC). Bile samples were assayed by direct LSC. Selected tissues were assayed for parent tilmicosin by HPLC.

   vi. Results: Results of assay for radioactivity in tissues are summarized in Table 6.3.

   Table 6.3. Total radioactivity in tissues of swine treated with 600 ppm

   (14)C-tilmicosin in feed for 5 days

Withdrawal Time	**Mean radioactivity of 14C-tilmicosin* (ppm)**					
	Liver	**Kidney**	**Muscle**	**Fat**	**Lung**	**Skin**
6 hours	10.615	12.282	1.085	0.407	4.705	1.036
14 days	1.584	0.576	NDR	NDR	0.307	0.214
28 days	0.318	0.150	NDR	NDR	NDR	NDR

   * Each tissue residue value represents the mean of three animals.

NDR = No detectable residues (less than 0.2 ppm muscle or 0.1 ppm fat)

vii. Conclusions: The pattern of residues in this study are consistent with the results from the other tissue residue studies cited above. Liver and kidney had the highest concentrations of radioactivity and liver was the tissue with the most slowly depleting residues. Muscle and fat residues declined to nondetectable concentrations (limit of quantitation for the assay was 0.2 ppm for muscle and 0.1 ppm for fat) by 14 days withdrawal.

HPLC assays indicated that most of the radioactivity in the primary edible tissues at zero-hour withdrawal was parent tilmicosin. The proportion of liver and kidney radioactivity which was parent tilmicosin declined over the 28-day withdrawal time. By 28 days withdrawal, the concentrations of tilmicosin in liver and kidney were 44 to 47 percent of the radioactivity concentrations.

D. Comparative Metabolism

Study ABC-0395 (described below) which was conducted for the NADA for MICOTIL® 300, is referenced in the application for PULMOTIL® 90 because it demonstrates the similarity of the metabolism of tilmicosin by cattle, swine, and rats. Therefore, rats exposed to tilmicosin during toxicology studies were auto-exposed to the same metabolites that are present in the edible tissues of cattle and swine:

Comparative Metabolism of (14)C-Tilmicosin in Cattle and Rats. Study ABC-0395.

Investigator:

A.L. Donoho, Ph.D.
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

Extraction and fractionation of radioactive residues from livers of (14)C-tilmicosin-treated cattle demonstrated that there were three primary components of the liver residue. These components were parent tilmicosin, Metabolite T-1, and Metabolite T-2. All three of these components were found in excreta or liver from rats which were fed the same lot of (14)C-tilmicosin as was used for dosing the cattle.

Metabolite T-1 was isolated from cattle feces and purified for analysis. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) analysis indicated that T-1 was N-desmethyl tilmicosin. It had a molecular weight of 854, with a composition of C45H78N2O13. This corresponds to tilmicosin minus CH2, and this loss appears to be on the mycaminose sugar of tilmicosin.

Metabolite T-2 was found by comparative HPLC to be identical with an impurity in the tilmicosin technical material. This impurity had a molecular weight of 1609 and consisted of components of two macrolide rings and one piperidine ring. This compound is not a likely metabolite of tilmicosin. Therefore, the residue in liver of treated cattle is probably due to injection of this impurity along with parent tilmicosin

rather than to metabolism of tilmicosin. This conclusion is further supported by the observation that rats which were dosed orally and calves which were injected with highly purified (14)C-tilmicosin had little or none of this compound in their livers.

E. Target Tissue, Marker Residue, and Tolerance (Rm)

The data from Study T5C619301 described in Section VI.G.1. (400 ppm unlabeled tilmicosin in feed for 21 days) suggested similar tilmicosin residue depletion patterns in weanling and finishing swine populations.

Based upon data from all of the total residue and metabolism studies described in Section VI.C., liver and the parent tilmicosin were selected as the target tissue and the marker residue, respectively, in swine dosed with tilmicosin in feed. The study described in VI.C.1. indicated that steady state tissue levels would be reached after approximately 4 days of dosing at 165 ppm tilmicosin in feed. The time to steady state and steady state residue level were not established for the 400 ppm or 600 ppm doses. Thus, the 400-ppm- and 600-ppm-dose studies did not support zero withdrawal for the 400 ppm tilmicosin dose in swine feed. However, taken together, these total residue data were considered adequate to establish a tolerance for tilmicosin in swine liver.

In the 600-ppm study, the ratio of parent tilmicosin to total residues was greater than 0.90. In the 165 ppm study, the parent to total residue ratio was approximately 0.66. In the 400-ppm-dose study, the parent to total ratio was approximately 0.50. The data from the 400-ppm-dose study were used to establish the tolerance because: a) the maximum proposed dose is 400 ppm tilmicosin; b) the regulatory HPLC method for parent tilmicosin was used in the 400-ppm-dose study, so these data are more accurate than the data obtained in the 165- and 600-ppm-dose studies; and c) the parent tilmicosin to total residue ratio of 0.5 observed in the 400-ppm-dose study yields the most conservative tolerance. Using the ratio of marker residue to total residues of 0.5, the tolerance for the parent tilmicosin in swine liver is calculated as:

Tolerance

= SC(Target Tissue) X Marker Residue / Total Residues

= 15 ppm X 0.5 = 7.5 ppm

F. Regulatory Methods for Tilmicosin Residues

1. Tilmicosin Determinative Assay Procedure

   The determinative assay for measuring tilmicosin residues in treated swine 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 swine liver is homogenized with methanol (MeOH) and centrifuged to separate the solids. The methanol extract is diluted with sodium chloride solution, and partitioned with hexane. The aqueous methanol phase is made basic with sodium carbonate solution, and tilmicosin is extracted into

chloroform ($CHCl_3$):hexane. The extract is evaporated to dryness, and the residue is reconstituted for analysis by HPLC, using a reversed-phase phenylsilyl stationary column, with UV detection at 280 nm. The main difference between this method and the original method for tilmicosin in cattle liver is that hexane is substituted for carbon tetrachloride in the initial partitioning step. Overall, this swine liver method is not significantly different from the determinative method (approved under NADA 140-929 for MICOTIL® 300) measuring tilmicosin level in the liver of treated cattle.

The limit of quantification of the method is 0.1 ppm.

2. Tilmicosin Confirmatory Assay Procedure

Ground swine liver is homogenized with methanol and centrifuged to separate the solids. The methanol extract is diluted with a sodium chloride solution and partitioned with hexane. The aqueous methanol phase is made basic with sodium carbonate solution, and tilmicosin is extracted into chloroform:hexane. The extract is evaporated to dryness and dissolved in chloroform. The chloroform extract is then applied to a silica gel cartridge, and the cartridge is washed with chloroform/methanol. Tilmicosin is eluted from the column with chloroform:methanol:concentrated ammonium hydroxide, and the solution is evaporated to dryness. Following reconstitution of the residue in methanol, the sample is subjected to analysis by reversed-phase high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (HPLC/APCI-MS). The mass spectrometer operating conditions are set up to monitor three structurally indicating fragment ions unique to tilmicosin.

3. Method Validation

A method trial of the determinative and confirmatory assays for cattle liver was satisfactorily completed by FDA and USDA laboratories (approved NADA for MICOTIL® 300, NADA 140-929). The sponsor has satisfactorily demonstrated that hexane may be substituted for carbon tetrachloride for partitioning and the methods are valid for swine liver analysis. Therefore, laboratory testing was not required for the determinative procedure used in the quantitation of tilmicosin in swine liver. The confirmatory method for tilmicosin residue in swine liver has been satisfactorily evaluated by the FDA laboratory.

4. Method Location

The validated regulatory analytical methods for detection and confirmation of residues of tilmicosin in swine liver are on display in the Docket's Management Branch (HFA-305), Parklawn Building (Room 1-23), 12420 Parklawn Drive, Rockville, Maryland 20857. They are attached to this FOI Summary.

G. Marker Residue Depletion and Withdrawal Time

The liver is the target tissue for regulation of residues. Therefore, the raw data for the liver obtained from the Study T5C619301 (pivotal unlabeled tilmicosin decline

study described below in Section VI.G.1.) were analyzed to determine the withdrawal period for the treatment of swine with PULMOTIL® 90 Type A Medicated Article. FDA's statistical tolerance method (95% confidence interval on the 99th percentile), which is programmed according to the FDA *Guideline for Establishing a Withdrawal Period*, was employed in the calculation of the withdrawal time. The time required to reach the tolerance of 7.5 ppm tilmicosin level in treated swine liver was calculated to be 6 days. However, a 7-day withdrawal period has been assigned for this product so that kidneys from treated swine would be negative in USDA's Swab Test On Premises (STOP) at slaughter (see the Section VI.G.2. for a description of the STOP results).

1. Tilmicosin Tissue Residue Decline Study in Swine. Study T5C619301:

   Investigators:

   R.S. Readnour, Ph.D. and J.M. Darby, B.S.
   Lilly Research Laboratories
   Division of Eli Lilly and Company
   Greenfield, Indiana 46140

   The purpose of this study was to determine the concentration of tilmicosin residues in tissues of swine at various withdrawal times after dosing with a ration containing 400 ppm of tilmicosin phosphate. Thirty crossbred swine with a mean initial weight of 85.6 kg were fed ad libitum the medicated feed for 21 days. Twelve pigs, six castrated males and six females, were slaughtered 6 hours (practical "zero-hour" withdrawal) after withdrawal of the medicated feed. Six pigs, three castrated males and three females, were slaughtered at 7, 14, and 21 days after withdrawal of the medicated feed. Tissues were collected and assayed by high performance liquid chromatography (HPLC) using a method that had a limit of detection of 0.02 ppm. Results from the tissue residue analysis are summarized in Table 6.4.

   Table 6.4. Mean residues of tilmicosin (ppm) in tissues of swinetreated with 400 ppm tilmicosin in feed for 21 days

Withdrawal Time (days)	**n**	**Mean ppm tilmicosin concentration**				
		Liver	**Kidney**	**Muscle**	**Fat**	**Skin**
0	12	4.16	4.14	0.32	0.09	0.08
7	6	0.71	0.34	(2)0.03	<0.02	0.12
14	6	0.19	0.08	<0.02	<0.02	(2)0.04
21	6	0.06	0.06	*	*	0.04

   * Tissues were not assayed since 14 day results were <0.02 ppm.

2. Residue Screening Method:

   STOP Results in Swine Fed Tilmicosin at 200 or 400 ppm for 7 Consecutive Days. Pilot Study T5CAX9305

   Investigators:

   T.D. Thomson, V.M.D., Ph.D. and J.M. Darby, B.S.

Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

This study was conducted to assess the ability of USDA's Swab Test On Premises* (STOP) residue

* "Performing the Swab Test On Premises (STOP): For Detection of Antibiotic Residues in Livestock Kidney Tissue". United States Department of Agriculture, Food Safety and Inspection Service. Revised August, 1991.

screening method to detect residual tilmicosin antibacterial activity in kidneys from swine fed rations containing 200 or 400 ppm tilmicosin phosphate following various withdrawal periods. Thirty-six clinically normal, cross bred, castrated male pigs, weighing approximately 42 kg, were fed grower rations containing 0, 200, or 400 ppm tilmicosin phosphate *ad libitum* for 7 consecutive days. Feed assays and feed consumption data indicated the study animals received a mean (±SD) tilmicosin phosphate dose of 10.20 (1.34) and 21.10 (0.95) mg/kg/day for the two treatment levels, respectively. Standard STOP analyses were performed on fresh kidney tissue from 2 pigs of each treatment group at withdrawal periods of 0, 2, 4, 7, 10, and 14 days. For each animal, five STOP tests were performed on each kidney, for a total of 10 replicates per animal. The STOP results were negative (i.e., all 10 replicates per animal were negative for both animals) by withdrawal days 4 and 7 for the 200 and 400 ppm treatment levels, respectively, i.e., the STOP will detect kidney residues of tilmicosin in pigs fed 400 ppm tilmicosin phosphate and slaughtered less than 7 days of drug withdrawal.

V. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that PULMOTIL® 90 Type A medicated article is effective for the control of swine respiratory disease associated with *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* when administered in feed for 21 days at levels of 200 to 400 ppm.

Based on a battery of toxicology tests, the safe concentrations for total tilmicosin-related residues are 5 ppm in muscle, 15 ppm in liver, 30 ppm in kidney, and 30 ppm in fat. Based on metabolism studies in swine, a tolerance (Rm) of 7.5 ppm for the marker residue, parent tilmicosin, has been established in liver. The tolerance (Rm) refers to the residue measured by the regulatory method described herein.

A pre-slaughter withdrawal period of 6 days was calculated from a residue depletion study of tilmicosin residues in swine, following the administration of PULMOTIL® 90 Type A medicated article at a level of 400 ppm for 21 consecutive days. The Agency agreed with the sponsor that a 7-day withdrawal is appropriate in order to avoid false positive tests when using the USDA STOP (Swab Test On Premises) residue screen in slaughter facilities.

Tilmicosin is a Category II drug as defined in 21 CFR 558.3(b)(1)(ii). As provided in 21 U.S.C. 360b(m), as amended by the Animal Drug Availability Act of 1996 (ADAA), a feed mill license is required for making a Type B or C medicated feed containing tilmicosin phosphate.

Tilmicosin phosphate is the first Veterinary Feed Directive (VFD) drug to be approved since the enactment of the ADAA. Animal feed bearing or containing this VFD drug shall be fed to animals only by or upon a lawful veterinary feed directive issued by a licensed veterinarian in the course of the veterinarian's professional practice. In addition, veterinary feed directives issued for this drug are not refillable. These decisions were based on the following factors: (a) the product contains a new antimicrobial entity intended only for therapeutic purposes, (b) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product to treat swine respiratory disease, and (c) the frequency of violative tissue residues and rate of emergence of resistant organisms will be reduced by the involvement of veterinarians in product use.

PULMOTIL® 90 does not fall within the scope of 21 CFR 558.15 based on the following factors: (a) the product is available by veterinary feed directive only, (b) the claim is for control of disease, (c) the claim is supported by data demonstrating that the product is effective, (d) the product is intended for use in a limited portion of the indicated species or production class, and (e) there is a rational and demonstrated benefit associated with administering the product for more than 14 days.

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 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 (HFA-305), Parklawn Building (Room 1-23), 12420 Parklawn Dr., Rockville, Maryland 20857.

Under Section 512(c)(2)(F)(iii) of the Act, this approval for food-producing animals qualifies for THREE (3) years of marketing exclusivity beginning on the date of approval because the application contains substantial evidence of the effectiveness of the drug involved, any studies of animal safety, or in the case of food producing animals, human food safety studies (other than bioequivalence or residue studies) required for the approval of the application and conducted or sponsored by the applicant.

PULMOTIL® 90 is under U.S. patent number 4,820,695 which expires April 11, 2006.

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.