Date of Approval: December 23, 2014

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-068

BAYTRIL 100 Injectable Solution

Enrofloxacin

Swine

1) For the control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with *Escherichia coli* has been diagnosed.

2) For intramuscular injection in swine.

Sponsored by:

Bayer HealthCare LLC
Animal Health Division

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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

BAYTRIL 100 Injectable Solution

D. Established Name

Enrofloxacin

E. Pharmacological Category

Antimicrobial

F. Dosage Form

Injectable Solution

G. Amount of Active Ingredient

100 mg/mL

H. How Supplied

100, 250, and 500 mL bottles

I. Dispensing Status

Rx

J. Dosage Regimen

Administer, either by intramuscular or subcutaneous (behind the ear) injection, a single dose of 7.5 mg/kg of body weight (3.4 mL/100 lb). Administered dose volume should not exceed 5 mL per injection site. For the control of colibacillosis, administration should be initiated within the first 60 days post-weaning when clinical signs are present in at least 2% of the animals in the group.

K. Route of Administration

Intramuscular or subcutaneous injection

L. Species/Class

Swine

M. Indication

Baytril® 100 is indicated for the control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with *Escherichia coli* has been diagnosed.

N. Effect of Supplement

This supplement provides for 1) the control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with *Escherichia coli* has been diagnosed, and 2) for intramuscular injection in swine.

II. EFFECTIVENESS

A. Dosage Characterization

This supplemental approval does not change the previously approved dosage. The Freedom of Information (FOI) Summary for the supplemental approval of NADA 141-068 dated March 14, 2008, contains dosage characterization information for swine.

B. Substantial Evidence

1. Addition of IM Route of Administration - Pharmacokinetic Study

BAYTRIL 100 Injectable Solution was approved March 14, 2008, for the treatment of swine respiratory disease (SRD) at a dose of 7.5 mg enrofloxacin/kg body weight (BW) when administered by subcutaneous (SC) injection. To support the addition of an intramuscular (IM) route of administration, a parallel design bioequivalence study was conducted, comparing serum concentrations obtained when enrofloxacin injectable solution was administered at 7.5 mg/kg BW either IM or SC in healthy pigs.

a. Title: "Bioequivalence of BAYTRIL Max 10% Injectable Solution after Single Intramuscular or Subcutaneous Application of 7.5 mg/kg Body Weight to Pigs." AHD Study 145.515, Bayer Study no. V07-008, Report ID 31812 (August 2007).

b. Study Director and Location: Dr. G. Daube, Bayer HealthCare AG, Animal Health Division, Leverkusen, Germany

c. Study Design:

1) Objective: To demonstrate the bioequivalence of BAYTRIL Max 10% Injectable Solution (a formulation of enrofloxacin identical to BAYTRIL 100 Injectable Solution) when administered by subcutaneous and intramuscular injection into the neck of swine.

2) Study Animals: A total of 32 healthy, male and female, German Landrace Hybrid fattening pigs were used in the study. Of these, 28 were included in the determination of bioequivalence.

   Pigs were approximately 4 months old and ranged in weight from 52.5 to 64.5 kg at the start of the study. Animals were housed in an indoor facility; each pen contained two pigs of the same gender. Animals were fed a commercially available ration once daily. Water was available *ad libitum*.

3) Treatment Groups: Pigs were randomized to one of two enrofloxacin treatment groups (IM or SC, 14 pigs per group) or a control group (4 pigs).

4) Drug Administration: The test article was of BAYTRIL Max 10% Injectable Solution, manufactured by Bayer Vital GmbH. Pigs in the enrofloxacin treatment groups received a single SC or IM injection of enrofloxacin in the neck at 7.5 mg/kg BW.

5) Measurements and Observations: Pigs were blocked by gender and randomly assigned to pen and treatment using a randomization table. A clinical examination was performed on Day -1 to ensure pigs were healthy and acceptable for inclusion. Clinical examinations were performed again on Day 6. General health observations were conducted from Day -1 through Day 6. Body weights were recorded on Day -1 and Day 6.

   Blood samples were collected from each animal at 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 10, 12, 32, 48, and 72 hours after injection. Serum samples were analyzed for enrofloxacin and ciprofloxacin concentrations using a validated turbulent flow chromatography/ tandem mass spectrometry (TFC-MS/MS) method (analytical limit of quantification is 0.025 mg/L for ciprofloxacin and enrofloxacin).

d. Statistical Analysis: For the individual and combined analytes, drug pharmacokinetics was quantified via the use of a noncompartmental analysis. Parameters measured included the area under the concentration/time profile from time zero to the last quantifiable concentration ($AUC_{0\text{-last}}$), the peak observed concentration (C_{max}), the time to C_{max} (T_{max}) and the terminal elimination depletion half-life (T_{half}) (Phoenix, V6.3). The log-transformed $AUC_{0\text{-last}}$ and C_{max} values obtained after IM and SC injection were compared using a one-way analysis of variance procedure (SAS V9.3), and the results of that analysis used in the estimation of the 90% confidence limits about the ratio of treatment means.

e. Results:

1) Clinical and General Health Observations: At study inclusion, five pigs were noted to have bilateral conjunctivitis, one pig had small nodules in the neck region, and one pig had swellings on all four legs. These pigs were considered within the definition of healthy and were included

in the study. One pig had a temporary protrusion of the third eyelid and one pig had an abscess in the right neck at 6 days post-injection. No other abnormal observations were recorded during the post-injection period.

2) PK Parameters: When comparing drug absorption profiles, it is the parent compound that typically provides the most sensitive treatment comparison. Therefore, for the purpose of this bridging study, only the serum enrofloxacin concentrations were considered. The PK results are provided in Table 1.

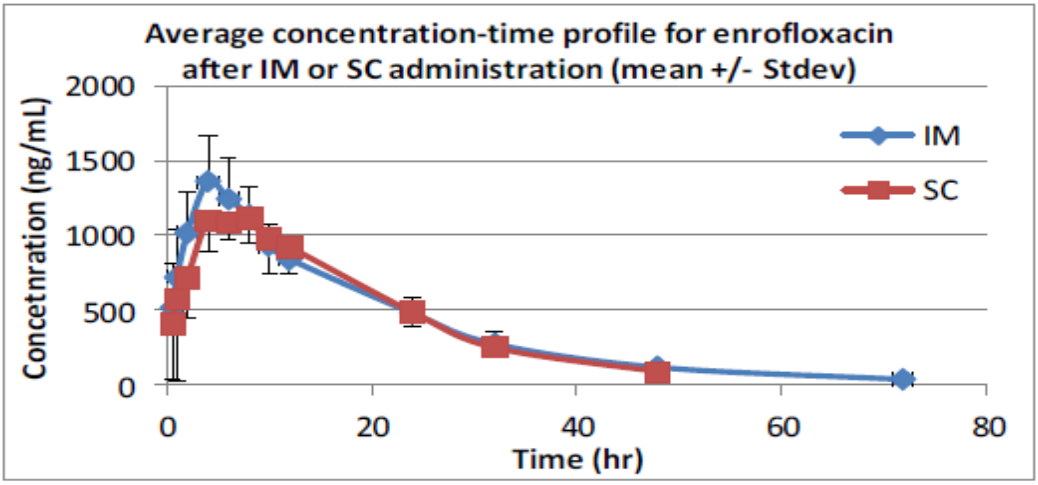
Table 1. Summary of PK parameters following IM or SC injection of enrofloxacin in swine.

	Arithmetic mean (%CV), IM	**Arithmetic mean (%CV), SC**	**Ratio of average* IM/SC**	**LCL****	**UCL*****
AUC (ng·hr/mL)	28285 (13)	26234 (11)	1.08	1.0	1.16
C_{max} (ng/mL)	1459 (22)	1296 (27)	1.13	0.97	1.31
T_{max} (hr)	4.9 (38)	5.8 (63)	--	--	--
T_{half} (hr)	13.4 (31)	10.4 (30)	--	--	--

* Ratio of arithmetic means
**LCL = lower confidence limit for the 90% interval about the ratio of treatment means.
***UCL = upper confidence limit for the 90% interval about the ratio of treatment means

The corresponding plot of the serum enrofloxacin concentrations obtained after IM or SC injection of enrofloxacin (7.5 mg/kg BW) is provided in Figure 1.

Figure 1. Serum enrofloxacin concentrations following IM or SC injection of enrofloxacin in swine.



f. Adverse Reactions: No test-article related adverse events were observed during the study.

g. Conclusions: Based upon the results of this study, comparable effectiveness will be achieved when BAYTRIL 100 Injectable Solution is administered at a dose of 7.5 mg/kg by either SC or IM injection into the neck of swine.

2. Addition of Control of Colibacillosis – Field Study

The effectiveness of BAYTRIL 100 Injectable Solution for the control of colibacillosis in weaned pigs associated with *Escherichia coli* was demonstrated in the field study summarized below. The field study was conducted using an intramuscular (IM) route of administration. Based on the results of the pharmacokinetic study summarized above and the following field study, BAYTRIL 100 Injectable Solution is considered effective for the control of colibacillosis when administered as a single dose of 7.5 mg/kg BW by IM or SC injection.

1) Title: "A Clinical Efficacy Study of BAYTRIL 100 Injectable Solution for the Control of Colibacillosis in Weaned Pigs". Study Number 200282. September 2012 to November 2012.

2) Investigators and Study Locations:

Lyle Kesl DVM, PhD. Veterinary Resources, Inc., Ames, IA (Study location: Schwartz Facility, Story City, IA)
Lindy Miller, PhD. LFM Quality Laboratories, Inc., Terre Haute, IN
Kelly Lechtenberg, DVM, PhD. Midwest Veterinary Services, Inc., Oakland, NE
Lynn Raab, DVM. Thomas D. Morris, Inc., Reisterstown, MD
Daren Miller, DVM. Swine Health Services, Inc., Frankfurt, IN (Study location: LFM Quality Laboratories, Inc., Terre Haute, IN)

3) Study Design:

a) *Objective:* To evaluate the effectiveness of BAYTRIL 100 Injectable Solution for the control of naturally-acquired colibacillosis in weaned pigs associated with *Escherichia coli*.

b) *Test Animals:* Weaned pigs (intact or castrated male and female) were sourced from U.S. commercial swine operations and shipped to the study sites. Pigs were sourced from herds with a history of colibacillosis, ongoing colibacillosis outbreaks, and/or were commingled at the study site with pigs with colibacillosis. Pigs were between 16 and 33 days of age and weighed between 4.7 and 26.5 lbs on Day 0. A total of 1500 pigs were enrolled across the 5 sites. Pigs were housed in pens containing 5 pigs each.

In addition to the study candidate pigs, each site acquired between 30 and 75 additional beta-hemolytic *E. coli* positive (bHEC+) pigs. These pigs were sourced from one or more commercial swine operations with either a history of colibacillosis outbreaks or a current outbreak.

Rectal swabs were collected and evaluated, and the bHEC+ pigs were commingled at each study site with the candidate pigs to naturally induce the pathogen at that site. The bHEC+ pigs were not enrolled in the study.

c) *Treatment Groups:* The test article was BAYTRIL 100 injectable solution, as the currently marketed formulation containing 100 mg enrofloxacin/mL, administered as a single dose of 7.5 mg enrofloxacin/kg body weight (BW). The control article was physiological normal saline solution, 0.9% NaCl w/v, administered as a single dose volume equivalent to the enrofloxacin-treated group. At each site, 150 pigs (30 pens) were enrolled in each treatment group. Treatment was assigned by pen.

d) *Drug Administration:* Pigs were dosed with their assigned treatment once on the day of enrollment (Day 0), using Day 0 body weight and a dosing chart. Injections were administered intramuscularly in the neck.

e) *Randomization*: Candidate pigs were observed daily for general health and clinical signs of colibacillosis. When a minimum of 5% of the candidate pigs were considered clinically affected (diarrhea score ≥ 2 on a severity scale of 0 [normal] to 3 and either a depression score ≥ 1 on a severity scale of 0 [normal] to 3 and/or a gauntness score ≥ 1 on a severity scale of 0 [normal] to 2), all candidate pigs were individually scored for colibacillosis, a rectal swab was collected for culture, and pigs were weighed and randomly allocated to pens such that clinically affected pigs were evenly distributed across pens. Treatment was randomly assigned to pens within blocks of two.

f) *Measurements and Observations:* Enrolled pigs were observed at least once after test material administration on Day 0 for adverse events. General health observations were conducted twice daily from Days 1 to 6 and once on Day 7. Pigs that became moribund due to colibacillosis and pigs that developed conditions not related to colibacillosis were removed and euthanized. On Study Day 7 all remaining enrolled pigs were clinically scored for colibacillosis, weighed, and had a rectal swab collected for culture. Individuals performing clinical assessments were masked to treatment and did not participate in treatment administration.

4) Statistical Analysis: Pen was the experimental unit. The primary variable was treatment success. Success was defined as a pig that had a diarrhea score of ≤ 1, AND a depression score of 0, AND a gauntness score of ≤ 1 on Day 7. The primary variable was based on the comparison of percent pen success in the two treatment groups. Treatment success was evaluated using the GLIMMIX procedure in SAS (SAS Institute, Cary, NC). A binomial distribution was assumed and a logit link was used. The statistical model included treatment as a fixed effect and site and treatment x site as random effects. Percent success and 95% confidence intervals were used to summarize the results.

5) Results: A total of 299 (of 300) enrolled pens were included in the analysis. One pen was removed from the study and analysis due to reasons not associated with colibacillosis.

   a) *Treatment Success:* The percentage of pens classified as a treatment success was statistically significantly higher (p=0.0350) in the BAYTRIL 100-treated group (61.5%) compared to the saline-treated group (44.7%).

   b) *Microbiology:* At least 30 isolates of beta-hemolytic *E. coli* were isolated from at least 30 individual pigs enrolled in the study across the five study sites.

6) Adverse Reactions: No adverse reactions attributable to the test article were observed during the study.

7) Conclusion: Based on the results of this study, BAYTRIL 100 Injectable Solution administered as a single IM dose of 7.5 mg/kg BW was effective for the control of colibacillosis associated with *Escherichia coli* in weaned pigs.

III. TARGET ANIMAL SAFETY

A. Margin of Safety Study:

a. Title: "Safety of BAYTRIL 100 (Enrofloxacin 10%) Following Intramuscular Administration to Weaned Pigs". Study Number 200287. January 2013 to September 2013.

b. Study Director and Location: Teresa Schieber, DVM. Midwest Veterinary Services, Inc. (MVS), Oakland, NE

c. Study Design:

   1) Objective: To evaluate the safety of BAYTRIL 100 Injectable Solution administered by intramuscular (IM) injection in weaned pigs at 7.5, 22.5, and 37.5 mg/kg body weight (BW) on Days 0, 7, and 14.

   2) Study Animals: Forty-eight healthy, crossbred, weaned pigs (24 females and 24 intact males). At study initiation (Day 0), pigs were 20-22 days old and weighed between 5.1 and 9.1 kg. Study pigs were housed in an environmentally controlled room, with *ad libitum* access to a commercially available feed ration and water.

3) Treatment Groups: Pigs were randomized to one of four treatment groups, as follows:

Table 2. Treatment Groups.

Group	Treatment regimen	Total number of pigs
0X	saline; 0.0375 mL/kg BW IM (equivalent to 5X dose volume) on Days 0, 7, and 14	6M, 6F
1X	BAYTRIL 100; 7.5 mg enrofloxacin/kg BW IM on Days 0, 7, and 14	6M, 6F
3X	BAYTRIL 100; 22.5 mg enrofloxacin/kg BW IM on Days 0, 7, and 14	6M, 6F
5X	BAYTRIL 100; 37.5 mg enrofloxacin/kg BW IM on Days 0, 7, and 14	6M, 6F

4) Drug Administration: The test article was BAYTRIL 100 (enrofloxacin) Injectable Solution, currently marketed formulation, 100 mg enrofloxacin/mL. The control article was physiological saline solution. Pigs were separated into two cohorts (with Day 0 for each cohort staggered by one calendar day) to facilitate data collection and necropsy. Pigs were dosed with their assigned treatment in the neck on Days 0, 7, and 14. A maximum of 3 mL was injected per injection site.

5) Measurements and Observations: Body weights were recorded on Days -3 (or -2), -2 (or -1), 6, 13, and 15. Daily feed and water consumption were measured for each pen on Days 0 to 15. Physical examinations (including pupillary light reflex and menace response) were conducted on Days -3 (or -2), 6, and 15. Clinical evaluations (attitude, locomotion, respiration, abdominal fill, hydration, injection site, and adverse events) were conducted twice daily on Days 1-6 and Days 8-13; four times daily on Days 0, 7, and 14; and once on Day 15.

Blood was collected for hematology, coagulation, and clinical chemistry evaluations on Days -8 (or -7), 7, and 14. Urine sample collection was attempted on Days -2 (or -1) and 7 using individual cage pans, and at necropsy on Day 15 via cystocentesis. Fecal samples were collected on Days -8 (or -7), 7, and 14. Reference ranges for hematology, coagulation, and clinical chemistry were generated from contemporaneous samples collected from similar age pigs from the same facility and using American Society for Veterinary Clinical Pathology (ASVCP) guidelines. Baseline values were used as the reference range for urinalysis and fecal analysis.

Blood was collected for serum enrofloxacin levels before the first dose, at 4 hours and 48 hours after the first and second doses, and at 4 hours after the third dose.

On Day 15, all pigs were humanely euthanized and a complete necropsy was conducted. Organs and tissues from all groups were examined grossly and sampled for histopathology.

d. Statistical Analysis: All continuous variables were analyzed using a mixed model analysis. Variables measured multiple times were analyzed using a repeated measures analysis of covariance using the best fitted covariance structure with the following fixed effects: pre-treatment values as a covariate, treatment group, gender, time, and two- and three-way interactions. Cohort was included in the model as a random effect; pen and animal nested within pen were also included as random effects where consistent with the covariance structure being modelled. Statistical comparisons of treatment effects and comparison of treatment by time were performed at the 0.1 level of significance. Comparison of treatment by time by sex was performed at the 0.05 level of significance. Continuous variables measured once were analyzed using an analysis of variance with similar fixed and random effects. Data that were binary in nature were only evaluated if abnormalities were reported using exact tests with an alpha of 0.1.

e. Results:

1) Clinical Observations and Physical Examinations: There were no mortalities or unscheduled removals. Injection site swelling was recorded in one 5X pig after the first treatment, in two 5X pigs after the second treatment, and in eight 5X pigs following the third treatment. Swelling was characterized as mild in severity and resolved without intervention within 24 to 48 hours of onset (first and second treatments). Other than injection site swelling, there were no test article-related clinical observations or abnormal physical examination findings.

2) Body Weight: Body weight increases were observed in all individual pigs and in all treatment groups. Although not statistically significant, weight gain per treatment group was inversely proportional to dose.

3) Feed and Water Consumption: Over the course of the study, daily feed and water consumption generally increased in all treatment groups. However, each treatment was associated with a transient (~72 hours), dose-related, decrease in feed and water consumption.

4) Clinical Pathology: Serum concentrations of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and creatinine kinase were statistically significantly higher in enrofloxacin-treated groups compared to the control group. Although these results were considered test article-related, they were not considered to be clinically relevant to safety because all pigs remained clinically healthy during the study (other than injection site swelling) and the elevations were consistent with muscle damage related to injection site injury. No clinically relevant test article-related differences in hematology, coagulation, urinalysis, or fecal analysis parameters were detected.

5) Post-Mortem Findings: The only post-mortem findings attributed to administration of the test article were injection site lesions in the enrofloxacin-treated pigs. Observations varied from no visible lesions to the presence of moderately dark or red, firm or edematous tissue, and their appearance seemed to correspond to day of injection and dose

volume. The macroscopic findings generally correlated microscopically with areas of necrotic muscle surrounded by inflammation.

6) Pharmacokinetics: Pharmacokinetic evaluation indicated that negligible accumulation of enrofloxacin or its active metabolite, ciprofloxacin, occurred with a once weekly dosing regimen. Although peak enrofloxacin and ciprofloxacin concentrations were dose proportional, the terminal elimination half-life (as reflected in C_{min} values) tended to be higher than predicted in the 3X and 5X dose group. This prolonged terminal exposure likely reflects drug precipitation (and therefore longer absorption phase) after the intramuscular administration of 22.5 and 37.5 mg/kg.

f. Conclusions: Based upon the results of this study, BAYTRIL 100 Injectable Solution is safe in swine when administered at a dose of 7.5 mg/kg by IM injection in the neck.

IV. HUMAN FOOD SAFETY

A. Antimicrobial Resistance

1. Addition of IM Route of Administration

The impact of the addition of an intramuscular (IM) injection route of administration to the current BAYTRIL 100 Injectable Solution label on microbial food safety was carefully considered by the Agency. For this supplemental approval, Bayer provided information to CVM through a microbial food safety *hazard characterization*.

Bayer identified the hazard of concern as human illness caused by fluoroquinolone-resistant foodborne bacteria (*Campylobacter* spp., *Salmonella* spp., or MDR *Salmonella* spp.). The *hazard characterization* demonstrates 1) that the use of enrofloxacin for the currently approved swine indication is unlikely to result in an increased use of the drug in swine and 2) that the intramuscular use of enrofloxacin would pose no greater risk than the subcutaneous route of administration when considering the hazard of human illness caused by fluoroquinolone-resistant foodborne bacteria (*Campylobacter* spp., *Salmonella* spp., or MDR *Salmonella* spp.).

Considering that 1) the use of enrofloxacin is unlikely to increase as a result of this new route of administration, and 2) the IM route of administration is comparable to the subcutaneous route of administration when considering the pharmacokinetics and pharmacodynamics of the drug within the animal, the Agency determined that the addition of an IM injection route of administration to the already approved indication in swine will not pose any additional risks to public health from fluoroquinolone-resistant organisms originating from treated swine.

2. Addition of Control of Colibacillosis

The impact of the addition of control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with *Escherichia coli* has been diagnosed was carefully considered by the Agency. For this supplemental approval, Bayer

provided information to CVM through a qualitative microbial food safety risk assessment.

The firm provided a qualitative microbial food safety risk assessment that included:

1) A *release assessment* to describe the probability that the use of enrofloxacin in swine production systems will result in emergence or selection of fluoroquinolone-resistant *Campylobacter* and *Salmonella* spp., a thorough assessment of the spectrum of antibacterial activity of enrofloxacin, mechanisms of fluoroquinolone resistance in *Campylobacter* and *Salmonella*, and the current prevalence of *Campylobacter* and *Salmonella* in swine and retail pork.
2) An *exposure assessment* to describe the likelihood of human foodborne exposure to *Campylobacter* and *Salmonella* following consumption of pork chops from treated swine.
3) A *consequence assessment* to describe potential human health consequences arising from exposure to the defined foodborne pathogens or resistance determinants by considering the human medical importance of fluoroquinolones in the treatment of human gastrointestinal diseases.

The Agency evaluated the information submitted by the firm, considered the current fluoroquinolone and quinolone susceptibility profiles of *Salmonella* spp., *E.coli*, and *Campylobacter* spp., including their prevalence in the food commodity of concern (pork chops) and target animal (swine), and worked with the firm to develop the following extent-of-use mitigating factors:

- Addition of a CAUTION statement, "*To assure responsible antimicrobial drug use, enrofloxacin should only be used as a second-line drug for colibacillosis in swine following consideration of other therapeutic options,"* to appear on all labels, inserts and marketing materials.
- Administration of enrofloxacin only within the first 60 days post-weaning.
- Administration of enrofloxacin only when clinical signs are observed in at least 2% of the animals in the group.
- If clinical resolution is not observed within 48 hours post-treatment, the diagnosis should be reevaluated.

Based upon this evaluation and mitigating factors, the Agency concludes that the use of enrofloxacin in swine for the control of colibacillosis will not result in a significant risk with respect to the development of fluoroquinolone resistance in foodborne *Campylobacter* and *Salmonella* originating from treated swine.

Decision Statement:
The overall risk estimation associated with the use of enrofloxacin injectable in swine under the proposed conditions of use is high, based on individual rankings of high for the *release assessment*, high for the *exposure assessment*, and high for the *consequence assessment*. The latter ranking of high for the consequence assessment is based on fluoroquinolones being *critically important* in human medicine as it is the empiric drug class of choice to treat a majority of clinical gastrointestinal infections. However, the use of risk management strategies such as prescription only (Rx) marketing status (under the direction of a veterinarian), use of an injectable route of administration in individual animals, extra-label use prohibition, isolate monitoring by the National Antimicrobial Resistance Monitoring

System (NARMS), and conditions of use including appropriate use parameters to determine if swine are eligible to receive enrofloxacin for the control of colibacillosis have allowed the Agency to conclude that antimicrobial resistance concerns for fluoroquinolone-resistant *Campylobacter* and *Salmonella* originating from treated swine are minimized.

B. Impact of Residues on Human Intestinal Flora

The impact of enrofloxacin residues and metabolites on human intestinal flora was previously assessed, and a microbiological acceptable daily intake (mADI) was determined under supplemental approvals under NADA 141-068 (February 13, 2008, and July 24, 2012). The assessment of the effects of enrofloxacin residues and metabolites on human intestinal flora is revised and summarized below.

1. Determination of the need for establishing a microbiological ADI

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

      Yes, based on *in vitro* susceptibility data, it was determined that enrofloxacin residues are active against representative human intestinal bacteria. The relevant MIC study is summarized below.

      Study: Minimum inhibitory concentration of enrofloxacin against bacterial isolates of human gut origin.

      Study Number: 112.718

      Study Period: September, 1996

      Study Director: Andrew Pridmore, BSc, Ph.D.

      Study Location: Don Whitely Scientific Limited, Shipley, United Kingdom

      Study Summary:
      This series of studies investigated MICs for enrofloxacin against bacterial groups collected from human intestinal flora, and the influence of pH on MICs following Clinical and Laboratory Standards Institute (CLSI) recommended agar dilution methodology. The pH of the test medium was adjusted to three different values similar to the *in vivo* pH in the large intestine (5.2, 6.2, and 7.2). Standard reference bacterial strains were used between experiments for quality control purposes to ensure that MIC variations in the testing were not more than one double-dilution.

      Results and Conclusions:
      It was found that acidic pH caused a decrease in enrofloxacin activity (*i.e.*, increased MIC values) for some isolates of *Bacteroides*, *Fusobacterium*, *Peptostreptococcus*, *Clostridium*, and *Eubacterium*. These results might be due to poor growth conditions of these strains at pH 5.2, causing an apparent increase in MICs for enrofloxacin. *E. coli* was the most sensitive bacteria with average MIC values of 0.031, 0.049, and 0.27 µg/mL at pH

values of 7.2, 6.2, and 5.2, respectively. For the purpose of determination of *in vitro* activity of enrofloxacin, MICs at standard pH (7.2) were used to derive MIC_{50} values. Table 3 lists the MIC_{50}s of representative bacterial groups of human intestinal flora. A calculated MIC (MIC_{calc}) of 0.189 µg/mL was determined based on MIC_{50}s of relevant bacterial species/groups.

Table 3. MIC_{50}s of enrofloxacin against representatives of human intestinal flora.

Genus/Group	**MIC_{50} (µg/mL)**
Escherichia coli	0.031
Enterococcus spp.	1
Lactobacillus spp.	0.5
Bacteroides spp.	1
Bifidobacterium spp.	0.5
Fusobacterium spp.	0.125
Eubacterium spp.	0.25
Peptostreptococcus spp.	0.25
Clostridium spp.	0.5

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

Yes, by default, it was assumed that enrofloxacin residues enter the human colon.

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

Yes, based on a fecal binding study data generated by the firm, it was concluded that up to 2% of enrofloxacin residues entering the human colon remain microbiologically active. The fecal binding study is summarized below.

Study: Effect of fecal binding on antibacterial activity of enrofloxacin.

Study Number: DWS Study 02004

Study Period: December 10, 2004 to February 4, 2005

Study Director: Andrew Pridmore, BSc, Ph.D.

Study Location: Don Whitely Scientific Limited, Shipley, United Kingdom

Study Summary:
The objective of the study was to determine the effects of fecal binding on the antibacterial activity of enrofloxacin. Stock solutions of enrofloxacin were freshly prepared on the day of experiment. Test solutions were prepared from stock solutions to obtain final tested concentrations of 0, 0.1, 0.2, 0.5, 1, 2, and 5 µg/mL of enrofloxacin. Each enrofloxacin dilution was mixed with corresponding fecal suspensions (at final concentrations of 0, 10, 25, and 50% w/v, respectively). Fecal samples were derived from

three, separate, healthy male and female donors. The mixtures of drug and fecal material were incubated for 0, 0.5, 1, 2, 6, and 12 hours. Fecal solids from each mixture were removed by centrifugation at each time period. The supernatant of the mixtures from each test were inoculated with *Escherichia coli* ATCC 25922 (at a final bacterial density of 5×10^5 cfu/mL) and incubated for 24 hours to assess antibacterial activity of the compound in the supernatant. Growth of the test strain was estimated by turbidity or presence of a cell pellet in inoculated cells compared to non-inoculated control cells of each fecal/drug mixture.

An estimate of the bound enrofloxacin was performed applying the approach for assessing the binding of an antimicrobial to serum, which is calculated as follows:

$$\% \text{ bound} = \frac{\text{MIC in feces} - \text{MIC in broth alone}}{\text{MIC in feces}} \times 100$$

Equation 1. Percentage bound equals (Minimum Inhibitory Concentration (MIC) in feces minus Minimum Inhibitory Concentration (MIC) in broth alone) divided by (Minimum Inhibitory Concentration (MIC) in feces) multiplied by 100.

Results and Conclusions:
Concentrations of fecal suspensions from 10% to 50% showed apparent effects of solids on binding. It was estimated that the percentage of enrofloxacin bound to feces (25% and 50% dilutions) at 0 time of incubation is approximately 80%. The percentage of binding increases with incubation time. Binding in one donor required 6 or 12 hours of incubation (10% mixture), while in the other two donors, binding to 10% feces occurred in the first 30 minutes. Binding to the 25% mixture occurred at 50 to 80% at 0 time of incubation, and was dependent on individual fecal samples. Binding to the 50% mixture occurred at 95% at 0 incubation time in one sample, and increased at 95 to 98% after 1 hour of incubation in all three samples. At 6 hours, all samples reached 98% binding. It was concluded that binding depends on both the interaction time and the concentration of feces. At high feces concentration, binding can reach 98% in 30 minutes. Thus, approximately 2% of the fraction of enrofloxacin residues in the fecal environment were unbound and are considered biologically active.

d. Step 4: Determination if there is any scientific justification to eliminate testing for either one or both endpoints of concern:

- Colonization barrier disruption

It was determined that a mADI should be determined based on this endpoint.

- Increase in populations of resistant intestinal bacteria

CVM determined that there was justification to eliminate testing for the endpoint of increases in resistant bacterial populations in the human colon. This conclusion was made a) based on results from a previously-funded Agency study (titled "Effects of Low Levels of Ciprofloxacin on a Chemostat Model of the Human Colonic Microflora," Regulatory Toxicology and Pharmacology 2001; 33(3):276–284), where findings suggested that *Bacteroides fragilis* group is a suitable index bacteria group for use in assessing this endpoint, b) based on a survey by the firm of baseline MICs of this bacterial group derived from the intestinal tracts of healthy human subjects that found that there are existing populations in healthy fecal donors with MICs as high as 128 µg/mL against ciprofloxacin, and c) knowledge that because of the presence of the existing resistant subpopulation and its wide variation in healthy human subjects associated with the index bacterial group, it would be difficult to determine a no observable adverse effect concentration (NOAEC) or no observable adverse effect level (NOAEL) for use in setting a mADI for this endpoint.

2. Determination of the final microbiological ADI

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

      Based on the fecal binding study data described above, there is a 2% fraction of the oral dose available to microorganisms.

   b. Determination of the microbiological ADI using MIC_{calc}

      A mADI was determined using MIC data to determine an effect by enrofloxacin residues according to the formula:

$$\text{ADI } (\mu\text{g/kg BW/day}) = \frac{MIC_{calc} \text{ X Mass of colon content}}{\text{Fraction of dose available X 60 kg}}$$

      Equation 2. Acceptable Daily intake (ADI) equals (Minimum Inhibitory Concentration (MIC) calculated times mass of colon content) divided by (fraction of dose available times 60 kg).

      Where MIC_{calc} is 0.189 (µg/gram), the mass of colon contents is 220 (gram), the fraction of oral dose available 0.02, leading to a calculated mADI of 34.65 (µg/kg BW/day).

Decision statement:
The Agency did not require additional information for the impact of residues on human intestinal flora for this supplemental approval. The above updated information replaces information found in the previous FOI Summaries under NADA 141-068 pertaining to establishment of microbiological ADI.

C. Toxicology

Reassessment of the toxicological acceptable daily intake (ADI) was not needed for this supplemental approval. The FOI Summary for the supplemental approval of NADA 141-068, dated February 13, 2008, contains summaries of all toxicology studies and information.

D. Assignment of the Final ADI

The final ADI is the toxicological ADI of 3.0 µg/kg BW/day derived from a 3-month subchronic oral toxicity study in dogs. The codified ADI is listed under 21 CFR 556.226.

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

The safe concentrations of total enrofloxacin residues in each edible tissue of growing and finishing pigs are 0.6 ppm for muscle, 1.8 ppm for liver, 3.6 ppm for kidney, 3.6 ppm for fat, and 6 ppm for the injection sites.

F. Residue Chemistry

1. Summary of Residue Chemistry Studies

   a. Total Residue and Metabolism Studies

   CVM did not require total residue and metabolism studies for this supplemental approval. The FOI Summary for the supplemental approval of NADA 141-068, dated March 14, 2008, contains summaries of total residue and metabolism studies for enrofloxacin in swine.

   The sponsor provided a written discussion for why the subcutaneous route of administration represents a worst-case scenario to address Agency concerns for the human food safety of intramuscular residues. The sponsor provided a summary of Bayer Study 145.515 that was conducted to show bioequivalence between BAYTRIL Max 10% Injectable Solution when administered either by subcutaneous or intramuscular routes of administration. In addition, the sponsor focused on the metabolism and residue depletion studies conducted in swine following subcutaneous administration that are summarized in the FOI Summary for NADA 141-068, dated March 14, 2008. Lastly, the sponsor compared the enrofloxacin concentrations in tissues by subcutaneous *vs.* intramuscular administration at 7.5 mg/kg BW. These results are presented in Table 4. The data at 3 days post-dose show residues in the edible tissues of swine (liver and muscle) that received enrofloxacin *via* intramuscular injection are less than those in swine that received enrofloxacin *via* subcutaneous injection.

   Table 4. Mean Enrofloxacin Concentrations (ppm ± S.D.) in Swine Tissues After SC (Bayer Report Nos. 74502 and 74763) *vs.* IM Administration (Bayer Report No. 200860).

Withdrawal Time (days)	Edible Tissue	SC injection ± S.D.	IM injection ± S.D.
1	Liver	2.470 ± 0.79	Not measured
1	Muscle	1.79 ± 0.50	Not measured
1	Injection Site	223 ± 165.44	Not measured
2	Liver	0.552 ± 0.307	Not measured
2	Muscle	0.375 ± 0.173	Not measured
2	Injection Site	40.3 ± 50.3	Not measured

Withdrawal Time (days)	Edible Tissue	SC injection ± S.D.	IM injection ± S.D.
3	Liver	0.260 ± 0.180	0.197 ± 0.07
3	Muscle	0.146 ± 0.135	0.0851 ± 0.03
3	Injection Site	3.25 ± 3.49	2.908 ± 3.4
4	Liver	0.232 ± 0.121	Not measured
4	Muscle	0.146 ± 0.076	Not measured
4	Injection Site	2.38 ± 1.91	Not measured
5	Liver	Not measured	<LOQ[1]
5	Muscle	Not measured	<LOQ[2]
5	Injection Site	Not measured	<LOQ[2]

[1]LOQ in liver is 0.12 ppm
[2]LOQ in muscle and injection site is 0.05 ppm

Taken together, we conclude that the subcutaneous route of administration remains the worst-case scenario for residues in the edible tissues of swine. It is reasonable to apply the existing tolerances to the intramuscular use of enrofloxacin.

b. Comparative Metabolism Study

CVM did not require comparative metabolism studies for this supplemental approval. The FOI Summary for the supplemental approval of NADA 141-068, dated March 14, 2008, contains a summary of the comparative metabolism studies for enrofloxacin in swine.

c. Study to Establish Withdrawal Period

Tissue Residue Depletion Study

"Depletion of Enrofloxacin in Swine Tissues Following a Single Intramuscular Treatment of Baytril 100 Antimicrobial Injectable Solution" (Bayer Study No. 200860)

Study Dates: July 30, 2012, to September 23, 2013

Study Director: Heasook Kim-Kang, Ph.D.

In-Life Testing Facility: Southwest Bio-Labs, Inc., Las Cruces, NM

Analytical Facility: XenoBiotic Laboratories, Inc., Plainsboro, NJ

Test Animals: Twenty-seven (14 male and 13 female) Berkshire-cross swine weighing 42 to 61.5 kg at initiation of the study

Test Article Administration: Animals were randomly assigned to one of five treatment groups (n=5 animals/group) and two animals (1 male and 1 female) were assigned to the control group. Animals were dosed in the

left side of the neck with a single intramuscular injection of BAYTRIL 100 Injectable Solution at 7.5 mg/kg BW.

Sampling: The control animals were slaughtered at 2 days post-dose. Test animals were slaughtered at 3, 5, 7, 10 and 14 days post-dose. Samples of liver, composite muscle (shoulder, loin and round) and injection site muscle were collected.

Liver, Injection Site and Muscle Assay Results: The mean enrofloxacin residues in swine liver, injection site and muscle measured by HPLC-MS/MS are presented in Table 5. Mean liver, injection site and muscle residues fall below the LOQ by Day 5.

Table 5. Mean Enrofloxacin Concentrations (ppm ± S.D.) in the Liver, Injection Site and Muscle of Swine Treated Intramuscularly with 7.5 mg/kg BW BAYTRIL 100 Injectable Solution.

Withdrawal Time (days)	Liver ± S.D.	Injection Site ± S.D.	Muscle ± S.D.
3	0.197 ± 0.07	2.908 ± 3.4	0.0851 ± 0.03
5	<LOQ[1]	<LOQ[2]	<LOQ[2]
7	<LOQ	<LOQ	<LOQ
10	<LOQ	<LOQ	<LOQ
14	<LOQ	<LOQ	<LOQ

[1]LOQ in liver is 0.12 ppm
[2]LOQ in muscle and injection site is 0.05 ppm

2. Target Tissue and Marker Residue

The target tissue for residue monitoring is liver. The marker residue in edible tissues is enrofloxacin. The studies supporting the target tissue and marker residue assignments can be found under NADA 141-068 FOI Summary, dated March 14, 2008.

3. Tolerance

Based on previously submitted information and the data summarized under F1a (above), we conclude that the currently assigned tolerance is consistent with the public health. The liver tolerance in swine is 0.5 ppm enrofloxacin (21 CFR 556.226). See the FOI Summary for the supplemental approval of NADA 141-068, dated March 14, 2008.

4. Withdrawal Period

Tissue residue data from Study No. 200860 (F1c, above) support a 5-day withdrawal period for BAYTRIL 100 Injectable Solution when used according to label directions in swine.

G. Analytical Method for Residues

The FOI Summary for the supplemental approval of NADA 141-068, dated March 14, 2008, contains the analytical method summaries for enrofloxacin in swine.

V. USER SAFETY

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

Not for use in humans. Keep out of 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 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 Injectable Solution, when used according to the label, is safe and effective for 1) the control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with *Escherichia coli* has been diagnosed, and 2) for intramuscular injection in swine. Additionally, data demonstrate that residues in food products derived from species treated with BAYTRIL 100 Injectable Solution will not represent a public health concern when the product is used according to the label.

A. Marketing Status

Labeling restricts this drug to use by or on the order of a licensed veterinarian. 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 control colibacillosis in weaned pigs and (b) restricting this drug to use by or on the order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues.

B. Exclusivity

This supplemental approval for BAYTRIL 100 Injectable Solution qualifies for THREE years of marketing exclusivity under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act because the supplemental approval included safety and effectiveness studies. This exclusivity begins as of the date of our approval letter and only applies to 1) the control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with *Escherichia coli* has been diagnosed, and 2) for intramuscular injection in swine.

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:

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