Date of Approval: March 14, 2008

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-068

BAYTRIL 100

Enrofloxacin
Injectable Solution
Swine

For the treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*, and *Streptococcus suis*.

Sponsored by:

Bayer HealthCare LLC
Animal Health Division

TABLE OF CONTENTS

I. GENERAL INFORMATION:

A. File Number:	NADA 141-068
B. Sponsor:	Bayer HealthCare LLC Animal Health Division P.O. Box 390 Shawnee Mission, KS 66201 Drug Labeler Code: 000859
C. Proprietary Name(s):	BAYTRIL 100
D. Established Name(s):	Enrofloxacin
E. Pharmacological Category:	Antimicrobial
F. Dosage Form(s):	Injectable solution
G. Amount of Active Ingredient(s):	100 mg/mL
H. How Supplied:	100 and 250 mL bottles
I. How Dispensed:	Rx
J. Dosage(s):	Cattle: Single-Dose Therapy: 7.5 - 12.5 mg/kg body weight (3.4 - 5.7 mL/100 lb). Multiple-Day Therapy: 2.5 - 5.0 mg/kg body weight (1.1 - 2.3 mL/100 lb). Treatment should be repeated at 24-hour intervals for three days. Additional treatments may be given on Days 4 and 5 to animals that have shown clinical improvement but not total recovery. Swine: 7.5 mg/kg body weight (3.4 mL/100 lb) once.
K. Route(s) of Administration:	Subcutaneous injection, behind the ear
L. Species/Class(es):	Cattle (beef and non-lactating dairy) and swine

M. Indication(s):

Cattle:
For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida*, and *Histophilus somni* (previously *Haemophilus somnus*) in beef and non-lactating dairy cattle.

Swine:
For the treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis,* and *Streptococcus suis*.

N. Effect(s) of Supplement:

This supplement provides a new indication, for the treatment and control of swine respiratory disease, in swine.

II. EFFECTIVENESS:

A. Dosage Characterization:

Two dose determination studies were conducted in 1994 to identify the most suitable dose of enrofloxacin injectable solution for the treatment of swine respiratory disease (SRD). SRD was induced in healthy pigs by commingling them with "seeder" pigs that had been inoculated intranasally with a known virulent strain of *Actinobacillus pleuropneumoniae*. A total of 120 pigs that exhibited clinical signs of respiratory disease (rectal temperatures ≥ 104 ºF; increased respiratory rate, labored or dyspneic breathing; depressed attitude; and decreased appetite) were enrolled in the studies. Enrofloxacin was administered at 0, 2.5, 5.0, 7.5, or 10.0 mg/kg body weight (BW) as a single subcutaneous (SC) injection on the day of enrollment (Day 1).

Pigs were evaluated for treatment success (normal temperature and respiration scores, with normal attitude and/or normal appetite scores) on Day 5 or Day 6 and for continued treatment success on Day 15. Mortality, pneumonic lung scores, and average weight gain were also evaluated. In both studies, enrofloxacin, administered at all dose levels, resulted in higher treatment success rates, fewer mortalities, lower average pneumonic lung scores, and higher average weight gains compared to the untreated group. Based on the results of these studies, 7.5 mg/kg BW was selected as the enrofloxacin dose for the pivotal SRD field studies.

B. Substantial Evidence:

Two field studies were conducted to evaluate the effectiveness of 7.5 mg enrofloxacin per kg BW, given once as a SC injection, for the treatment and control of SRD. Both studies were conducted using enrofloxacin 10% injectable formula BAY Vp 2674, which is the formula marketed as BAYTRIL 100.

1. Natural Infection Field Effectiveness Study

a. Title: "Clinical Safety and Effectiveness of Enrofloxacin (BAY Vp 2674) Administered as a Single Injection, at 7.5 mg/kg BW, for the Treatment and Control of Naturally Occurring Bacterial Respiratory Disease in Pigs." Study Number 150.058 (Report No. 74846). May 1996 to June 1996.

b. Investigators: Kent J. Schwartz, DVM, MS, and Jeff Meister, TEAM Associates, Story City, IA.

c. Study Design:

1) Objective: To confirm the effectiveness of enrofloxacin 10% injectable solution administered once at a dosage of 7.5 mg/kg BW SC for the treatment and control of naturally-occurring SRD.

2) Test Animals: A total of 280 female and castrated male, 10-week old, healthy, white composite (Cotswald) pigs, originating from a Minnesota farm with a history of pleuropneumonia were included in the study. Pigs were randomly assigned to one of two treatment groups - enrofloxacin (10 pens, 137 pigs) or saline (10 pens, 138 pigs). Five additional (sentinel) pigs were necropsied at the beginning of the study to confirm an SRD infection.

3) Test Article Administration: The test article was a sterile injectable aqueous solution containing 100 mg enrofloxacin per milliliter. Enrofloxacin was administered once at a dosage of 7.5 mg/kg BW by SC injection behind the ear on the day of enrollment (Day 0). Pigs in the control group received a sterile saline injection at a dose volume equivalent to the enrofloxacin dosage.

4) Measurements and Observations: Pigs were examined twice per day for signs of respiratory disease. When a respiratory disease outbreak appeared imminent, respiratory and attitude scores were recorded and rectal temperatures were taken. A pig with a rectal temperature of $\geq$ 104.0 ºF, increased respiratory rate, labored or dyspneic breathing, and depressed attitude was considered sick and febrile. When at least three pigs in a pen were sick and febrile, the pen was enrolled in the study.

Pens containing three or more sick and febrile pigs on Day 4 were considered failures, removed from the study, and were not further evaluated. Attitude scores, respiratory scores, and mortality were recorded daily for pigs in the remaining pens through Day 14. Rectal temperature was measured at enrollment, on Day 4, and any time a relapse was suspected. Body weight was recorded on Days 0 and 14.

Primary decision variables for evaluating control of SRD were morbidity, mortality, and rectal temperature (Day 4). Morbidity rates were

determined by dividing the number of sick and febrile pigs by the total number of pigs within a pen.

The primary variable for evaluating treatment of SRD was treatment success for the pigs that were classified as sick and febrile on Day 0. A pig was considered a treatment success if it had a rectal temperature of < 104.0 ºF, normal respiratory character, and no or mild depression on Day 4. Individual parameters (attitude score, respiratory score, and rectal temperature) were analyzed as secondary variables. Attitude was scored using a criteria-based scale of 1 to 3, where a score of 1 was normal. Respiratory character was scored using a criteria-based scale of 1 to 4, where a score of 1 was normal.

d. Statistical Analysis: The study was designed as a randomized complete block with the pen as the experimental unit.

For the control indication, data were analyzed by analysis of variance and repeated measures analysis of variance using a mixed model. A 0.05 level of significance was used for all tests. Morbidity rates were transformed using the arcsine formula. Mortality was evaluated for untransformed, log-transformed, rank-transformed, and arcsine-transformed pen mortality rates. Nonparametric analysis was also used.

For the treatment indication, the binomial proportion of sick and febrile pigs (as defined on Day 0) classified as successes on Day 4 was transformed prior to the analyses using the arcsine square root transformation. An analysis of variance assuming a completely randomized design was used to test for treatment group differences at the 0.05 level of significance, with classification variables of treatment group and pen. Rectal temperatures were analyzed including Day 0 rectal temperature as a covariate. Median Day 4 pen attitude and respiration scores were analyzed using Wilcoxon's Rank Sum test.

e. Results:

1) Control of SRD: A total of 275 pigs were included in the analyses. The results are shown in Table 2.1.

Table 2.1. Day 4 morbidity, mortality, and rectal temperature results (Study Number 150.058).

	Enrofloxacin	Saline	P-value
Morbidity	7.4% (10/137)	51.7% (71/138)	< 0.0001
Mortality	0% (0/137)	6.5% (9/138)	0.0325
Rectal Temperature	104.1 ºF	105.2 ºF	< 0.0001

2) *Treatment of SRD:* A total of 98 pigs classified as sick and febrile on Day 0 were included in the analyses. The results are shown in Table 2.2.

Table 2.2. Day 4 treatment success and individual parameter results analyzing only pigs classified as sick and febrile on Day 0 (Study Number 150.058).

	Enrofloxacin	**Saline**	**P-value**
Treatment Success	33.0% (16/49)	0% (0/49)	0.0001
Median Attitude Score	1	2	< 0.01
Median Respiratory Score	1	2	< 0.01
Rectal Temperature	104.2 ºF	105.2 ºF	< 0.01

3) *Postmortem Findings:* Necropsy and laboratory results revealed macroscopic lesions including hemorrhagic consolidation, congestion, and pleuritis. *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*, *Bordetella bronchiseptica*[1], and *Streptococcus suis* were cultured from the lungs of sentinel pigs and saline-treated pigs that died or were euthanized during the study.

f. Adverse Reactions: No adverse reactions resulting from enrofloxacin administration were observed during this study.

g. Conclusion: The results of this study indicate that enrofloxacin 10% injectable solution is effective for the treatment and control of naturally-occurring SRD associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*, and *Streptococcus suis*, when administered to pigs as a single SC dosage of 7.5 mg/kg BW.

2. Natural Infection Field Effectiveness Study

a. Title: "Clinical Safety and Effectiveness of Enrofloxacin (BAY Vp 2674) Administered as a Single Injection, at 7.5 mg/kg BW, for the Treatment and Control of Naturally Occurring Bacterial Respiratory Disease in Pigs." Study Number 150.059 (Report No. 74839). April 1996.

b. Investigators: Kelly F. Lechtenberg, DVM, PhD, and Mark Sunderman, Midwest Veterinary Services, Inc., Oakland, NE.

c. Study Design:

1) *Objective:* To confirm the effectiveness of enrofloxacin 10% injectable

[1]*Bordetella bronchiseptica* was isolated in this study, but not in sufficient numbers to support the product indication.

solution administered once at a dosage of 7.5 mg/kg BW SC for the treatment and control of naturally-occurring SRD.

2) *Test Animals:* A total of 320 female and castrated male, three-month old, healthy, crossbred pigs, originating from a Nebraska farm with a history of pleuropneumonia were included in the study. Pigs were randomly assigned to one of two treatment groups - enrofloxacin (10 pens, 157 pigs) or saline (10 pens, 158 pigs). Five additional (sentinel) pigs were necropsied at the beginning of the study to confirm an SRD infection.

3) *Test Article Administration:* The test article was a sterile injectable aqueous solution containing 100 mg enrofloxacin per milliliter. Enrofloxacin was administered once at a dosage of 7.5 mg/kg BW by SC injection behind the ear on the day of enrollment (Day 0). Pigs in the control group received a sterile saline injection at a dose volume equivalent to the enrofloxacin dosage.

4) *Measurements and Observations:* Pigs were examined twice per day for signs of respiratory disease. When a respiratory disease outbreak appeared imminent, respiratory and attitude scores were recorded and rectal temperatures were taken. A pig with a rectal temperature of $\geq$ 104.0 ºF, increased respiratory rate, labored or dyspneic breathing, and depressed attitude was considered sick and febrile. When at least three pigs in a pen were sick and febrile, the pen was enrolled in the study.

   Pens containing three or more sick and febrile pigs on Day 4 were considered failures, removed from the study, and were not further evaluated. Attitude scores, respiratory scores, and mortality were recorded for pigs in the remaining pens daily through Day 14. Rectal temperature was measured at enrollment, on Day 4, and any time a relapse was suspected. Body weight was recorded on Days 0 and 14.

   Primary decision variables for evaluating control of SRD were morbidity, mortality, and rectal temperature (Day 4). Morbidity rates were determined by dividing the number of sick and febrile pigs by the total number of pigs within a pen.

   The primary variable for evaluating treatment of SRD was treatment success for the pigs that were classified as sick and febrile on Day 0. A pig was considered a treatment success if it had a rectal temperature of < 104.0 ºF, normal respiratory character, and no or mild depression on Day 4. Individual parameters (attitude score, respiratory score, and rectal temperature) were analyzed as secondary variables. Attitude was scored using a criteria-based scale of 1 to 3, where a score of 1 was normal. Respiratory character was scored using a criteria-based scale of 1 to 4, where a score of 1 was normal.

d. Statistical Analysis: The study was designed as a randomized complete block

with the pen as the experimental unit.

For the control indication, data were analyzed by analysis of variance and repeated measures analysis of variance using a mixed model. A 0.05 level of significance was used for all tests. Morbidity rates were transformed using the arcsine formula. Mortality was evaluated for untransformed, log-transformed, rank-transformed, and arcsine-transformed pen mortality rates. Nonparametric analysis was also used.

For the treatment indication, the binomial proportion of sick and febrile pigs (as defined on Day 0) classified as successes on Day 4 was transformed prior to the analyses using the arcsine square root transformation. An analysis of variance assuming a completely randomized design was used to test for treatment group differences at the 0.05 level of significance, with classification variables of treatment group and pen. Rectal temperatures were analyzed including Day 0 rectal temperature as a covariate. Median Day 4 pen attitude and respiration scores were analyzed using Wilcoxon's Rank Sum test.

e. Results:

1) Control of SRD: A total of 315 pigs were included in the analyses. The results are shown in Table 2.3.

Table 2.3. Day 4 morbidity, mortality, and rectal temperature results (Study Number 150.059).

	Enrofloxacin	**Saline**	**P-value**
Morbidity	5% (8/157)	61.4% (97/158)	< 0.0001
Mortality	0% (0/157)	1.9% (3/158)	0.2105
Rectal Temperature	103.1 ºF	104.0 ºF	< 0.0001

2) *Treatment of SRD:* A total of 75 pigs classified as sick and febrile on Day 0 were included in the analyses. The results are shown in Table 2.4.

Table 2.4. Day 4 treatment success and individual parameter results analyzing only pigs classified as sick and febrile on Day 0 (Study Number 150.059).

	Enrofloxacin	**Saline**	**P-value**
Treatment Success	89.7% (35/39)	10.3% (3/36)	< 0.0001
Median Attitude Score	1	2	< 0.01
Median Respiratory Score	1	2.5	< 0.01
Rectal Temperature	103.2 ºF	104.1 ºF	< 0.01

3) *Postmortem Findings:* Necropsy and laboratory results revealed macroscopic lesions including hemorrhagic consolidation, congestion, and pleuritis. *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, and *Haemophilus parasuis* were cultured from the lungs of sentinel pigs and saline-treated pigs that died or were euthanized during the study.

f. Adverse Reactions: No adverse reactions resulting from enrofloxacin administration were observed during this study.

g. Conclusion: The results of this study indicate that enrofloxacin 10% injectable solution is effective for the treatment and control of naturally-occurring SRD associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, and *Haemophilus parasuis* when administered to pigs as a single SC dosage of 7.5 mg/kg BW.

III. TARGET ANIMAL SAFETY:

A. Drug Tolerance Study (Acute Toxicity)

1. Title: "Drug Tolerance Test for the Subcutaneous Use of an Enrofloxacin 10% Injectable Solution in Swine." Study Report Number 74548. April 1994.

2. Study Director: M. L. Kohlenberg, BS, Bayer Corporation, Animal Health, Shawnee Mission, KS

3. Study Design:

a. *Objective:* To evaluate the safety of enrofloxacin 10% injectable solution in swine following SC administration of 50 mg/kg BW (6.67X the labeled dose), once daily for five consecutive days (5X the labeled duration). This study was conducted in accordance with Good Laboratory Practice (GLP) regulations.

b. *Test Animals:* Three Yorkshire x Hampshire pigs (two female and one castrated male) weighing 42 to 52 lbs (19 to 24 kg) at the time of initial treatment were used in the study. One female pig served as a control pig.

c. *Test Article Administration:* The test article was a sterile injectable aqueous solution containing 100 mg enrofloxacin per milliliter (the same formulation marketed as BAYTRIL 100). Enrofloxacin was administered at a dosage of 50 mg/kg BW by SC injection into the lateral neck at two adjacent sites for five consecutive days, beginning on Day 0. The control pig received SC sterile physiologic saline injections at a dose volume equivalent to the enrofloxacin dose once a day for five consecutive days. Treatments were administered on alternating sides of the neck each day. The maximum volume administered per SC site was 5.9 mL (enrofloxacin) or 5 mL (saline).

d. *Measurements and Observations:* Pre-treatment blood samples were taken for clinical chemistry and hematology analysis on Days -6 and -4. On Day 0, pigs were weighed, examined, assigned to groups, and treatment was initiated. Clinical observations were conducted twice a day during the treatment phase of the study, and once a day for the remainder of the study. On Day 7 (3 days following administration of the final treatment), blood samples for clinical chemistry and hematology were drawn, and pigs were weighed, euthanized, and necropsied. Tissue samples were obtained for histopathology evaluation. Variables evaluated included abnormal clinical signs of toxicity, body weight, clinical chemistry, hematology, gross pathology, and histopathology.

4. Statistical Analysis: none

5. Results:

a. *Mortality:* No deaths occurred during the study.

b. *Clinical Observations:* No clinical signs of toxicity were observed. Diarrhea was noted in one treated animal on the afternoon of Day 1, which resolved by Day 2. Swelling of the injection site (that resolved by Day 5) was noted in the treated pigs.

c. *Body Weight:* Average daily body weight gain was similar for the treated and control pigs.

d. *Hematology/Clinical Chemistry:* Pre-treatment and post-treatment hematology and clinical chemistry variables were similar for the treated and control pigs. Both treated and control animals pigs had elevated creatinine phosphokinase and lactate dehydrogenase in the post-treatment sample, compared to the pre-treatment sample.

e. *Gross Pathology/Histopathology:* No test article-related lesions were observed at necropsy and there were no histopathologic lesions in any tissues examined, including articular cartilages.

6. Conclusion: Administration of enrofloxacin 10% solution at 50 mg/kg BW (6.67X the labeled dose), SC once daily for five consecutive days (5X the labeled duration) did not result in drug-induced clinical signs, gross pathologic abnormalities, or histopathologic lesions.

B. Margin of Safety Study

1. Title: "General Safety Study for the Subcutaneous Use of an Enrofloxacin 10% Injectable Formulation in Swine." Study Report Number 74575. August 1994 to October 1994.

2. Study Director: James A. Shmidl, DVM, MS, Bayer Corporation, Animal Health, Shawnee Mission, KS

3. Study Design:

   a. *Objective:* To determine the safety margin of enrofloxacin 10% injectable solution when administered to swine subcutaneously at 5, 15, and 25 mg/kg BW per day (0.67X, 2X, and 3.34X the labeled dose) for 15 consecutive days (15X the labeled duration). This study was conducted in accordance with GLP regulations.

   b. *Test Animals:* Healthy female and castrated male, crossbred (Large White X Landrace X Duroc X Hampshire) pigs, weighing 94 to 155 lbs (42.7 to 70.4 kg) at the time of the initial treatment. A total of 32 pigs (16 barrows and 16 gilts) were used for the study. There were eight pigs (four barrows and four gilts) per treatment group (0, 5, 15, or 25 mg enrofloxacin/kg BW).

   c. *Test Article Administration:* The test article was a sterile injectable aqueous solution containing 100 mg enrofloxacin per milliliter (the same formulation marketed as BAYTRIL 100). Enrofloxacin was administered at a dosage of 5, 15, or 25 mg/kg BW per day for 15 consecutive days by SC injection over the neck, shoulder, and rib regions. Control pigs received SC physiological saline injections at a dose volume equivalent to 25 mg enrofloxacin per kg BW. When necessary, the dose was divided between multiple sites to administer a maximum volume of 5 mL per injection site.

   d. *Measurements and Observations:* Pre-treatment blood samples were taken for clinical chemistry and hematology analysis on Day -4. On Day -1, pigs were weighed, examined, and assigned to groups. Treatment was initiated on Day 0. Clinical signs (appearance, depression, muscle tremors, abnormal stool, abnormal stance/gait, and any other abnormal observations) were observed and scored twice daily during the treatment period and once daily afterward. Clinical chemistry and hematology samples were taken on Days 5 and 17. Body weights were recorded on Days 6 and 17, and feed intake was recorded during the entire study. Two pigs from each group were necropsied on Days 18, 20, 31, and 32. Variables evaluated included clinical observations, body weight, feed intake, hematology/clinical chemistry, gross pathology, and histopathology.

4. Statistical Analysis: Clinical chemistry values were evaluated by making comparisons of means between the control and treated groups using T-tests. Means were compared at each day. Comparisons of occurrences of abnormal histological readings and lameness were made using Fisher's Exact Tests. A 0.10 level of significance was used for all tests.

5. Results:

   a. *Mortality:* No deaths occurred during the study.

   b. *Clinical Observations/Lameness:* A dose-related increase in the incidence and severity of depression, lameness, and stiffness occurred in the pigs receiving the 2X and 3.34X treatments during the second week of treatment. Four animals in the 2X group and all eight animals in the 3.34X group were lame or stiff in one or more limbs. Clinical signs improved after treatment was stopped and most lame animals were clinically normal at necropsy. One animal in the 2X group was lame at necropsy and two animals from the 3.34X group were lame at the time of necropsy. The proportions of animals which were clinically lame during or immediately after the treatment period are presented in Table 3.1 below.

   c. *Body Weight:* Pigs in the 0.67X group showed body weight gains slightly greater than the non-treated controls. Pigs in the 2X group achieved a slightly lower mean body weight gain than the non-treated controls, due to weight loss of one animal in the 2X group during the final weigh period. A clinically significant (1 lb/day) decrease in average daily body weight gain was recorded in pigs in the 3.34X group compared to the non-treated controls, probably due to stiffness seen in this group. Feed intake generally correlated with body weight gain.

   d. *Hematology/Clinical Chemistry:* No test article-related abnormalities were observed in pigs in the 0.67X or 2X groups. Some abnormalities, non-clinically relevant and unrelated to the drug administration, were seen in the 3.34X group.

   e. *Gross Pathology/Histopathology*: Detailed gross observation and histological evaluation of the articular cartilage revealed an incidence of lesions in all groups, including the control pigs. Pathologic lesions consisted of histological formation of chondrones, described as an abnormal clustering of chondrocytes. This lesion is also described with osteochondrosis and has been associated with fluoroquinolone treatments in other species. There was no correlation between gross and histological articular cartilage abnormalities and either dose level or clinical lameness. Histological differences between control and treated groups were not statistically significant. The proportion of animals with articular cartilage damage is presented in Table 3.1.

      Table 3.1. Incidence of clinical lameness and articular cartilage lesions in pigs treated with enrofloxacin injectable solution for 15 consecutive days.

Enrofloxacin dose (mg/kg BW)	Proportion of clinically lame pigs	Proportion of pigs with cartilage abnormalities
0	2/8	2/8
5 (0.67X)	1/8	1/8
15 (2X)	4/8	5/8
25 (3.34X)	8/8	3/8

6. Conclusion: An adequate margin of safety is demonstrated for enrofloxacin 10% solution administered once by SC injection at 7.5 mg/kg BW. The articular lesions and lameness seen in pigs in this study demonstrate that BAYTRIL 100 has an adverse effect on joints and articular cartilage in rapidly growing swine when administered at doses of 2X and 3.34X the labeled dosage for 15X the labeled duration.

C. Injection Site Tolerance Study

1. Title: "Local Tolerance for the Subcutaneous Use of an Enrofloxacin 10% Injectable Formulation in Swine." Study Report Number 74561. July 1994 to September 1994.

2. Study Director: James A. Shmidl, DVM, MS, Bayer Corporation, Animal Health, Shawnee Mission, KS

3. Study Design:

   a. *Objective:* To evaluate the local (injection site) tolerance of swine following SC administration of enrofloxacin 10% injectable formulation at a dose of 5 mg enrofloxacin per kg BW (0.67X the labeled dose) per day for five consecutive days (5X the labeled duration). This study was conducted in accordance with GLP regulations.

   b. *Test Animals:* Fifteen Yorkshire x Hampshire swine (six females, nine castrated males), weighing 42 to 51 lbs (19 to 23 kg) at the time of initial treatment, were used in the study. Pigs were ranked by weight and randomly assigned to five necropsy groups. There were three pigs per group. Treated pigs served as their own controls, via saline injections in the right-side neck.

   c. *Test Article Administration:* The test article was a sterile injectable aqueous solution containing 100 mg enrofloxacin per milliliter (same formulation marketed as BAYTRIL 100). Enrofloxacin was administered at a dosage of 5 mg/kg BW per day for five consecutive days by SC injection over the neck (left-side), axillar (fold behind elbow), and inguinal (flank fold) regions; a different site was used for each day's injection. Each pig also received SC injections of physiologic saline in the opposite side of the body, at a volume equivalent to the test article dose.

d. *Measurements and Observations:* The initial injection day was Day 0. Each injection site was observed, palpated, and scored for swelling prior to treatment on Day 0, and on Days 1, 2, 3, 4, 7, 14, 24, 44, and 64. Three pigs (per time point) were necropsied on Days 7, 14, 24, 44, and 64. Injection sites were examined grossly and microscopically at necropsy.

4. Statistical Analysis: none

5. Results: There were no mortalities. All saline injection sites were clinically normal throughout the study. Injection site reactivity to enrofloxacin was minimal (17 of 75 sites) with all swelling scores being slight or mild. No swelling was noted at the injection site after Day 14. Grossly visible lesions, described as subcutaneous discoloration and tissue alterations (pale areas with cystic or red stippled appearance or firmness) in the superficial musculature were observed in pigs necropsied between Days 7 and 24. Microscopically, there was no evidence of purulent reaction (abscess formation), and changes were more pronounced in muscle tissue than in adipose (fat) tissue. By Day 24, lesion samples only exhibited evidence of healing and slight scar formation.

6. Conclusion: Subcutaneous injection of BAYTRIL 100 may result in discoloration or firmness of the subcutaneous tissue and superficial musculature which persists beyond seven days. This may result in trim loss of edible tissue at slaughter.

IV. HUMAN FOOD SAFETY:

A. Toxicology:

CVM did not require toxicology studies for this supplemental approval. The FOI Summary for the supplemental approval of NADA 141-068 dated February 13, 2008, contains a summary of all toxicology studies.

B. Residue Chemistry:

1. Summary of Residue Chemistry Studies

a. Total Residue Depletion and Metabolism Study: 106548

1) Study Director: Abraham E. Mathew, PhD, Bayer Research Park, Stilwell, KS

2) Animals Used: Swine, Chester cross; 53 to 61 kg (118 to 135 lb) at the time of dosing

3) Test Substance: 10% enrofloxacin injectable formulation containing ^{14}C-enrofloxacin. The label was at the C-4 position of the quinolone ring, which is not susceptible to metabolism.

4) Treatment Regimen: Subcutaneous injections for five consecutive days at a dose rate of 5 mg enrofloxacin per kg BW per day.

5) Number of Animals Per Sacrifice Interval: 3 animals (2 barrows + 1 gilt or 2 gilts + 1 barrow)

6) Sacrifice Intervals: 0 (12 hours), 1, 2, and 5 days after the administration of the fifth (final) dose

7) Tissues Collected: Liver, kidney, muscle, fat, injection sites

8) Total Residue Depletion: Tissue samples from each of the animals at all the sampling intervals were analyzed to determine the [^{14}C] total residue depletion. The results are summarized in Table 4.1.

Table 4.1. Average total ^{14}C-residues (ppm ± standard deviation) in the edible tissues of swine treated with [^{14}C] enrofloxacin at 5 mg enrofloxacin per kg BW per day for five consecutive days by subcutaneous injection.

Tissue	**Sacrifice Interval; Days Post-Final Dose**			
	0[1]	**1**	**2**	**5**
Liver	3.422 (±0.909)	1.787 (±0.260)	1.005 (±0.558)	0.277 (±0.034)
Kidney	4.549 (±0.915)	2.080 (±0.300)	0.786 (±0.362)	0.141 (±0.039)
Muscle	1.925 (±0.413)	0.863 (±0.114)	0.199 (±0.069)	0.028 (±0.007)
Fat	0.381 (±0.067)	0.174 (±0.019)	0.048 (±0.004)	0.010 (±0.000)
Injection Site	184.9 (±59.6)	80.4 (±18.33)	64.3 (±36.98)	3.82 (±4.81)

[1]0-Day interval is actually 12 hours after the last injection.

9) Metabolism of [^{14}C] Enrofloxacin: Samples of the edible tissues of one barrow and one gilt from the Day 0 (12 hours after the administration of the fifth [final] dose) sacrifice interval were analyzed. The extraction schemes removed 94 to 100% of the total radioactive residue from the Day 0 liver, kidney, muscle, and fat tissues. The extracts were analyzed, and the parent compound was the major residue (79-99%) in all the tissues. Two metabolites, ciprofloxacin and desethylene enrofloxacin, were found in much smaller amounts. The results are summarized in Table 4.2.

Table 4.2. Amounts of enrofloxacin, ciprofloxacin, and desethylene enrofloxacin as percentages of the total radioactive residue (TRR) in liver, kidney, muscle, and fat approximately 12 hours after the last dose.

Compound	Tissue			
	Liver	**Kidney**	**Muscle**	**Fat**
Enrofloxacin	79-81	77-83	84-93	92-99
Ciprofloxacin	8-9	7-14	6-15	0-8
Desethylene enrofloxacin	4	4-7	nd[1]	nd

[1]nd = nondetectable

Each of the five injection sites from two of the Day 0 animals (one gilt and one barrow) were extracted and analyzed. The extraction scheme removed 77 to 99% of the total radioactive residue. The only component identified was enrofloxacin which represented 74 to 99% of the radioactive residue in the injection sites.

b. Comparative Metabolism Study in the Rat: 106547

The metabolism of enrofloxacin in Wistar Furth rats, treated via oral gavage doses (Bayer Report No. 106547), was previously described in the FOI Summary for BAYTRIL (enrofloxacin) 3.23% Concentrate Antimicrobial Solution, NADA 140-828, dated October 4, 1996. That information is being provided again in this FOI Summary because approval of NADA 140-828 was withdrawn by the Agency on August 1, 2005 (70FR 44105), for reasons not related to this study.

Wistar Furth rats (WF/NHsd: males and nulliparous females) were treated with five daily oral gavage doses of ^{14}C-enrofloxacin at a rate of 15 mg/kg BW/day. Urine samples were collected and assayed by HPLC. Enrofloxacin and the metabolites ciprofloxacin and desethylene enrofloxacin were observed in both the urine of rats orally dosed with ^{14}C-enrofloxacin (see Table 4.3) and the edible tissues from swine subcutaneously treated with ^{14}C-enrofloxacin. Therefore, the rats used in the toxicity tests were exposed to the same enrofloxacin metabolites as those observed in the edible tissues of swine.

Table 4.3. Percent of total radioactivity of enrofloxacin-related compounds in rat urine.

Compound	Percent of Total Radioactivity in Rat Urine
enrofloxacin	17
ciprofloxacin	31
oxociprofloxacin	5

enro-conjugate (enro amide)	23
dioxociprofloxacin	9
desethylene ciprofloxacin	3
desethylene enrofloxacin	2
N-formyl ciprofloxacin	<2
oxoenrofloxacin	<2
hydroxy oxoenrofloxacin	3

c. Study to Establish Withdrawal Period (Single-Dose Therapy at 7.5 mg/kg BW Administered Once): 74502, 74763, and 74823

1) Study Directors:
In-life Phase: J. A. Shmidl, DVM, MS, Bayer Corporation, Animal Health, Shawnee Mission, KS
Analytical Phase: Shailesh Vengurlekar, MS, MBA, ABC Laboratories, Inc., Columbia, MO

2) Animals Used: 21 pigs (barrows and gilts; commercial crossbreeds); 51 to 66 kg at dosing

3) Number of Animals per Sacrifice Interval: 5 animals (3 gilts and 2 barrows or 3 barrows and 2 gilts); one barrow was used as the non-treated control.

4) Sacrifice Interval Days: 1, 2, 3, and 4 days after administration of dose

5) Tissues Collected at Sacrifice: Liver, muscle, and injection sites

6) Results: The liver samples from the 7.5 mg/kg BW treated animals were analyzed using the determinative procedure (Section D below) to determine the depletion of enrofloxacin. The average enrofloxacin residue levels in swine liver are presented in Table 4.4.

Table 4.4. Enrofloxacin residues in swine liver.

Withdrawal Interval (Days)	**Enrofloxacin Average ppm (± Standard Deviation)**
1	2.47 (±0.703)
2	0.552 (±0.275)
3	0.224 (±0.162)
4	0.156 (±0.121)

The injection site tissues from each of the 7.5 mg/kg BW treated animals were analyzed for both enrofloxacin and ciprofloxacin. No ciprofloxacin residues were detected in the injection site tissues. The average enrofloxacin residue levels in the injection site tissues are presented in Table 4.5.

Table 4.5. Enrofloxacin residues in injection sites of swine.

Withdrawal Interval (Days)	Enrofloxacin Average ppm
1	223.0
2	40.3
3	1.99
4	1.47

The muscle samples from the 7.5 mg/kg BW treated animals were analyzed using the determinative procedure (Section D below) to determine the depletion of enrofloxacin. The average enrofloxacin residue levels in swine muscle are presented in Table 4.6.

Table 4.6. Enrofloxacin residues in swine muscle.

Withdrawal Interval (Days)	Enrofloxacin Average ppm
1	1.790
2	0.375
3	0.146
4	0.098

2. Target Tissue and Marker Residue Assignment

The amount of time required for the average total ^{14}C-residue levels in the edible tissues to deplete to the safe concentration was less than 5 days for all edible tissues. The edible tissue in which the total residue was slowest to deplete to the safe concentration was the injection site. As the injection site is not a practical target tissue, the liver was selected as the target tissue.

Analysis of the liver samples collected at intervals greater than 12 hours after the administration of the final enrofloxacin dose indicated that enrofloxacin comprised the largest portion of the extractable radioactivity. Enrofloxacin was observed in the 0-, 1-, 2-, and 5-day liver samples. Thus, enrofloxacin was selected as the marker substance in swine liver.

3. Tolerance Assignments

Using the determinative procedure developed for the extraction and determination of enrofloxacin residues in swine liver (Section D below), the enrofloxacin content in the liver samples of swine treated with ^{14}C-enrofloxacin was measured. These values were used for comparison to the enrofloxacin residues in injection site tissues. When the total residue in injection site tissues depleted to the safe concentration, the corresponding residue value for enrofloxacin in liver was approximately 0.5 ppm. Thus, the tolerance for enrofloxacin in swine liver is 0.5 ppm.

4. Withdrawal Time

Liver residue depletion data from the single dose studies were individually analyzed using CVM's statistical tolerance limit algorithm. Liver and injection site residue data following the subcutaneous administration of a single dose of 10% injectable solution, 7.5 mg/kg BW, support a 5-day withdrawal time. Therefore, a withdrawal period of 5 days is being established for the single use of the 10% enrofloxacin injectable solution in swine.

C. Microbial Food Safety:

A series of studies was conducted by the sponsor to assess microbial food safety relating to the administration of enrofloxacin (10% injectable solution) to pigs in a typical swine production facility on the development of resistance in a challenge strain of *Salmonella typhimurium*, and among resident *Escherichia coli*.

Bayer study 150.779 titled, "The Effect of Enrofloxacin Administration on Ciprofloxacin Minimum Inhibitory Concentrations of Colonized *Salmonella typhimurium* and Resident *Escherichia coli* Isolates Shed in the Feces of Feeder Pigs," was conducted at Colorado Animal Research Enterprises. The study director was Diane J. Fagerberg, PhD.

A total of twenty-four young pigs were divided into three equal groups (three gilts and three barrows) consisting of a control group and two treatment groups. Pigs were individually housed in designated isolation spaces in the same facility, and they were tested and shown to be free of *Salmonellae* prior to procurement. The control group received sterile saline subcutaneously, repeated for three regimens 14 days apart (Days 0, 14, and 28). The two treated groups received injectable 10% enrofloxacin at 2.5 mg/kg BW once a day for 5 days, and 7.5 mg/kg BW once, respectively, repeated for three regimens 14 days apart (Days 0, 14, and 28).

Prior to treatment, all pigs were challenged with a test strain of *S. typhimurium*, shown to be highly susceptible to ciprofloxacin with a minimum inhibitory concentration (MIC) of 0.015 µg/mL. Colonization was shown to be successful, as evidenced by prolonged shedding in feces in untreated control animals. Fecal samples were collected on selected days following each treatment regimen, and cultured for both *Salmonella* and *E. coli* with appropriate selective media and identification schemes for each organism.

For each sample obtained from individual animals, up to five pure colonies of *E. coli* and/or the recoverable test strain of *S. typhimurium* were selected and subject to ciprofloxacin susceptibility testing using broth micro-dilution method with concentrations ranged between 0.015 and 16 μg/mL. The susceptibility testing was performed according to guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI).

Results indicated that there was no increase in *Salmonella* shedding as a result of enrofloxacin treatment. No remarkable change was observed in the susceptibility of the test strain of *S. typhimurium* to ciprofloxacin following exposure to enrofloxacin as judged by no shifts in MIC_{50} and MIC_{90} between treatment and control groups following each treatment regimen. There were few isolates of *Salmonella* recovered from a single pig on Days 2 and 3 during the first regimen from the group treated with 2.5 mg/kg BW showing an MIC increase up to 0.5 μg/mL (which is within the defined susceptible range established by CLSI); however, there was no additional recovery of *Salmonella* isolates with similar MICs during the remaining 56-day observation period, suggesting that the increase in MICs among those few isolates appeared to be unstable.

The MIC_{50} and MIC_{90} of resident *E. coli* did not change between treatment and control groups. Results indicated that the use of enrofloxacin at the proposed conditions of use was safe with respect to microbial food safety, as determined by a negligible impact on ciprofloxacin resistance development in a challenge strain *Salmonella* and among resident *E. coli* in treated pigs.

The microbial food safety assessment was based on a qualitative risk assessment, and included a release assessment to describe the probability that enrofloxacin and its use in swine production will result in the emergence of resistant bacteria or resistance determinants in treated pigs under the proposed conditions of use; an exposure assessment to describe the likelihood of human exposure to resistant bacteria or resistance determinants through consumption of edible products from treated pigs; and a consequence assessment to describe potential human health consequences arising from exposure to defined resistant bacteria or resistance determinants by considering the human medical importance of antimicrobials (e.g., ciprofloxacin) used in the treatment of human infectious diseases.

Based upon 1) the Agency's evaluation of the prospective study data submitted by the sponsor, consideration of the therapeutic use in swine for treating individual animals, relatively short treatment regimens, and an overall low extent of use, and 2) Agency findings on individual rankings of **medium** for the release assessment, **medium** for the exposure assessment, and **high** for the consequence assessment, it is reasonably determined that the overall risk estimation associated with the use of the enrofloxacin in swine under the proposed conditions is **high**[2], corresponding with mitigation strategies assigned to Category 1 antimicrobial drugs for food animal use. Risk

[2] See FDA's Guidance for Industry #152, Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern (http://www.fda.gov/cvm/Documents/fguide152.pdf).

management steps for a Category 1 antimicrobial drug include prescription marketing status, extra-label use restriction, use in individual diseased animals, and continued monitoring by the national Antimicrobial Resistance Monitoring System (NARMS). These are all applicable to the use of enrofloxacin in swine as described above.

D. Analytical Method for Residues:

1. Determinative Method

Determinative Procedure for Enrofloxacin in Swine Liver: 75505*

Report Preparer: Harish Chopade, PhD, Bayer Corporation, Animal Health, Shawnee Mission, KS

[*Report based on the original investigations by Lorianne Fought, PhD, and Abraham E. Mathew, PhD; Bayer Corporation; Bayer Report No. 106958]

The determinative procedure for measuring the marker residue, enrofloxacin, in treated swine uses acidic organic solvent extraction to extract enrofloxacin from swine liver (the target tissue). The quantification of the enrofloxacin in the extract is by high performance liquid chromatography (HPLC) and fluorescence detection.

In the determinative procedure, ground swine liver sample is homogenized in aqueous acidic acetonitrile (acetonitrile/water/acetic acid, 75:25:0.1, v/v), and the slurry is centrifuged. The supernatant is decanted, and the extraction is repeated on the pellet. The two supernatants are combined. Aqueous 5% trichloroacetic acid is added to the supernatant, the pH is adjusted to approximately 4 with 50% NaOH, and the sample is centrifuged to remove the precipitate. The supernatant is analyzed by HPLC with fluorescence detection.

The limit of quantitation for the determinative procedure is approximately 0.05 ppm.

2. Confirmatory Method

Confirmatory Procedure for Enrofloxacin in Swine Liver: 75504**

Report Preparer: Harish Chopade, PhD, Bayer Corporation, Animal Health, Shawnee Mission, KS

[**Report based on the original investigations by Mr. Robert A. Bethem and Mr. Robert G. Peterson, Alta Analytical Laboratory, Inc., El Dorado Hills, CA; Bayer Report No. 106581-1]

In the confirmatory procedure, a liver sample is processed as described above for the determinative procedure. The procedure uses HPLC for separation and electrospray ionization tandem mass spectrometry (ESI/MS/MS) with selective ion monitoring (SIM) to monitor three major product ions, which are characteristics of enrofloxacin.

3. Availability of Method

The validated regulatory method for detection and confirmation of residues of enrofloxacin is available from the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to BAYTRIL 100:

For use in animals only. Keep out of the reach of children. Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight. For customer service or to obtain product information, including a Material Safety Data Sheet, call 1-800-633-3796. For medical emergencies or to report adverse reactions, call 1-800-422-9874.

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514. The data demonstrate that BAYTRIL 100, when used according to the label, is safe and effective for the treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*, and *Streptococcus suis*. Additionally, data demonstrate that residues in food products derived from swine treated with BAYTRIL 100 will not represent a public health concern when the product is used according to the label.

A. Marketing Status:

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). This decision was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product to treat SRD, and (b) restricting this drug to use by or on order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues.

B. Exclusivity:

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

C. Supplemental Applications:

This supplemental NADA did not require a reevaluation of the safety or effectiveness data in the original NADA (21 CFR §514.106(b)(2)).

D. Patent Information:

BAYTRIL 100 is under the following U.S. patent number:

U.S. Patent Number	Date of Expiration
5,756,506	June 27, 2015

VII. ATTACHMENTS:

Facsimile Labeling:

A. BAYTRIL 100 – multiple insert
B. BAYTRIL 100 – 100 mL unit label
C. BAYTRIL 100 – 100 mL unit carton
D. BAYTRIL 100 – 100 mL case shipping label
E. BAYTRIL 100 – 100 mL case storage label
F. BAYTRIL 100 – 250 mL unit label
G. BAYTRIL 100 – 250 mL unit carton
H. BAYTRIL 100 – 250 mL case shipping label
I. BAYTRIL 100 – 250 mL case storage label