Date of Approval: April 9, 2024

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-550

Pradalex™

(pradofloxacin injection)

Injectable Solution

Cattle: For use in cattle intended for slaughter (beef calves 2 months of age and older, growing beef steers, growing beef heifers, and beef bulls intended for slaughter), and in cattle intended for breeding less than 1 year of age (replacement beef and dairy heifers less than 1 year of age and beef and dairy bulls less than 1 year of age). Not for use in cattle intended for breeding 1 year of age and older (replacement beef and dairy heifers 1 year of age and older, beef and dairy bulls 1 year of age and older, and beef and dairy cows), beef calves less than 2 months of age, dairy calves, and veal calves.

Swine: For use in weaned swine intended for slaughter (nursery, growing, and finishing swine, boars intended for slaughter, barrows, gilts intended for slaughter, and sows intended for slaughter). Not for use in swine intended for breeding (boars intended for breeding, replacement gilts, and sows intended for breeding) and in nursing piglets.

Cattle: Pradalex™ is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis* in cattle intended for slaughter, and in cattle intended for breeding less than 1 year of age.

Swine: Pradalex™ is indicated for the treatment of swine respiratory disease (SRD) associated with *Bordetella bronchiseptica, Glaesserella (Haemophilus) parasuis, Pasteurella multocida, Streptococcus suis,* and *Mycoplasma hyopneumoniae* in weaned swine intended for slaughter.

Sponsored by:

Elanco US Inc.

Executive Summary

Pradalex™ (pradofloxacin injection) solution is approved in cattle for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*; and in swine for the treatment of swine respiratory disease (SRD) associated with *Bordetella bronchiseptica*, *Glaesserella (Haemophilus) parasuis*, *Pasteurella multocida*, *Streptococcus suis*, and *Mycoplasma hyopneumoniae*.

Pradalex™ is in the fluoroquinolone class of antimicrobial drugs and is administered as a single injection (subcutaneous in cattle and intramuscular in swine). The drug is for use in cattle intended for slaughter and in cattle intended for breeding less than 1 year of age; it is not for use in cattle intended for breeding 1 year of age and older, beef calves less than 2 months of age, dairy calves, and veal calves. The drug is also for use in weaned swine intended for slaughter; it is not for use in swine intended for breeding and nursing piglets.

Safety and Effectiveness

Cattle

The sponsor conducted a multi-site natural infection field study to demonstrate that Pradalex™ is effective at treating BRD in cattle. Commercial-type calves were sourced from three geographic regions of the United States (U.S.) and were enrolled in the study when they showed clinical signs of BRD based on elevated depression and/or respiratory scores and an elevated rectal temperature. On Day 0, calves received a subcutaneous injection of Pradalex™ or saline, and on Day 10, they were classified as either a treatment success or failure based on their depression and respiratory scores and rectal temperature. More calves in the treated group were treatment successes compared to calves in the control group, and the difference was statistically significant. Isolates of the pathogens commonly associated with BRD were identified in the study calves in sufficient numbers to be included on the labeling. No adverse reactions were reported.

The sponsor conducted a margin of safety study in young, healthy, Angus-cross steers and heifers. Each calf was administered Pradalex™ subcutaneously at 0X, 1X, 3X, or 5X the labeled dose for a total of three doses, each one given 4 days apart. The 0X group received saline at the 5X dose volume. There were no clinically significant adverse findings in any of the treatment groups. The study demonstrates that Pradalex™ is safe in steers, female beef cattle, and replacement dairy heifers when used according to the labeling.

The sponsor also conducted a margin of safety study in young, healthy, Angus-cross bull calves. Each calf was administered Pradalex™ subcutaneously at 0X, 1X, 3X, or 5X the labeled dose for a total of three doses, each one given 4 days apart. No macroscopic or microscopic abnormalities were seen in the testes or epididymis of any bull calf, and there was no effect on testicular or epididymal weights. This study and the margin of safety study mentioned above demonstrate that Pradalex™ is safe in beef bulls intended for slaughter and beef and dairy bulls intended for breeding less than one year of age when used according to the labeling. This study did not evaluate the reproductive safety of Pradalex™ in beef and dairy bulls intended for breeding over one year of age.

Swine
The sponsor conducted a multi-site natural infection field study to demonstrate that Pradalex™ is effective at treating SRD in swine. Commercial, female and castrated male crossbred pigs were enrolled in the study when they showed clinical signs of SRD based on elevated depression and/or respiratory scores and an elevated rectal temperature. On Day 0, pigs received an intramuscular injection of Pradalex™ or saline, and on Day 7, they were classified as either a treatment success or failure based on their depression and respiratory scores and rectal temperature. More pigs in the treated group were treatment successes compared to pigs in the control group, and the difference was statistically significant. Isolates of the pathogens commonly associated with SRD were identified in the study pigs in sufficient numbers to be included on the labeling. No adverse reactions were reported.

The sponsor conducted an induced infection study to demonstrate that Pradalex™ is effective against *M. hyopneumoniae* in swine. Healthy, female and castrated male crossbred pigs that were serologically negative for *M. hyopneumoniae* were challenged with an inoculum of *M. hyopneumoniae* for three consecutive days. When sufficient clinical signs and lung lesions associated with *M. hyopneumoniae* were seen, the pigs received an intramuscular injection of Pradalex™ or saline (considered Day 0) and lung lesions were evaluated on Day 10. Pigs in the treated group had decreased lung lesions associated with *M. hyopneumoniae* compared to pigs in the control group, and the difference was statistically significant. No adverse reactions were reported.

The sponsor also conducted a margin of safety study in healthy, weaned, male and female crossbred piglets. Each piglet was administered Pradalex™ intramuscularly at 0X, 1X, 3X, or 5X the labeled dose for a total of three doses, each one given 2 days apart. The 0X group received saline at the 5X dose volume. There were no clinically significant adverse findings in any of the treatment groups. The study demonstrates that Pradalex™ is safe in nursery, growing, and finishing swine; gilts, sows, and boars intended for slaughter; and barrows when used according to the labeling.

Human Food Safety
The Food and Drug Administration (FDA) evaluated the microbial food safety of pradofloxacin injection for its intended uses in cattle and swine using a qualitative risk assessment. The hazard was defined as a foodborne illness in a person caused by bacteria that are resistant to fluroquinolones and originated from food products derived from cattle or swine treated with Pradalex™.

The qualitative risk assessment described the antimicrobial characteristics of pradofloxacin injection with respect to 1) promoting the emergence or selection of fluroquinolone-resistant bacteria of public health concern in or on treated cattle and swine; 2) the relative consumption quantities and bacterial contamination rates for food products derived from treated cattle and swine; and 3) its importance in human clinical medicine. FDA used the results from these components to derive an overall risk estimation of high for the intended uses of pradofloxacin injection in cattle and swine, and the Agency applied corresponding risk mitigators to the labeling and conditions of use for Pradalex™.

FDA reviewed data from pivotal toxicology studies covering systemic toxicity, reproductive toxicity, genotoxicity, carcinogenicity, photoirritation/photoimmunogenicity,

and immunotoxicity and from studies that assessed the impact of pradofloxacin injection on human intestinal flora. Based on the data, FDA established a toxicological acceptable daily intake (ADI) for pradofloxacin injection as 2 µg/kg body weight/day and a microbiological acceptable daily intake (mADI) as 58 µg/kg body weight/day. The toxicological ADI is the final ADI for total residue of pradofloxacin injection. The Agency also calculated the safe concentrations of total residue of pradofloxacin injection in cattle and swine as 0.4 parts per million (ppm) in muscle, 1.2 ppm in liver, 2.4 ppm in kidney, 2.4 ppm in skin/fat, and 4.0 ppm in injection site muscle.

The sponsor conducted one total residue and metabolism study and one residue depletion study in cattle and one total residue and metabolism study and one residue depletion study in swine to assess the quantity and nature of the residues in tissues derived from these species treated with pradofloxacin injection. FDA used the information from these studies, in combination with the ADI and safe concentrations, to establish a tolerance of 30 parts per billion of pradofloxacin injection in cattle kidney and 1 ppm in swine kidney, and a withdrawal period of 4 days in cattle and 2 days in swine.

FDA determined that there is a reasonable certainty of no harm for residues of pradofloxacin injection in the edible tissues of treated cattle and swine following human consumption when Pradalex™ is used according to the labeling.

User Safety
The labeling for Pradalex™ contains information for people who handle, administer, or are exposed to the drug. There is a risk of photosensitization within a few hours after excessive exposure to quinolones.

Conclusions
Based on the data submitted by the sponsor for the approval of Pradalex™, FDA determined that the drug is safe and effective when used according to the labeling.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-550

B. Sponsor

Elanco US Inc.
2500 Innovation Way
Greenfield, IN 46140

Drug Labeler Code: 058198

C. Proprietary Name

Pradalex™

D. Drug Product Established Name

pradofloxacin injection

E. Pharmacological Category

Antimicrobial

F. Dosage Form

Injectable solution

G. Amount of Active Ingredient

200 mg/mL

H. How Supplied

100 mL and 250 mL bottles

I. Dispensing Status

Prescription (Rx)

J. Dosage Regimen

Cattle: single dose of 10 mg/kg (2.3 mL/100 lb) body weight

Swine: single dose of 7.5 mg/kg (1.7 mL/100 lb) body weight

K. Route of Administration

Cattle: subcutaneous

Swine: intramuscular

L. Species/Classes

Cattle: For use in cattle intended for slaughter (beef calves 2 months of age and older, growing beef steers, growing beef heifers, and beef bulls intended for slaughter), and in cattle intended for breeding less than 1 year of age (replacement beef and dairy heifers less than 1 year of age and beef and dairy bulls less than 1 year of age). Not for use in cattle intended for breeding 1 year of age and older (replacement beef and dairy heifers 1 year of age and older, beef and dairy bulls 1 year of age and older, and beef and dairy cows), beef calves less than 2 months of age, dairy calves, and veal calves.

Swine: For use in weaned swine intended for slaughter (nursery, growing, and finishing swine, boars intended for slaughter, barrows, gilts intended for slaughter, and sows intended for slaughter). Not for use in swine intended for breeding (boars intended for breeding, replacement gilts, and sows intended for breeding) and in nursing piglets.

M. Indication

Cattle: Pradalex™ is indicated for the treatment of BRD associated with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis* in cattle intended for slaughter (beef calves 2 months of age and older, growing beef steers, growing beef heifers, and beef bulls intended for slaughter), and in cattle intended for breeding less than 1 year of age (replacement beef and dairy heifers less than 1 year of age and beef and dairy bulls less than 1 year of age). Not for use in cattle intended for breeding 1 year of age and older (replacement beef and dairy heifers 1 year of age and older, beef and dairy bulls 1 year of age and older, and beef and dairy cows), beef calves less than 2 months of age, dairy calves, and veal calves.

Swine: Pradalex™ is indicated for the treatment of SRD associated with *Bordetella bronchiseptica, Glaesserella (Haemophilus) parasuis, Pasteurella multocida, Streptococcus suis,* and *Mycoplasma hyopneumoniae* in weaned swine intended for slaughter (nursery, growing, and finishing swine, boars intended for slaughter, barrows, gilts intended for slaughter, and sows intended for slaughter). Not for use in swine intended for breeding (boars intended for breeding, replacement gilts, and sows intended for breeding) and in nursing piglets.

II. EFFECTIVENESS

A. Dosage Characterization

1. Cattle

A dose titration study (Study Number 141.375) was conducted in California in 1999 to determine an effective dose of pradofloxacin to administer subcutaneously for the treatment of BRD. One hundred fifty calves exhibiting clinical signs of BRD randomly received one of the following: no treatment (negative control), 5 mg pradofloxacin/kg body weight (BW), 7.5 mg

pradofloxacin/kg BW, 10 mg pradofloxacin/kg BW, or 7.5 mg enrofloxacin/kg BW (active control). The animals were scored (respiration rate, attitude, and appetite) and rectal temperature was measured daily from Study Day (SD) 1 through SD 10. More pradofloxacin-treated animals were classified as treatment successes than saline-treated animals. Among animals treated with pradofloxacin, the best response was observed at the 10 mg pradofloxacin/kg BW dosage. Therefore, the 10 mg pradofloxacin/kg BW dosage was used for a pilot effectiveness study.

A pilot effectiveness study (Study Number 201218) conducted in Idaho in 2013 evaluated the effectiveness of pradofloxacin at 10 mg/kg BW for the treatment of BRD. Ninety (90) crossbred beef cattle met study inclusion criteria of exhibiting clinical signs of BRD at enrollment (SD 0). After randomization, 45 cattle received 10 mg/kg BW of pradofloxacin and 45 cattle received an equivalent volume of physiological saline. All injections were administered once subcutaneously in the neck region. Effectiveness was based on the percentages of animals classified as treatment successes on SDs 7, 10, and 14. A treatment success was defined as any treated animal not previously determined to be a treatment failure (elevated attitude score, respiratory score, and rectal temperature). On SDs 7, 10, and 14, more pradofloxacin-treated animals were classified as treatment successes than saline-treated animals. On SD 7, treatment success was 71.1% (32/45 animals) for pradofloxacin compared with 25.6% for saline (11/43). On SD 10, treatment success was 60.0% (27/45) for pradofloxacin compared with 20.9% (9/43) for saline. On SD 14, treatment success for pradofloxacin was 44.4% (20/45) compared with 16.3% (7/43) for saline. The results of this study supported the decision to use a dosage of 10 mg pradofloxacin/kg BW in the study conducted to demonstrate substantial evidence of effectiveness.

2. Swine

A study (Study 201219) was conducted in Nebraska to evaluate the effectiveness of pradofloxacin for the treatment of naturally-occurring SRD associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Glaesserella (Haemophilus) parasuis*, and *Streptococcus suis*. One hundred forty (140) pigs with clinical signs of SRD, defined as a respiratory score ≥ 2 (on a scale from 0 [normal] to 3 [severe]), a depression score ≥ 2 (on a scale from 0 [normal] to 3 [severe]), and a rectal temperature of ≥ 104.0 °F, were randomly allocated to pens. Pigs were administered a solution containing 20% w/v pradofloxacin at 7.5 mg/kg BW or an equivalent volume of saline as a one-time intramuscular (IM) injection (SD 0). The primary effectiveness variable was treatment success, defined as pigs with a respiratory score ≤ 1, and a depression score ≤ 1, and a rectal temperature < 104.0 °F on SDs 3, 5, and 7. There was a significant difference in overall treatment successes ($p < 0.05$) in favor of the pradofloxacin-treated animals when compared with the saline-treated animals. The results of this study supported the decision to use a dosage of 7.5 mg pradofloxacin/kg BW in the studies conducted to demonstrate substantial evidence of effectiveness.

B. Substantial Evidence

1. Natural Infection Field Study - Cattle

Title: A Pivotal Clinical Efficacy Study of Pradofloxacin Injectable Solution for the Treatment of Naturally Occurring Bovine Respiratory Disease Associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis* in Cattle. (Study No. 202518)

Study Dates: December 2014 to December 2015

Study Locations: Oakland, NE (two sites); Canyon, TX; Tulare, CA; Manhattan, KS

Study Design:

Objective: To evaluate the effectiveness of pradofloxacin injection for the treatment of naturally occurring BRD associated with *M. haemolytica, P. multocida, H. somni,* and *M. bovis.*

Study Animals: A total of 630 bull, steer, and heifer calves, at least 4 months of age, with body weights ranging from 340 to 602 lbs, were enrolled across five study sites. Each site obtained a sufficient number of study candidates to enroll 100 to 140 animals per site. Study candidates were commercial-type calves available in the geographic area, sourced from commercial stockyards, auctions, and sale barns, and transported to a study site. Calves were individually identified with duplicate uniquely numbered ear tags at each site. Calves received vaccines, antiparasitics (including coccidiostats), and implants at arrival, according to the normal incoming processing procedures typical for that study site.

Experimental Design: The study was conducted according to Center for Veterinary Medicine (CVM) Guidance for Industry (GFI) #85 “Good Clinical Practice” (Veterinary International Conference on Harmonization (VICH) Guideline (GL) 9), as well as site-specific standard operating procedures (SOPs) and forms. The study was conducted at five sites in three different geographic regions. Candidate animals were examined daily for clinical signs of BRD, including respiratory character, depression, and body temperature. The Respiratory and Depression Score scales are found in Table II.1 and Table II.2 below. Animals were enrolled when they exhibited clinical signs of BRD based on the following criteria:

Depression Score of ≥ 2 AND Rectal Temperature ≥ 104.0 °F

OR

Respiratory Score ≥ 2 AND Rectal Temperature ≥ 104.0 °F.

At each site, enrolled calves were randomized in a ratio of 1:1 to receive either pradofloxacin injection or saline. Five animals from each treatment group were assigned per pen. Each site enrolled 10 to 14 pens.

Study personnel involved in the collection, recording, or interpretation of clinical observations, and the observing or recording of any adverse events were masked to treatment assignment of animals. Personnel dispensing and/or administering treatments and any study personnel witnessing treatment administration were unmasked to study treatments. Unmasked study personnel were not involved in collection or recording of clinical observations, and the observing and recording of any adverse events.

Drug Administration: The test article was pradofloxacin injection (200 mg pradofloxacin/mL). The negative control article was sterile saline (0.9% NaCl). Calves received either pradofloxacin injection once at 10 mg/kg BW by subcutaneous (SC) injection or saline once at 0.05 mL/kg BW by SC injection at the time of enrollment (SD 0). Across the study, 315 animals received pradofloxacin and 315 received saline.

Measurements and Observations: Animals were observed daily for signs of BRD from SD 3 until SD 10. From SD 3 through SD 10, animals that had a Respiratory or Depression Score ≥ 2 had their clinical scores recorded and their temperature measured and recorded. The following scales were used to evaluate respiratory character (Table II.1) and depression (Table II.2):

Table II.1. Respiratory character clinical scoring scale

Clinical Score	Respiratory Score
0	Normal: No abnormal respiratory symptoms present. Respiratory rate and effort appropriate for the environment.
1	Mild Respiratory Distress: Serous nasal or ocular discharge and/or cough.
2	Moderate Respiratory Distress: Mucous or mucopurulent nasal or ocular discharge and/or increase in respiratory rate or effort.
3	Severe Respiratory Distress: Marked increase in respiratory rate or effort including one or more of the following: open-mouth breathing, abdominal breathing, or head extended.

Table II.2. Depression clinical scoring scale

Clinical Score	Depression Score
0	Normal: Bright, alert, and responsive.
1	Mild Depression: May stand isolated with head down or ears drooping, but quickly responds to minimal stimulation.
2	Moderate Depression: May remain recumbent or stand isolated with head down and/or show signs of muscle weakness (standing cross-legged or knuckling or swaying when walking). Depression obvious when stimulated.

Clinical Score	Depression Score
3	Severe Depression: May be recumbent and reluctant to rise, or if standing isolated, reluctant to move. When moving is ataxic, knuckling, or swaying. Head carried low with ears drooping. Eyes dull, possible excess salivation and/or lacrimation, obvious gauntness.
4	Moribund: Unable to stand; approaching death; highly unlikely to respond to any antimicrobial therapy.

Microbiologic samples were collected from all enrolled animals during incoming processing procedures (pretreatment) and from all treatment failures (posttreatment) via a double-guarded, deep nasopharyngeal swab. Lung tissue samples were collected from mortalities (found dead or euthanized) on SDs 1 through 10. The swabs and lung tissue samples were submitted to the diagnostic laboratory to determine the presence of *M. haemolytica, P. multocida, H. somni,* and *M. bovis*. Target pathogens were identified using biochemical methods.

Statistical Methods: The individual animal was the experimental unit of analysis. 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, pen nested within site, treatment by site interaction, and treatment by pen nested within site interaction as random effects. Treatment success was evaluated using a two-sided test at alpha = 0.05.

The primary variable for determining effectiveness was treatment success. Animals that became moribund or were suffering severe respiratory distress were removed from the study and euthanized at any time posttreatment. These animals were classified as treatment failures if removed for reasons related to BRD on SDs 1 through 10. An animal was also classified as a treatment failure if on any day from SD 3 through SD 9 it exhibited either of the following:

Depression score ≥ 2 AND rectal temperature ≥ 104.0 °F

OR

Respiratory score ≥ 2 AND rectal temperature ≥ 104.0 °F.

A treatment success was defined as any animal that had not previously been determined to be a treatment failure on SDs 1 to 9 and that was scored as follows on SD 10:

Depression score ≤ 1 AND

Respiratory score ≤ 1 AND

Rectal temperature < 104.0 °F.

Results: Six calves were removed from the study and excluded from the effectiveness analysis: one for non-BRD illness, one for an inclusion criteria

protocol deviation, and four for data collection protocol deviations. A statistically significantly different (p = 0.0089) and numerically greater success rate (based on back-transformed least squares means) was detected for the pradofloxacin-treated group (49.7%) compared with the saline-treated group (25.6%). Counting only a single isolate of a given pathogen from each enrolled calf, a total of 365 isolates of *M. haemolytica*, 248 isolates of *P. multocida*, 106 isolates of *H. somni*, and 159 isolates of *M. bovis* were recovered.

Adverse Reactions: No test article-related adverse reactions were reported in this study.

Conclusions: This study demonstrates that pradofloxacin injection administered once as a SC injection at 10 mg/kg BW is effective for the treatment of BRD associated with *M. haemolytica*, *P. multocida, H. somni,* and *M. bovis* in weaned beef cattle, weaned dairy bulls, and weaned replacement dairy heifers.

2. Natural Infection Field Study - Swine

Title: A Pivotal Clinical Efficacy Study of Pradofloxacin Injectable Solution in Swine for the Treatment of Naturally-Occurring Swine Respiratory Disease Associated with *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, *Haemophilus parasuis*, *Pasteurella multocida*, *Streptococcus suis*, and *Mycoplasma hyopneumoniae.* (Study No. 203574)

Study Dates: October 2016 to November 2019

Study Locations: Manhattan, KS; Dalhart, TX; Oakland, NE; Rice, MN; Tulare, CA; Ames, IA; and Terre Haute, IN

Study Design:

Objective: To evaluate the effectiveness of pradofloxacin administered as a single IM injection for the treatment of naturally-occurring SRD associated with *A. pleuropneumoniae*, *B. bronchiseptica*, *G. parasuis*, *P. multocida*, *S. suis*, and *M. hyopneumoniae.*

Study Animals: A total of 1200 commercial crossbred weaned barrows and gilts, 3.5 to 13 weeks of age and weighing 5.9 to 86.0 lbs, were enrolled across 10 study sites. Pigs were subjected to the normal environmental conditions, feeding methods, and management practices of their location.

Experimental Design: The study was conducted in accordance with GFI #85 “Good Clinical Practice” (VICH GL9). The study was a randomized, masked, multisite, natural infection field study. Pigs that met inclusion criteria (see description in Measurements and Observations below) were assigned to the first available pen, randomized to treatment groups, and administered their assigned treatment on SD 0. At each site, pigs were randomly assigned to pens and treatment groups in a 1:1 ratio (5 pradofloxacin-treated and 5 saline-treated pigs per pen). Each site enrolled 10 to 14 pens.

Drug Administration: The test article was pradofloxacin injection (22.7% w/v pradofloxacin trihydrate for a 20% w/v solution of pradofloxacin), as the final

intended market formulation. The control product was physiological normal saline (0.9% NaCl) for injection. The treatment groups are detailed in Table II.3 below.

Table II.3. Treatment groups

Treatment Group	Treatment Regimen	Number Treated
pradofloxacin	7.5 mg/kg (0.017 mL/lb) BW administered once as an IM injection in the neck on SD 0	600
saline	0.017 mL saline/lb BW (volume equivalent to the test article) administered once as an IM injection in the neck on SD 0	600

Measurements and Observations: General health observations were conducted on all pigs on the day of arrival at the study facility, then twice daily until the end of the study (SD 7). From arrival to SD 0, candidate pigs were observed twice daily for signs of SRD. Candidate pigs were enrolled if they had a respiratory score ≥ 2 (on a scale from 0 [normal] to 3 [severe]), a depression score ≥ 2 (on a scale from 0 [normal] to 3 [severe]), and a rectal temperature of ≥ 104.0 °F. Body weights were recorded on SD 0 and used to determine the dose of test article or volume of saline to administer. All pigs were evaluated for treatment success on SD 7 and then euthanized. Microbiologic samples were collected from five pigs that met enrollment criteria at each site on or just prior to SD 0, from all pigs that died or were removed prior to SD 7, and from all remaining pigs on SD 7. Pleural swabs and duplicate lung tissue samples were collected at necropsy from all pigs that were found dead or euthanized. Additionally, lung samples were cultured for *M. hyopneumoniae* and confirmed positive for *M. hyopneumoniae* by polymerase chain reaction.

Statistical Methods: The experimental unit of analysis was the individual animal. The primary effectiveness variable was treatment success. Pigs were classified as a treatment success if on SD 7 they had a respiratory score ≤ 1, and a depression score ≤ 1, and a rectal temperature < 104.0 °F. Pigs that died or were removed prior to SD 7 were considered treatment failures and included in the effectiveness analysis unless the cause was shown to be unrelated to SRD.

Statistical evaluations were conducted using a two-sided test at an alpha = 0.05. 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, site, pen(site), treatment-by-site interaction, and treatment-by-pen(site) interaction as random effects. Percent success and 95% confidence intervals were estimated.

Results: Twenty-nine pigs were removed from the analysis because of a protocol deviation related to inclusion criteria. Effectiveness was evaluated in a total of 1171 pigs across ten sites (584 pigs in the pradofloxacin-treated group and 587 pigs in the saline-treated group). There was a significant difference in SD 7 treatment success ($p = 0.0274$) in favor of the pradofloxacin-treated pigs compared with the saline-treated pigs. The least squares means calculated

percent success was 45.2% and 34.2% for the pradofloxacin-treated groups and the saline-treated groups, respectively.

A total of 111 isolates of *B. bronchiseptica*, 93 isolates of *G. parasuis*, 212 isolates of *S. suis,* 99 isolates of *P. multocida,* and 37 isolates of *M. hyopneumoniae* were identified in study pigs. There were no isolates of *A. pleuropneumoniae* identified in study pigs.

Adverse Reactions: No test article-related adverse reactions were reported in this study.

Conclusion: This study demonstrated that pradofloxacin injection administered once at 7.5 mg pradofloxacin/kg BW as an IM injection is effective for the treatment of SRD associated with *B. bronchiseptica*, *G. parasuis*, *P. multocida*, *S. suis*, and *M. hyopneumoniae* in weaned swine.

3. Induced Infection Challenge Model Study – Swine

Title: The Efficacy of Pradofloxacin Injectable Solution in Swine for the Treatment of an Experimentally Induced *Mycoplasma Hyopneumoniae* Infection. (Study No. 202011)

Study Dates: February 2017 to November 2019

Study Location: Manhattan, KS

Study Design:

Objective: The objective of this study was to demonstrate that a single IM dose of pradofloxacin injectable solution administered at 7.5 mg/kg BW has a specific effect against *M. hyopneumoniae* by evaluating lung lesions following an experimentally-induced infection.

Study Animals: Seventy-two healthy female and castrated male crossbred pigs were enrolled in the study. Pigs were approximately 8 to 10 weeks of age and weighed 44.1 to 96.4 lbs at the time of the test article administration. All candidate pigs were serologically negative for *M. hyopneumoniae*. Pigs were subjected to the normal environmental conditions, feeding methods, and management practices of the location.

Experimental Design: The study was conducted in accordance with GFI #85 “Good Clinical Practice” (VICH GL9). This study was a single location, placebo-controlled, masked, randomized challenge model study. One hundred twenty-six candidate pigs arrived at the study site on SD -12 and were randomized to 12 pens (9 to 11 pigs per pen) to begin an acclimation period. The *M. hyopneumoniae* challenge inoculum was administered to all candidate pigs for 3 consecutive days (SDs -5, -4, and -3). When 5% of the inoculated pigs were observed with occasional coughing in a single day and four of five randomly selected sentinel pigs each had a total lung lesion score ≥ 5%, six pigs from each pen were randomized to treatment in a 1:1 ratio (three pradofloxacin-treated and three saline-treated pigs per pen) and the remaining pigs were removed from the pens (SD 0).

Infection Challenge Administration: Pigs were administered, by endotracheal and intranasal administration, an inoculum which contained an isolate of *M. hyopneumoniae* that had been previously demonstrated to induce lung lesions representative of those expected with natural infection. The inoculum consisted of crude lung homogenate obtained from a healthy, cesarean section-derived, colostrum-deprived pig.

Drug Administration: The test article was pradofloxacin injection (22.7% w/v pradofloxacin trihydrate for a 20% w/v solution of pradofloxacin), as the final intended market formulation. The control product was physiological normal saline (0.9% NaCl) for injection. The treatment groups are detailed in Table II.4 below.

Table II.4. Treatment groups

Treatment Group	Treatment Regimen	Number Treated
pradofloxacin	7.5 mg/kg BW (0.017 mL/lb) BW administered once as an IM injection in the neck on SD 0	36
saline	0.017 mL saline/lb BW (volume equivalent to the test article) administered once as an IM injection in the neck on SD 0	36

Measurements and Observations: During the acclimation, treatment, and posttreatment period, pigs were observed twice daily for general health observations. Body weights were recorded on SD 0 to determine the dose/volume of the test and control articles. Coughing, depression, and respiratory scores were recorded once on SDs -6, 0, and 10, and twice daily on SDs 3 through 9, but were not used in the analysis. All pigs were euthanized and necropsied at 10 days posttreatment (SD 10) for evaluation of lung lesions.

Statistical Methods: The experimental unit of analysis was the individual animal. The primary variable was total lung lesion score, calculated as the sum of the lung lesion percentage observed in each lobe multiplied by the approximate volume that each lobe contributes to the entire lung volume (left apical lobe – 10%, left cardiac lobe – 10%, left diaphragmatic lobe – 25%, right apical lobe – 10%, right cardiac lobe – 10%, right diaphragmatic lobe – 25%, and accessory lobe – 10%). Statistical evaluations were conducted using a two-sided test at an alpha = 0.05. For the primary variable, a linear mixed model with fixed effect of treatment and random effects of pen and pen-by-treatment interaction was used. The estimated mean total lung lesion scores were obtained from the reversed transformation of the arcsine square root of the least squared means.

Results: One pig in the saline-treated group became non-ambulatory, was removed from the study on SD 4, and excluded from the analysis. There was a significant difference ($p = 0.0002$) in the mean total lung lesion score in favor of the pradofloxacin-treated pigs (11.7%) compared with the saline-treated pigs (33.1%).

Adverse Reactions: No test article-related adverse reactions were reported in this study.

Conclusion: This study demonstrates that pradofloxacin injection administered once at 7.5 mg/kg BW as an IM injection decreased lung lesions associated with *M. hyopneumoniae* in swine.

III. TARGET ANIMAL SAFETY

A. Margin of Safety Study - Cattle

Title: Pivotal Safety of a 20% Pradofloxacin Injectable Solution Following Subcutaneous Administration to Cattle. (Study No. 202460)

Study Dates: December 2014 to January 2016

Study Location: Oakland, NE

Study Design:

Objective: To evaluate the acute clinical and pathological effects associated with the subcutaneous administration of pradofloxacin injection to crossbred steers and heifers at doses of 0, 10, 30, and 50 mg/kg BW on SDs 0, 4, and 8.

Study Animals: A total of 32 (16 steers and 16 heifers) healthy, weaned, acclimated, Angus-cross calves, sourced from a single, commercial Midwestern cow/calf producer, were selected from a candidate pool of 40 calves for study enrollment. On SD 0, calves were 4 to 5 months old with body weights between 158-207 kg.

Experimental Design: The study was conducted in compliance with the Good Laboratory Practice (GLP) Regulations set forth in 21 CFR Part 58. This pivotal, placebo-controlled, masked target animal safety study was based upon a randomized design. The study was conducted in 2 cohorts. Calves were randomly assigned to treatment groups by gender and randomized to individual pens within each cohort. On SDs 0, 4, and 8 of each cohort, calves were subcutaneously injected with a dose of test article (pradofloxacin) or control article (saline).

Drug Administration: The test article was pradofloxacin injection. Sterile saline (0.9% NaCl), dosed at 0.25 mL/kg BW (equivalent volume to the 5X treated group), served as the control article. Calves were randomized to 4 gender-balanced treatment groups: 0X (saline control), 1X (10 mg pradofloxacin/kg BW), 3X (30 mg pradofloxacin/kg BW) and 5X (50 mg pradofloxacin/kg BW). A total of three SC doses were administered, each one 4 days apart, at the following locations: right neck (SD 0), left neck (SD 4), and over the right ribs (SD 8). Treatment dosages were based on body weights from the day prior to each treatment. A maximum of 15 mL was administered at each injection site.

Measurements and Observations: Clinical observations were conducted once daily until SD -1, then twice daily on SDs 1 through 8. On treatment days (SDs 0, 4, and 8), clinical observations were conducted pretreatment and 2, 4, and 6 hours posttreatment. On SD 9, clinical observations were conducted once prior to euthanasia. Physical examinations, including evaluation of injection sites, were performed on SDs -7, 1, 5, and 9. Injection sites were evaluated for general appearance, swelling, and erythema, and were palpated for pain and heat. Body weights of all 40 study candidates were measured and recorded on several

occasions between SD -21 and -7. Thereafter, body weights for the 16 enrolled calves were measured on SDs -1, 3, 7, and 9. Feed and water consumption were measured once daily for each pen from SD 0 to SD 9.

Clinical pathology samples for hematology, coagulation, chemistry, urinalysis (females only), and fecal analysis were collected on several occasions pretreatment between SD -21 and -3. Thereafter, on SDs 0, 4, and 8, clinical pathology samples (with the exception of urine on SD 8) were collected 1 to 3 hours posttreatment. Urine was obtained from each calf via cystocentesis at necropsy on SD 9. For toxicokinetic (TK) analysis, whole blood was collected from all calves on SDs 0, 4, and 8 immediately pretreatment and 1, 2, 6, 10, and 24 hours after each treatment. Plasma pradofloxacin drug concentrations were measured using a validated liquid chromatography with mass spectrometry detection (LC-MS/MS) method. A noncompartmental analysis was performed using an extravascular dosing model.

On SD 9, calves were humanely euthanized and necropsied. All organ systems were grossly examined. Tissue specimens were collected, fixed, processed, stained, and examined microscopically.

Statistical Methods: The experimental unit of analysis was the individual calf for all analyzed data. Continuous variables measured multiple times were analyzed using a repeated measures analysis of covariance with the following fixed effects: pretreatment values as a covariate, treatment group, gender, time, and two- and three-way interactions. Animal identification was included in the models as a random effect. Covariance structure with the minimum value of the Akaike's Information Criterion was selected in the final analysis. Treatment effects, treatment by time, and treatment by sex were tested at the 0.1 level of significance. Treatment by time by sex was tested at the 0.05 level of significance, and if significant, no further statistical evaluations were performed. Continuous variables measured once were analyzed using an analysis of variance with treatment, sex, and sex by treatment interaction as fixed effects. Adjusted least square means (LSM) were presented to represent the magnitudes for various factors in the above model. Unless otherwise noted, all central tendencies are reported as adjusted least square means.

Results:

Clinical Observations: All saline-treated and most pradofloxacin-treated calves remained clinically normal throughout the study. Right shoulder swelling was reported in four pradofloxacin-treated calves and was attributable to trauma from the chute. Injection site swelling was documented in three pradofloxacin-treated calves from the 1X and 5X groups during the in-life period. The severity of injection site swelling showed a dose-related trend on pathology, but was not accompanied by observable pain, erythema, or heat on physical examination.

Body Weight: Between study initiation (SD -1) and study conclusion (SD 9), body weights increased by 5 to 20 kg in all calves.

Daily Feed and Water Consumption: Mean daily feed consumption was statistically different ($p \leq 0.05$) and numerically lower in the 5X group (5.81 kg) compared with the control group (6.66 kg). Mean daily water intake was numerically lower in the 5X group (2.75 gallons) as compared with the other treatment groups (0X = 3.82

gallons, 1X = 3.42 gallons, and 3X = 3.18 gallons), but was not statistically different. Changes in feed and water consumption were associated with injection site discomfort and inflammation and were not considered clinically relevant due to continued weight gain, acceptable body condition score, and evidence of adequate hydration.

Clinical Pathology: Most clinical pathology results were within the established reference intervals; statistically different changes were detected for the following variables:

Absolute Neutrophils - Compared with controls, neutrophil counts were significantly different ($p < 0.05$) and numerically higher in the 5X group and higher but not significantly different in both the 3X and 1X groups. The higher counts in the pradofloxacin-treated groups were considered to be related to injection site inflammation and were not considered clinically significant. Individuals' values outside the reference interval were very mild elevations and not associated with any trend.

Absolute Monocytes - Compared with controls, monocyte counts were significantly different ($p < 0.1$) and numerically higher in the 1X and 5X groups on SD 0 and in the 1X group on SD 4 ($p < 0.05$). The increased monocyte counts in the pradofloxacin-treated groups were considered to be related to injection site inflammation and were not considered clinically significant. Individuals' values outside the reference interval were very mild elevations and not associated with any trend.

Activated Partial Thromboplastin Time (APTT) - For females only, APTT values in the 1X group were significantly different ($p < 0.1$) and numerically higher (55.22 seconds) than the corresponding gender controls (46.28 seconds). Though statistically significant, the change was not considered clinically significant. There was no dose-related trend and there was no evidence of coagulopathy on physical examination.

Creatinine - Compared with the control creatinine values, the creatinine results of the 3X ($p < 0.01$) and 5X groups ($p < 0.05$) were statistically different and numerically higher. With the exception of some instances of transient and mild elevations above the reference range in individual animals, the values remained well within the reference range. These changes were not clinically significant and were associated with the decreased water consumption in the elevated dose groups.

Creatine Kinase - On SDs 0, 4, and 8, the creatine kinase results of most pradofloxacin-treated groups were significantly different ($p < 0.1$) and numerically greater than the corresponding control group results. This was associated with inflammation in the underlying muscle at the injection sites and was not clinically significant.

Potassium - On SD 4, potassium values were significantly different ($p \leq 0.05$) and numerically higher in the 1X group compared with the control group. On SD 8, all three pradofloxacin-treated groups had statistically different ($p \leq 0.1$) and numerically higher potassium values compared with the control group. These differences were not considered clinically significant. All LSMs were numerically comparable and were

within or slightly below the reference range, and there were no dose-related trends observed.

Urinalysis: Results were consistent with those expected for cattle and the methods of collection used in the study. There were no changes attributed to the test article.

Fecal analysis: No abnormal changes were observed during the posttreatment period.

Toxicokinetics: No accumulation occurred at a dosing interval of 4 days. Maximum plasma concentration (C_{max}) and area under the plasma concentration versus time curve from the time of dosing to the time of the last measurable concentration (AUC_{last}) were dose-proportional.

Table III.1. Plasma pradofloxacin pharmacokinetic parameters in weaned calves (N = 8 per group) following a 10, 30 or 50 mg/kg BW SC dose of pradofloxacin on SD 0

Pharmacokinetic Parameter	Pradofloxacin (10 mg/kg BW)	Pradofloxacin (30 mg/kg BW)	Pradofloxacin (50 mg/kg BW)
C_{max} (µg/mL)	1.9 (1 – 3.4)	5.2 (4.0 – 6.8)	9.0 (6.2 – 13.2)
T_{max}[a] (hours)	1 (1 to 2)	2 (1 to 2)	1.5 (1 to 2)
AUC_{last} (hr*µg/mL)	11.0 (8.6 – 13.9)	35.2 (27.1 – 45.6)	65.5 (48.6 – 88.2)
$t_{1/2}$ (hours)	3.0 (2.7 – 3.3)	3.2 (2.6 – 3.9)	3.7 (2.7 – 5.1)

[a] Values provided for T_{max} are median (and range). Values for the other parameters are geometric mean (95% confidence limits).
C_{max} = maximum concentration
T_{max} = time to maximum concentration
AUC_{last} = area under the curve from the time of dosing to the time of the last measurable concentration
$t_{1/2}$ = elimination half-life

Gross Necropsy and Histopathology: Macroscopic test article-related effects were limited to dark or red discoloration with or without edema at the three injection sites. The calculated lesion volumes showed a clear dose-related trend. Microscopically, the test-article injection site response was characterized by hemorrhage, tissue necrosis, and inflammation that progressed from acute to chronic. These changes were consistent with lesions typical for injectable drugs and were not considered clinically significant.

Macroscopic and/or microscopic lesions of fluoroquinolone-induced arthropathy were not found on the articular surfaces of stifle and elbow joints in cattle treated with any dose level of the test article.

Conclusions: The study demonstrates that pradofloxacin injection is safe for use in steers, female beef cattle, and replacement dairy heifers when administered once as a subcutaneous injection at 10 mg/kg BW.

B. Margin of Safety Study - Intact Male Cattle

Title: Pivotal Safety of a 20% Pradofloxacin Injectable Solution Following Subcutaneous Administration to Intact Male (Bull) Calves. (Study No. 2040006)

Study Dates: May 2017 to January 2019

Study Location: Oakland, NE

Study Design:

Objective: To evaluate the safety to the testes and epididymes of subcutaneous administration of pradofloxacin injection of weaned bull calves at doses of 0, 10, 30, and 50 mg/kg BW on SDs 0, 4, and 8.

Study Animals: A total of 16 healthy, weaned, Angus-cross bull calves, sourced from a single, commercial Midwestern cow/calf producer, were selected for study enrollment from a candidate pool of 23 calves. On SD 0, calves were approximately 5 months old with body weights between 228 and 319 kg.

Experimental Design: The study was conducted in compliance with the GLP Regulations set forth in 21 CFR Part 58. This controlled, masked margin of safety study was based upon a complete randomized design. Calves were randomly assigned to treatment groups and individual pens. On SDs 0, 4, and 8, calves were subcutaneously injected with a dose of test article (pradofloxacin) or control article (saline).

Drug Administration: The test article was pradofloxacin injection. Sterile saline (0.9% NaCl), dosed at 0.25 mL/kg BW (equivalent volume to the 5X treated group), served as the control article. Calves were randomized to 4 treatment groups: 0X (saline control), 1X (10 mg pradofloxacin/kg BW), 3X (30 mg pradofloxacin/kg BW) and 5X (50 mg pradofloxacin/kg BW). A total of three SC doses were administered, each one 4 days apart, at the following locations: right neck (SD 0), left neck (SD 4), and over the right ribs (SD 8). Treatment dosages were based on body weights from the day prior to each treatment. A maximum of 15 mL was administered at each injection site.

Measurements and Observations: Clinical observations were conducted once daily until SD -1, then twice daily on SDs 1 through 8. On treatment days (SDs 0, 4, and 8), clinical observations were conducted pretreatment and 2, 4, and 6 hours posttreatment. On SD 9, clinical observations were conducted once prior to euthanasia. Physical examinations, including evaluation of injection sites, were performed on SDs -3, 1, 5, and 9. Injection sites were evaluated for general appearance, swelling, and erythema, and were palpated for pain and heat. Body weights for the 16 enrolled calves were measured and recorded on SDs -7, -3, -1, 3, 7, and 9.

Plasma samples were collected from each calf predose, and at 1, 2, 6, 10, and 24 hours postdose on each treatment day. Plasma drug concentrations were measured using a validated LC-MS/MS method. A noncompartmental analysis was performed using an extravascular dosing model.

On SD 9, calves were castrated and testes and epididymes were collected and grossly examined. Tissue specimens were fixed, processed, stained, and examined microscopically.

Statistical Methods:

The experimental unit of analysis was the individual calf for all analyzed data. Appropriate descriptive statistics were presented for all applicable variables. Body weights were analyzed by a repeat measures analysis of covariance (pretreatment body weights served as the covariate) with treatment, day, treatment-by-day in the model as fixed effects, and animal identified as the subject in the repeated statement. The covariance structure with the minimum value of the Akaike's Information Criterion was selected in the final analysis. Organ weights were analyzed using an analysis of covariance with treatment in the model as a fixed effect. SD 9 body weights served as the covariate.

For organ weights, all pairwise comparisons of active treatment groups with the control were evaluated at an unadjusted alpha = 0.10 if the overall treatment effect was significant. For body weights, if treatment x day interactions were significant at alpha = 0.10 level, then pairwise comparisons of each treatment group against control for each day at an unadjusted alpha = 0.10 were performed. If the treatment x day interactions were not significant, then the main effect of treatment group was evaluated at alpha = 0.10 level.

Data collected from histopathologic evaluation of testicular and epididymal tissues was evaluated, listed, and summarized using frequency tables arranged by treatment group and animal identification.

Results:

Clinical Observations: All saline-treated and pradofloxacin-treated calves remained clinically normal throughout the study. There were seven instances of immediate response during injection of either bellowing and/or flinching in pradofloxacin-treated groups. Injection site swelling was documented in fifteen instances for pradofloxacin-treated calves from the 1X, 3X, and 5X groups during the study. The injection site swelling was not accompanied by observable pain, erythema, or heat on physical examination. In the 1X group, 3 of 4 calves developed mild to moderate subcutaneous swellings, one of which resolved by 6 hours posttreatment and two of which were not resolved by 5 days posttreatment at the end of the study. The injection-related pain and swelling observed during the study was consistent with that seen following administration of other injectable drugs and was not considered clinically significant.

Body Weight: Between study initiation (SD -1) and study conclusion (SD 9), body weights increased in all calves in all four treatment groups except one calf (1X group), whose weight at the end of the study was the same as on SD -1. The pattern of weight gain seen in the study calves was consistent with that expected in healthy untreated calves.

Anatomic pathology and organ weights: No macroscopic nor microscopic abnormalities were observed in the testes or epididymis of any animal. There were

no statistically significant differences in SD 9 testicular weights and epididymal weights between treatment groups.

Toxicokinetics:

Following multiple SC injections of pradofloxacin, mean pradofloxacin C_{max} and AUC_{last} values increased with increasing dose in an approximately dose proportional manner on SDs 0, 4 and 8 over the 10 to 50 mg/kg BW dose range. There was no accumulation following repeated SC injections of pradofloxacin on SDs 4 and 8. Following multiple SC injections of pradofloxacin, mean pradofloxacin half-life ($t_{½}$) values appeared similar between dose groups and days.

Table III.2. Plasma pradofloxacin pharmacokinetic parameters in weaned bull calves (N = 3 or 4 per group) following a 10, 30, or 50 mg/kg BW SC dose of pradofloxacin on SD 0

Pharmacokinetic Parameter	Pradofloxacin (10 mg/kg BW)	Pradofloxacin (30 mg/kg BW)	Pradofloxacin (50 mg/kg BW)
C_{max} (µg/mL)	1.9 (1.0 – 3.5)	5.6 (3.2 – 9.7)	8.7 (3.5 – 21.2)
T_{max}[a] (hours)	1 (1 to 2)	1.5 (1 to 2)	1.0 (1 to 2)
AUC_{last} (hr*µg/mL)	9.4 (8.3 – 10.5)	37.3 (22.5 – 62.0)	60.5 (30.0 – 122.0)
$t_{1/2}$ (hours)	2.3 (1.4 – 3.7)	3.2 (2.6 – 4.0)	3.2 (1.9 – 5.9)

[a] Values provided for T_{max} are median (and range). Values for the other parameters are geometric mean (95% confidence limits).
C_{max} = maximum plasma concentration
T_{max} = time to maximum plasma concentration
AUC_{last} = area under the curve from the time of dosing to the time of the last measurable concentration
$t_{1/2}$ = elimination half-life

Conclusions: This study demonstrates an adequate margin of safety of pradofloxacin injection for the reproductive tissues of male calves less than one year of age when administered once as a SC injection of 10 mg/kg BW. Together, this study and the margin of safety study summarized above, demonstrate that pradofloxacin injection is safe in beef bulls intended for slaughter and beef and dairy bulls intended for breeding less than one year of age when administered once as a SC injection of 10 mg/kg BW. This study did not evaluate the reproductive safety of pradofloxacin injection in beef and dairy bulls intended for breeding over one year of age.

C. Margin of Safety Study - Swine

Title: Safety of a 20% Pradofloxacin Injectable Solution Following Intramuscular Administration to Early Weaned Pigs. (Study No. 203637)

Study Dates: May 8, 2017 to November 5, 2018

Study Location: Oakland, NE

Study Design:

Objective: To evaluate the safety of IM administration of pradofloxacin injection to newly-weaned pigs at doses of 0, 7.5, 22.5, and 37.5 mg/kg BW on SDs 0, 2, and 4.

Study Animals: A total of 32 (16 boars and 16 gilts) healthy, weaned, crossbred piglets, sourced from a single farrowing facility were selected from a candidate pool of 40 piglets for study enrollment. On SD 0, piglets were 19 days old with body weights between 5.5 and 7.9 kg.

Experimental Design: The study was conducted in compliance with the GLP Regulations set forth in 21 CFR Part 58. This controlled, masked margin of safety study was based upon a complete randomized design blocked by gender. Piglets were randomly assigned to treatment groups and pens, with 4 pigs of a single gender per each of 8 pens. On SDs 0, 2, and 4, piglets were intramuscularly injected with a dose of test article (pradofloxacin) or control article (saline).

Drug Administration: The test article was pradofloxacin injection. Sterile saline (0.9% NaCl), dosed at 0.1875 mL/kg BW (equivalent volume to the 5X treated group), served as the control article. Piglets were randomized to 4 gender-balanced treatment groups: 0X (saline control), 1X (7.5 mg pradofloxacin/kg BW), 3X (22.5 mg pradofloxacin/kg BW) and 5X (37.5 mg pradofloxacin/kg BW). A total of three IM doses were administered, each one 2 days apart, at the following locations: cranial half of right neck (SD 0), center of left neck (SD 2), and caudal half of right neck (SD 4). Treatment dosages were based on body weights taken just prior to each treatment. A maximum of 5 mL was administered at each injection site.

Measurements and Observations: Clinical observations were conducted once daily until SD -1, then twice daily on SDs 1, 3, and 5 through 10. On treatment days (SDs 0, 2, and 4), clinical observations were conducted pretreatment and 2, 4, and 6 hours posttreatment. On SD 11, clinical observations were conducted once prior to euthanasia. Physical examinations, including evaluation of injection sites, were performed on SDs -2, 1, 3, 5, and 10. Body weights of all 40 study candidates were measured and recorded on several occasions between SD -18 and -1. Thereafter, body weights for the 32 enrolled piglets were measured on SDs 0, 2, 4, 6, 8, and 10. Feed and water consumption were measured once daily for each pen on SDs 0 through 11.

Pre-study clinical pathology samples for hematology, coagulation, and chemistry, were collected on SD -7, with hematology samples for 9 piglets redrawn on SD -4 due to clotting of the original samples on SD -7. On SDs 0, 2, and 4, clinical pathology samples were collected 2 to 4 hours posttreatment, and on SD 10 a final set of samples were collected. Urine samples were collected on SD -1 from as many

of the 40 study candidates as possible. On SDs 0, 2, and 4, posttreatment urine samples were collected from as many of the 32 study pigs as possible. On SD 11, all 32 enrolled animals had urine collected via cystocentesis at necropsy. On SDs 0, 1, 3, 5, and 10, fecal samples were collected from each pen. Toxicokinetic analysis was performed on whole blood collected from all piglets immediately pretreatment and 0.5, 1, 2, 6, 10, and 24 hours after each treatment on SDs 0, 2, and 4. On SD 4, samples at the 0.5 hour and 2 hour time points were not collected to reduce stress due to handling. Plasma samples collected were analyzed for pradofloxacin concentrations using a validated LC-MS/MS method. A noncompartmental analysis was performed using an extravascular dosing model.

On SD 11, piglets were humanely euthanized and necropsied. All organ systems were grossly examined and organ weights were measured. Tissue specimens were collected, fixed, processed, stained, and examined microscopically.

Statistical Methods: The experimental unit of analysis was the individual pig for all analyzed data except for feed and water consumption. All continuous variables were analyzed using a mixed model analysis. Variables measured multiple times were analyzed using a repeated measures analysis of covariance with treatment, gender, and time (and possible 2- and 3-way interactions) as fixed effects and animal identification as the subject in the repeated statement. Pretreatment values were used as a covariate and remained in the model regardless of significance.

Endpoints measured once posttreatment and without a pretreatment value (e.g., organ weights) were analyzed using analysis of covariance with treatment, gender, and the interaction of treatment x gender as fixed effects.

The covariance structure was investigated using four structural assumptions: compound symmetry (CS), CS heterogeneous (CSH), first order autoregressive [AR(1)], and heterogeneous first order autoregressive [ARH(1)]. The covariance structure giving the minimum value of the Akaike's Information Criterion was selected in the final analysis. For each outcome class, a representative variable within the class (e.g., glucose values for serum chemistry) was used in the assessment of the covariance structure. The structure appropriate for this selected variable was used for the remaining variables within that outcome class.

All pairwise comparisons treatment groups with controls were tested at the 0.10, 0.05, and 0.01 levels of significance with the 0.1 level considered statistically significant. Statistical comparison of treatment x time x gender was performed at the 0.05 level of significance. Statistical comparison of two-way interactions (treatment x gender and treatment x day) were performed at the 0.1 level of significance.

Results:

Clinical Observations and Physical Exams: All control and pradofloxacin-treated pigs remained clinically normal throughout the study. All injection sites remained clinically normal with no evidence of swelling or pain throughout the study.

Body Weight: Between study initiation (SD -1) and study conclusion (SD 11), body weights increased in all pigs in all four treatment groups, ranging from 0.2 to 2.9 kg.

Daily Feed and Water Consumption: Treatment did not have a statistically significant effect on feed consumption. However, on SDs 0 and 1, mean feed consumption was minimal (0.0 to 0.25 kg) in all pens. Thereafter, from SDs 2 to 10, mean feed consumption per pen generally increased each day (between 0.1 to 0.6 kg). The decreased feed consumption on SDs 0 and 1 was not considered related to the test article. The pigs were weaned on SD -1 and it is common for newly-weaned pigs to take 24 to 48 hours to become accustomed to a dry, pelleted ration. In addition, there was no evidence of pain or discomfort due to injections that could have contributed to the inappetence on SDs 0 and 1.

Treatment did not have a statistically significant effect on mean water consumption/usage per pen. Mean daily water consumption/usage per pen per day was fairly consistent from study initiation (3.25 to 4.8 kg) to study conclusion (5.75 to 7.0 kg).

Clinical Pathology: Statistically significant treatment main effects or interactions were detected for several clinical pathology variables. The treatment by day interaction was statistically significant for hematocrit, hemoglobin, mean corpuscular volume, and red blood cell count. These indicators did not show any negative effect of treatment and were not accompanied by any clinical signs. The main effect of treatment was statistically significant for absolute lymphocyte counts, absolute monocyte counts, and white blood cell counts. Individual values outside the reference interval were mild, transient decreases, and were not considered clinically significant.

Aspartate aminotransferase (AST) and creatine phosphokinase (CK) showed statistically significant treatment by day interactions. These findings were likely a transient change related to injection site injury. Attributing these results to the muscle's response to injection of pradofloxacin is corroborated by the gross and microscopic pathology results; both showed test article effects at the injection site but not in the liver. There were no other clinically relevant effects on the remaining hematology, chemistry, or coagulation indices.

Urinalysis: Throughout this study, the urinalysis results between the control and pradofloxacin-treated groups were similar. No treatment-related effects were observed.

Fecal analysis: Fecal evaluations were normal at all collection time points.

Toxicokinetics: Pradofloxacin was dose-proportional over a 7.5-37.5 mg/kg BW dose range. There was no accumulation when administered once every 2 days for 4 days. Following multiple IM injections of pradofloxacin, mean pradofloxacin $t_{1/2}$ values were similar between dose groups and days.

Table III.3. Plasma pradofloxacin pharmacokinetic parameters in early weaned pigs (N = 8 per group) following a 7.5, 22.5, and 37.5 mg/kg BW IM dose of pradofloxacin on SD 0

Pharmacokinetic Parameter	Pradofloxacin (7.5 mg/kg)	Pradofloxacin (22.5 mg/kg)	Pradofloxacin (37.5 mg/kg)
C_{max} (µg/mL)	2.5 (2.1 – 3.0)	7.4 (5.2 – 10.5)	11.2 (9.1 – 13.9)
T_{max}[a] (hours)	0.75 (0.5 to 2)	0.75 (0.5 to 2)	1.0 (0.5 to 2)
AUC_{last} (hr*µg/mL)	25.9 (18.8 – 35.7)	90.5 (71.9 – 11.1)	142 (107 – 188)
$t_{½}$ (hours)	8.2 (4.4 – 15.4)	10.8 (7.4 – 15.8)	8.9 (5.0 – 15.9)

[a] Values provided for T_{max} are median (and range). Values for the other parameters are geometric mean (95% confidence limits).
C_{max} = maximum concentration
T_{max} = time to maximum concentration
AUC_{last} = area under the curve from the time of dosing to the time of the last measurable concentration
$t_{1/2}$ = half-life

Gross Necropsy and Histopathology: Macroscopic test article-related effects were limited to the injection sites on the neck, which were described as firm and either pale (associated with treatments on SDs 0, 2) or dark / hemorrhagic (associated with treatments on SDs 2, 4). The calculated lesion volumes showed a clear dose-related trend. Only minimal reactions developed in controls, including a small abscess (0.25 cm^3) found at the IM injection site of a control pig.

Microscopically, test article-related effects were also found at the injection sites. Reactions were generally characterized by a central area of coagulative necrosis surrounded by a broad zone of fibrosis, inflammation (infiltration by lymphocytes, macrophages, scattered neutrophils), and hemorrhage. The microscopic lesions principally involved muscle tissue but occasionally spread to the intermuscular fascia. Microscopic pathology was similar at all dose levels of the test article but was more extensive (higher severity grades) at increasing doses. These changes were consistent with lesions typical for injectable drugs and were not considered clinically significant.

Macroscopic and/or microscopic lesions of fluoroquinolone-induced arthropathy were not found on the articular surfaces of stifle and elbow joints in pigs treated with any dose level of the test article.

There were no statistically significant effects of treatment on organ weight. A statistically significant treatment effect was found in the testes/brain weight ratio and a significant treatment by gender interaction was found in the adrenals (paired)/brain weight ratio. The differences were not attributable to test article exposure because they were not associated with adverse gross or microscopic observations for the involved organs.

Conclusion: This study demonstrates that pradofloxacin injection is safe in nursery, growing, and finishing swine; gilts, sows and boars intended for slaughter; and barrows, when administered once as an IM injection of 7.5 mg/kg BW.

IV. HUMAN FOOD SAFETY

A. Microbial Food Safety

Cattle

Background and outcome of risk assessment:

The Agency evaluated microbial food safety information and data for pradofloxacin, "*for treatment of bovine respiratory disease (BRD) associated with Mannheimia haemolytica, Pasteurella multocida, Histophilus somni and Mycoplasma bovis in weaned beef cattle, weaned dairy bulls and weaned replacement dairy heifers*". The hazard to human health is defined as a human illness caused by fluoroquinolone-resistant foodborne bacteria (*Campylobacter* spp*., Salmonella* spp*.* and multidrug resistant *Salmonella* spp.) attributable to consumption of contaminated beef originating from cattle injected with pradofloxacin for the treatment of BRD, and treated with a human antibiotic from the fluoroquinolone class of antimicrobials.

The microbial food safety assessment submitted to the Agency was based on a qualitative risk assessment, and included a *release* assessment to describe the probability that pradofloxacin and its use as a single injection at 10 mg/kg BW in cattle will result in the emergence of resistant bacteria or resistance determinants in treated cattle under 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 pradofloxacin-treated cattle; 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 fluoroquinolones and their use in the treatment of human infectious diseases.

The Agency evaluated the information submitted by the sponsor, considered the current fluoroquinolone and quinolone susceptibility profiles of *Salmonella* spp., *Escherichia coli*, and *Campylobacter* spp., including their prevalence in the food commodity of concern (retail beef) and target animal (cattle). Pradofloxacin is a third-generation fluoroquinolone, and in addition to broad activity against Gram-negative bacteria, it also demonstrates enhanced activity against Gram-positive organisms and anaerobes, differentiating this drug product from earlier generation fluoroquinolone compounds such as enrofloxacin. Pradofloxacin is similar in structure to moxifloxacin, an advanced generation fluoroquinolone approved for use in humans. Thus, inappropriate use of agents from this important class of antibiotics will likely worsen current problems with antimicrobial resistance. It is important that advanced generation fluoroquinolones such as pradofloxacin be used in a manner for the best clinical outcome while having the lowest risk possible for subsequent resistance development among organisms of human health concern. Thus, use of pradofloxacin in food producing animals should also be reserved for the treatment of clinical conditions which have responded poorly to other classes of antimicrobials.

Based upon this evaluation and the following antimicrobial resistance risk mitigating factors:

- Pradofloxacin will be a prescription only drug;
- Treatment via single dose of pradofloxacin at 10 mg/kg BW will be administered to cattle diagnosed with BRD;
- Extralabel use of fluoroquinolones is prohibited by law (21 CFR §530.21) in food producing animals;
- The addition of a CAUTION statement, "*To assure responsible antimicrobial drug use, use of pradofloxacin should be limited to treatment of BRD in cattle only after consideration of other non-fluoroquinolone therapeutic options*," to appear on all labels, inserts and marketing materials;
- Susceptibility to fluoroquinolones is already monitored by the National Antimicrobial Resistance Monitoring System (NARMS); thus, no changes in monitoring will be made for this new proposed use of pradofloxacin in cattle.

Considering these mitigating factors, the Agency concludes that the use of pradofloxacin in cattle for the treatment of BRD in cattle will not result in a significant risk to public health with respect to the development of fluoroquinolone resistance among foodborne *Campylobacter* and *Salmonella* originating from treated cattle.

Decision Statement:

The overall risk estimation associated with the use of pradofloxacin injectable in cattle under the proposed conditions of use is high, based on individual rankings of medium for the *release* assessment, medium 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 serious human infections. However, the use of risk management strategies such as prescription only (Rx) marketing status (under the direction of a veterinarian), an injectable route of administration in individual animals, extralabel use prohibition, isolate monitoring by NARMS, and conditions of use including appropriate use parameters to determine if cattle are eligible to receive pradofloxacin for the treatment of BRD have allowed the Agency to conclude that antimicrobial resistance concerns for fluoroquinolone-resistant *Campylobacter* and *Salmonella* originating from treated cattle are minimized.

<u>Swine</u>

Background and outcome of risk assessment:

The Agency evaluated microbial food safety information and data for pradofloxacin, "*for treatment of swine respiratory disease (SRD) associated with Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Glaesserella (Haemophilus) parasuis, Pasteurella multocida, Streptococcus suis, and Mycoplasma hyopneumoniae in weaned pigs, replacement gilts, and replacement boars*." The hazard to human health is defined as a human illness caused by fluoroquinolone-resistant foodborne bacteria (*Campylobacter* spp*.*, *Salmonella* spp*.* and multidrug resistant *Salmonella*

spp.) attributable to consumption of contaminated pork originating from swine injected with pradofloxacin for the treatment of SRD, and treated with a human antibiotic from the fluoroquinolone class of antimicrobials.

The microbial food safety assessment submitted to the Agency was based on a qualitative risk assessment, and included a *release* assessment to describe the probability that pradofloxacin and its use as a single injection at 7.5 mg/kg BW in swine will result in the emergence of resistant bacteria or resistance determinants in treated swine under 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 pradofloxacin-treated swine; 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 fluoroquinolones and their use in the treatment of human infectious diseases.

The Agency evaluated the information submitted by the sponsor, considered the current fluoroquinolone and quinolone susceptibility profiles of *Salmonella* spp., *Escherichia coli*, and *Campylobacter* spp., including their prevalence in the food commodity of concern (retail pork) and target animal (swine). Pradofloxacin is a third-generation fluoroquinolone, and in addition to broad activity against Gram-negative bacteria, it also demonstrates enhanced activity against Gram-positive organisms and anaerobes, differentiating this drug product from earlier generation fluoroquinolone compounds such as enrofloxacin. Pradofloxacin is similar in structure to moxifloxacin, an advanced generation fluoroquinolone approved for use in humans. Thus, inappropriate use of agents from this important class of antibiotics will likely worsen current problems with antimicrobial resistance. It is important that advanced generation fluoroquinolones such as pradofloxacin be used in a manner for the best clinical outcome while having the lowest risk possible for subsequent resistance development among organisms of human health concern. Thus, use of pradofloxacin in food producing animals should also be reserved for the treatment of clinical conditions which have responded poorly to other classes of antimicrobials.

Based upon this evaluation and the following antimicrobial resistance risk mitigating factors:

- Pradofloxacin will be a prescription only drug;
- Treatment via single dose of pradofloxacin at 7.5 mg/kg BW is to be administered to swine diagnosed with SRD;
- Extralabel use of fluoroquinolones is prohibited by law (21 CFR §530.21) in food producing animals;
- The addition of a CAUTION statement, "*To assure responsible antimicrobial drug use, use of pradofloxacin should be limited to treatment of SRD in swine only after consideration of other non-fluoroquinolone therapeutic options*," to appear on all labels, inserts and marketing materials;
- Susceptibility to fluoroquinolones is already monitored by the National Antimicrobial Resistance Monitoring System (NARMS); thus, no changes in monitoring will be made for this new proposed use of pradofloxacin in swine.

Considering these mitigating factors, the Agency concludes that the use of pradofloxacin in swine for the treatment of SRD in swine will not result in a significant risk to public health with respect to the development of fluoroquinolone resistance among foodborne *Campylobacter* and *Salmonella* originating from treated swine.

Decision Statement:

The overall risk estimation associated with the use of pradofloxacin injectable in swine under the proposed conditions of use is *high*, based on individual rankings of *medium* for the *release* assessment, *medium* for the *exposure* assessment, and *high* for the *consequence* assessment. The latter ranking of *high* for the *consequence* assessment is based on fluoroquinolones being ranked *critically important* for human medicine, as it is the empiric drug class of choice to treat serious human infections. However, the use of risk management strategies such as prescription only (Rx) marketing status (under the direction of a veterinarian), an injectable route of administration in individual animals, extralabel use prohibition, isolate monitoring by NARMS, and conditions of use including appropriate use parameters to determine if swine are eligible to receive pradofloxacin for the treatment of SRD have allowed the Agency to conclude that antimicrobial resistance concerns for fluoroquinolone-resistant *Campylobacter* and *Salmonella* originating from treated swine are minimized.

B. Toxicology

1. Summary of Toxicology Studies

   Toxicology testing was conducted on pradofloxacin (also referred to as BAY 14-1877). Toxicity studies conducted to determine the human food safety of pradofloxacin are summarized below:

   a. Subchronic Oral Toxicity Study in Rodents (Rats)

      Title: Study of Subchronic Toxicity in Wistar Rats - 13 Weeks Administration by Diet. (Study No. T 2076964)

      Report Date: April 10, 2008

      Study Location: Wuppertal, North Rhine–Westphalia, Germany

      Study Design: This GLP study was conducted according to Veterinary International Conference on Harmonization (VICH) Guideline No. 31 and the Organization for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals No. 408. Pradofloxacin was administered in the diet to Wistar rats (Hsd Cpb: WU), 10/sex/group, at dietary concentrations of 0, 1500, 2000, or 2500 ppm (equal to 0, 98.7, 130.2, or 166.7 mg per kg body weight per day (mg/kg bw/day) for males and 144.7, 180.0 or 229.4 mg/kg bw/day for females, respectively) for 13 weeks. In addition, rats (6/sex for the treatment groups and 3/sex for the control group) in a satellite study were administered the above dose levels for the determination of plasma concentrations of pradofloxacin after 13 weeks of treatment. Cage-side and detailed clinical observations, ophthalmologic examinations, body weights,

and food consumption were evaluated. Blood samples were taken from all animals at necropsy on day 92 to evaluate hematology and clinical chemistry (toxicology groups), and plasma concentrations of pradofloxacin (toxicokinetic groups). At day 92, organs and tissues were collected from all groups for gross necropsy and organ weight measurement, and full histopathology was conducted for control and high dose groups.

Results and Conclusion: No treatment-related mortality was observed. Loss of hair was observed at 2,500 ppm in males and females. No alterations in body weight, food consumption or other clinical signs were reported. The following changes in hematology and clinical chemistry were reported: The mean corpuscular hemoglobin concentrations (MCHC) were increased at ≥ 1,500 ppm in males and at ≥ 2,000 ppm in females; neutrophils were decreased at ≥ 1,500 ppm in females; alanine aminotransferase (ALAT) activity was decreased at 2,000 ppm in males and at ≥ 1,500 ppm in females; creatinine concentrations were increased at 1,500 and 2,500 ppm in males; albumin concentrations were increased at 2,500 ppm in males and 2,000 ppm in females; decreased phosphorus concentration at 1,500 and 2,500 ppm and potassium at 2,500 ppm in females. Decreases in relative liver weights were observed at ≥ 1,500 ppm in males and ≥ 2,000 ppm in females.

Histopathological examination showed an increase in incidences and grade of periportal hepatocellular lipid, which corresponded to the gross observation of distinct lobulation of the liver in males at 2,500 ppm. This finding was not accompanied by degenerative liver changes. In the kidneys of males, tubular dilation was observed at both 2,000 and 2,500 ppm. A positive administered dose of pradofloxacin and plasma pradofloxacin concentration relationship were demonstrated, with no significant difference between males and females.

A No-Observed-Effect Level/No-Observed-Adverse-Effect Level (NOEL/NOAEL) was not established due to effects (the increased MCHC and creatinine, decreased phosphorus, neutrophils and ALAT, and decreased liver weights) found at all doses in males and females. A Lowest-Observed-Effect Level/Lowest-Observed-Adverse-Effect Level (LOEL/LOAEL) was not identified due to the marked effects at all doses tested.

b. Subchronic Special Study on Liver Enzyme Induction in Female Rats

Title: Study for Sub-Chronic Oral Toxicity in Female Rats (Special Feeding Study on Liver Enzyme Induction) for 13 Weeks. (Study No. T 4067317)

Report Date: November 23, 1999

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: The objective of this GLP study was to evaluate the effect of pradofloxacin on the activities of metabolizing hepatic enzymes in a subchronic toxicity study in rats. Female Wistar rats of the strain Hsd

Cpb:WU (5/dose) were administered pradofloxacin in diets at dietary concentrations of 0, 10, 30, 50, or 100 ppm (equal to 0, 1.1, 3.0, 4.9 or 9.7 mg/kg bw/day, respectively) for 13 weeks. Clinical observations, morbidity, mortality, ophthalmoscopic examination, food consumption, and body weight were evaluated. At the interim (day 29) and terminal sacrifice (day 94), livers were taken, processed, and measured for the activity of the liver enzymes (7-ethoxycoumarin deethylation (ECOD), 7-7-ethoxyresorufin deethylation (EROD), ketose-phosphate aldolase (ALD), epoxide hydrolase (EH), UDP-glucuronide transferase (UDPGT), and glutathione transferase (GST)). The liver weight was obtained at the terminal sacrifice at day 94.

Results and Conclusion: No mortalities, clinical signs, or ophthalmoscopy changes were observed. Body weights and food consumption were not affected by the treatment. Absolute liver weight was decreased at 100 ppm. At day 29, significant reductions in hepatic EROD activity were observed at all doses; EH activity was decreased at 30 and 50 ppm. At day 94, only the GST activity was significantly elevated at 50 and 100 ppm.

A LOEL/LOAEL of 10 ppm (1.1 mg/kg bw/day) was established based on decreases of hepatic EROD activity noted at day 29 at all doses tested.

c. Subchronic Oral Toxicity Study in Rodents (Rats) with a (5-week) Recovery Period

Title: Study for Subchronic Oral Toxicity in Rats - Feeding Study for 14 Weeks Following with a 5-Week Recovery Period. (Study No. PH 31595)

Report Date: December 11, 2011

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL31 and OECD Test Guideline No. 408. Pradofloxacin was administered in the diet to Wistar rats (Hsd Cpb:WU), 20/sex/group, at dietary concentrations of 0, 300, 1000, or 3000 ppm (equal to 0, 21, 79 or 237 and 0, 25, 92 or 279 mg/kg bw/day in males and females, respectively) for 14 weeks. Two additional groups, 20 rats/sex/group, were observed for a 5-week recovery period after 13 weeks treatment at 0 and 3,000 ppm. Cage-side and detailed clinical observations, ophthalmologic examinations, body weights, and food and water consumption were evaluated. Hematology and clinical chemistry were evaluated at days 30, 65, and 92 (the main groups), and 114 (the recovery groups). Urinalysis was conducted during week 14. Necropsy was conducted at days 97-100 for the main groups and at days 125-126 for the recovery group, followed by determination of organ weights (all animals) and histopathology (control and high dose groups).

Results and Conclusion: No mortalities, ophthalmoscopic abnormalities, or changes in food consumption were observed in treated rats. Diarrhea and increases in feces excretion were seen at 1,000 and 3,000 ppm in both sexes. Mean water consumption was significantly increased at ≥ 1,000 ppm in males and females. The mean body weight and body weight gain were

reduced at 3,000 ppm in males. The followings changes in hematology and clinical chemistry were reported. There was a reduction in neutrophil numbers at ≥ 300 ppm, monocyte numbers at ≥ 1,000 ppm, leukocyte numbers at ≥ 1,000 ppm, and a transient increase in the incidence of Heinz bodies at 3,000 ppm in males after 1 month of treatment. All these treatment-related changes in these hematological parameters were returned to normal at the end of the recovery period, except that there was still a reduction in leukocyte numbers in females. At ≥ 1,000 ppm, there was a consistent reduction of MCHC in females throughout the treatment and a decrease in erythrocyte numbers in males at the end of the treatment. Aspartate aminotransferase (ASAT) activity was reduced in males at all doses throughout the entire treatment and was reduced in females at all doses at the end of the treatment. Alanine aminotransferase (ALAT) activity was decreased at 300 and 1,000 ppm in males and females at days 65 and at all doses at the end of the treatment. A significant reduction in glutamate dehydrogenase (GLDH) was seen at 3,000 ppm in females. Triglycerides were decreased at 1,000 and 3,000 ppm in females at day 30, day 65 and the end of the treatment at 3,000 ppm. Protein concentrations were decreased at the end of the treatment at all doses in males. Bilirubin concentration was decreased at all doses at day 65 in females and at 3,000 ppm in males, and at the end of the treatment at 3,000 ppm in males. The urinary volume was reduced, probably due to diarrhea. Increased urinary concentrations of protein (males and females at ≥ 1,000 ppm), presence of blood and ketone bodies in females (at 1,000 ppm) and increased urinary density at all doses in males and ≥ 1,000 in females were found.

Decreases in absolute and relative liver weights in females at 1,000 ppm and 3,000 ppm at terminal sacrifice, and at 3,000 ppm in males at the end of the recovery period were noted. A low incidence of knee joint cartilage lesions was reported in all animals at all dose groups in the histopathological examination.

A NOEL/NOAEL was not established because of observed effects such as reduced neutrophils, decreased plasma ALAT and ASAT activity, decreased urinary volume, increased urinary density, and low incidence of knee joint cartilage lesions at 300 ppm (equal to 21 mg/kg bw/day in males and 25 mg/kg bw/day in females). The establishment of NOEL/NOAEL did not consider the recovery phase.

A LOEL/LOAEL was established at 300 ppm, corresponding to 21 mg/kg bw/day in males and 28 mg/kg bw/day in females.

d. Subchronic Oral Toxicity Study in Rodents (Rats) with a (4-week) Recovery Period

Title: Study for Sub-Chronic Oral Toxicity in Rats - Feeding Study for 14 Weeks Following with a 4-Week Recovery Period. (Study No. PH 31596)

Report Date: December 11, 2001

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL31 and OECD Test Guideline No. 408. Pradofloxacin was administered in the diet to Wistar rats (Hsd Cpb: WU), 20 rats/sex/group, at dietary concentrations of 0, 300, 1000, or 3000 ppm for 14 weeks (equal to 0, 21, 71 or 231 mg/kg bw/day and 0, 28, 90 or 279 mg/kg bw/day for males and females, respectively). Two additional groups of 20 males and 20 females were observed for a 4-week recovery period after 13 weeks treatment at 0 and 3,000 ppm. Cage-side and detailed clinical observations, ophthalmologic examinations, body weights, and food and water consumption were evaluated. Hematology and clinical chemistry were evaluated at days 34, 60, 89 (the main groups) and at day 119 (the recovery groups). Drug hepatic metabolizing enzymes were analyzed in liver tissue samples obtained at days 96-98. Necropsy was conducted at days 96-98 and at day 131 for the recovery group followed by determination of organ weights (all animals) and histopathology (control and high dose groups).

Results and Conclusion: No mortalities or changes in food consumption were observed in treated rats. Clinical signs including diarrhea, soft feces, increased fecal and urinary excretion, and long claws were seen at doses ≥ 300 ppm in males and females. Increased urinary excretion was mainly observed at 3,000 ppm but also occurred at ≥ 300 ppm. Mean water consumption was increased in both sexes at ≥ 1,000 ppm during the first 3 weeks of the study. The mean body weight was reduced at 3,000 ppm in males and reduced at the beginning of the treatment at 1,000 ppm in males. The mean body weight gain was decreased at 3,000 ppm in males and females.

The number of neutrophils, monocytes, and atypical leukocytes were reduced at ≥ 300 ppm at days 60 and 89. ASAT activity was consistently reduced at ≥ 1,000 ppm at days 34 and 60 in males. A reduction in GLDH activity was seen at 1,000 and 3,000 ppm in females at the end of the treatment. Triglycerides concentrations were significantly decreased at day 60 (at ≥ 1,000 ppm in females) and at day 89 (at ≥ 1,000 in males and 3,000 ppm in females). Bilirubin concentration was decreased at 1,000 ppm in males at day 89. Glucose concentration was increased at ≥ 300 ppm at day 34 and at ≥ 1,000 ppm in males and females.

In the liver tissue samples, an increase of triglycerides was noted at 3,000 ppm. Relative liver weights were decreased at ≥ 1,000 ppm in males and females and relative adrenal weight was increased at 3,000 ppm in males. Histopathological findings were seen at 3,000 ppm, including thickening of the cecal wall with inflammatory reaction and increased mucus production in the cecum and rectum, and a slight reduction in the amount of periportal fat storage in the liver of males and less pronounced in the liver of females.

A NOEL/NOAEL could not be established due to clinical signs (diarrhea, soft feces, increased fecal and urinary excretion, and long claws) and reduced

neutrophil and monocyte numbers at ≥ 300 ppm. The establishment of NOEL/NOAEL did not consider the recovery phase.

A LOEL/LOAEL was established at 300 ppm, corresponding to 21 mg/kg bw/day in males and 28 mg/kg bw/day in females.

e. Subchronic Oral Toxicity Study in Rodents (Mice)

Title: 13-week Subchronic Dietary Toxicity Study in Mice. (Study No. T 8069346)

Report Date: November 6, 2001

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL31 and OECD Test Guideline No. 409. Pradofloxacin was administered in the diet to SPF-bred mice (CRL: CD1 (ICR)), 10/sex/group, for 13 weeks at dietary concentrations of 0, 500, 2000, or 7000 ppm (equal to 0, 137, 470, or 1,776 mg/kg bw/day for males and 0, 182, 787, or 2,486 mg/kg bw/day for females, respectively). Clinical observations, morbidity, mortality, ophthalmoscopic examination, food consumption, and body weight were evaluated. Hematology, clinical chemistry, and urine analysis were performed at termination. At the end of the 13 weeks, all surviving animals were anesthetized, exsanguinated, and subjected to organ weight measurements, macroscopic and histopathologic evaluations (control and high dose groups).

Results and Conclusion: No significant differences were noted in survival rates, body weights, food consumption, water consumption, or clinical signs. At 7,000 ppm, there were decreases of erythrocyte numbers, hemoglobin levels, and hematocrit in both sexes; an increase of albumin concentration in males and a decrease of cholesterol in females; decreases in relative organ weights of heart, testes, and epididymides in males and of thymus in females. A few enlarged and pleomorphic nuclei of the tubular epithelium of the inner renal cortex in females were observed at 7,000 ppm. Decreases in alkaline phosphatase activity were found at 2,000 and 7,000 ppm in males.

The NOEL/NOAEL was established at 500 ppm (equal to 137 and 182 mg/kg bw/day for males and females, respectively) based on decreases of alkaline phosphatase activity observed at 2,000 ppm.

f. Subchronic Oral Toxicity Study in Non-Rodents

Title: 13-week Subchronic Dietary Toxicity Study in Beagle Dogs. (Study No. T 8070047)

Report Date: April 10, 2002

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL31 and OECD Test Guideline No. 409. Pradofloxacin was administered in the diet to

beagle dogs (4/sex/group) for 13 weeks at dietary concentrations of 0, 50, 150, or 450 ppm (equal to 0, 2, 5, or 15 mg/kg bw/day for males and females, respectively). Cage-side and detailed clinical observations, ophthalmologic examinations, body weights, and food and water consumption were evaluated. Electrocardiograms (ECGs) and blood pressure measurements were performed at pretest, and weeks 2 and 13. Hematology, blood chemistry, and urine analysis were performed on all animals at pretest, and weeks 2, 6, and 13. At the end of the 13 weeks, all surviving animals were anesthetized, exsanguinated, and subjected to histopathology evaluations (control and high dose groups). Plasma samples were collected on days 1, 8 and 81 for measurement of pradofloxacin concentrations.

Results and Conclusion: There were no significant changes in body weight, food consumption, systolic and diastolic blood pressures, heart rate, and urinalysis. The occurrences of disintegration of carpal joint and disturbed attitude were higher at 450 ppm. Hemoglobin concentrations were increased at 150 and 450 ppm. Increased thromboplastin times were noted at 450 ppm. Decreased neutrophils, hematocrit, platelet, leukocyte, reticulocyte, and monocytes counts were identified at 450 ppm. Erythrocyte sedimentation rates (ESR1 and ESR2) at 450 ppm were higher than the control group. Total protein, chloride, triiodothyronine (T3), and thyroid-stimulating hormone (TSH) concentrations were increased at 450 ppm. Albumin, potassium, and thyroxine (T4) concentrations were increased at 150 and 450 ppm. Female heart and brain weights were increased at 450 ppm. Gall bladder weights were increased at 150 and 450 ppm.

At 150 ppm, increased synovial fluid with surface changes was found in the shoulder in one dog and knee joints in another dog. At 450 ppm, blisters and surface changes of the articular cartilage and increased synovial fluid were broadly found in the joints of dogs.

The NOEL/NOAEL was determined to be 50 ppm (equal to 2 mg/kg bw/day) based on synovial fluid found in the shoulder and knee joints with surface changes, changes in clinical pathology (increased hemoglobin, albumin, potassium, and T4 levels), and increased gall bladder weights observed at 150 ppm (5 mg/kg bw/day) and above.

g. Chronic Oral Toxicity Study in Rodents

Title: Chronic Oral Toxicity Study in Wistar Rats - 52 weeks Administration by Diet. (Study No. T0074621)

Report Date: May 9, 2008

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL37 and OECD Test Guideline No. 452. Pradofloxacin was administered in the diet to Wistar (Hsd Cpb: WU) rats (20/sex/group) at dietary concentrations of 0, 50, 250, 500 or 1,000 ppm (equal to 0, 2.5, 12.5, 24.6, or 48.7 mg/kg bw/day for males, and 3.4, 17.0, 35.0, or 67.7 mg/kg bw/day for females) for one year.

Cage-side and detailed clinical observations, ophthalmologic examinations, functional observational battery (FOB), body weights, and food consumption were evaluated. FOB was performed at the end of the treatment on week 51. Hematology and clinical chemistry were evaluated at days 95, 186 and 360 (terminal sacrifice). Necropsy was conducted at terminal sacrifice followed by determination of organ weights and histopathology.

Results and Conclusion: Body weight, food consumption, and survival were not affected by the treatment. Loss of hair was observed in most of the treated rats. Bloody eyes and redness of eyelids were observed in higher incidences in treated females. An increase in cornea abnormalities, lens opacity, and neovascularization of the cornea was observed at ≥ 500 ppm in males at the end of the treatment. Hematological examination revealed an increase of MCHC at 250, 500 and 1,000 ppm on day 360 in males and females. At day 186, MCHC was significantly increased at 250 and 1,000 ppm in males, and at 1,000 ppm in females. Differential leukocyte count decreased at 1,000 ppm in females on days 186 and 360 and at 50 and 250 ppm on day 186. A decrease in lymphocytes and leukocytes was found at 1,000 ppm in both sexes on day 360 and at 500 ppm in males on days 360 and 186. On day 360, the number of monocytes was decreased at ≥ 250 ppm in females, and at all doses in males. A slight decrease in protein concentration was noted at 1,000 ppm at day 360 in both sexes and at 250 ppm and 500 ppm in males at day 360. Some slight increases in sodium concentration in males at 250 ppm at days 186 and 360, and in females at 250 and 500 ppm at day 360 were observed. In males, the decrease in ASAT and ALAT activities was seen at 250, 500 and 1,000 ppm at day 360 and at 1,000 ppm at day 186. In females, ASAT and ALAT were decreased at all the doses at days 186 and 360.

Spleen showed a decrease in absolute and relative weight in males and females at 50 and 250 ppm. Liver and heart at 500 ppm in males showed decreases in relative organ weights. No histopathological finding could be correlated to the gross observation of cecal dilation in males at 1,000 ppm. In the mandibular lymph nodes, a decreased incidence of lymphoid activation was seen in males at 1,000 ppm. Serum/plasmacytosis (accumulation of serum/plasma cells in the medullary cords) of the mandibular lymph node was significant at 50 ppm in males and in all dose groups in females except 1,000 ppm. In the liver, the incidence of bile duct hyperplasia was increased at 50 ppm in males, at 500 ppm in males and females, and at 1,000 ppm in females. In addition, focal fatty changes of the liver were found at all doses in males and at 1,000 ppm in females. Females and males at 1,000 ppm showed spinal cord radiculoneuropathy. Alterations of the heart, such as cardiomyopathy and metaplasia, were also observed at 1,000 ppm in males and females. Mononuclear cell infiltration of the kidneys was observed in males at all doses, and transitional cell hyperplasia of kidneys at 50 and 250 ppm in males were noted. The following hyperplasia was observed: thyroid gland cell hyperplasia in males and females, parathyroid gland focal hyperplasia in males and females, adrenal gland focal medullary hyperplasia in males and females and focal cortical hyperplasia in males and females at

1,000 ppm. In the ovaries, the incidence of atrophy (presence of recent corpora lutea) was significantly reduced in females at 1,000 ppm.

A NOEL/NOAEL could not be established. A LOEL/LOAEL of 50 ppm (2.5 mg/kg bw/day in males and 3.4 mg/kg bw/day in females), the lowest dose tested, was established based on hematological changes (a decrease in leukocyte count in females at 50 ppm), clinical chemistry parameters (decreases in ALAT and ASAT activities at all doses), decreases in absolute and relative spleen weight in males and females at 50 ppm, and microscopic findings (bile duct hyperplasia, mononuclear cell infiltration and transitional cell hyperplasia in kidney) at 50 ppm.

h. Oral Prenatal Developmental Toxicity Study in Rodents

Title: Prenatal Developmental Toxicity Study in Wistar Rats. (Study No. T8062974)

Report Date: January 21, 2008

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL32 and OECD Test Guideline No. 414. Four groups of 25 sperm or copulatory plug positive female Wistar rats (Hsd Cpb: WU) per treatment group were administered: the vehicle only (0.9% aqueous sodium chloride solution) or pradofloxacin in vehicle at 5, 35 or 250 mg/kg bw/day via daily gavage from post coitus days 6 until 19 in 10 mL/kg bw dose volume. Additional female groups (5 inseminated females per treatment group) were included in a satellite toxicokinetic (TK) group. Each rat was observed once daily for clinical signs of toxicity and twice daily for viability/mortality, appearance and behavior, and excretory alterations from days 0 to 20 post coitus. Clinical signs as well as food and water consumption, and body weight were recorded. On post coitus day 20, females were sacrificed, and the fetuses removed by C-section and sacrificed. The reproductive organs were examined grossly and weighed; early and late resorption analyzed; and total implantations, number of corpora luteum, and appearance of the placentas recorded. Gravid uterine and mean body weights were recorded, and mean body weight changes calculated. Reproductive organs and other organs from all maternal animals were prepared for possible histopathological examination. The fetuses were counted, weighed, and sexed, and examined for external, visceral, and skeletal malformations and developmental variations.

For the TK group, blood samples for measurements of pradofloxacin concentrations were taken from all females on days 6 and 19 post coitus at pre-dose, 1, 2, 4, 7 and 24 hours after the 1st dose and after the 14th test article administration, respectively.

Results and Conclusions: Oral prenatal administration of pradofloxacin in pregnant Wistar rats (Hsd Cpb: WU) from days 6 to 19 post coitus induced systemic exposure that was dose-proportional in all groups. Females

administered 250 mg/kg bw/day exhibited impaired food consumption with a corresponding increase in body weight loss and impaired body weight gain as well as increased post-implantation loss and decreased placental weight. Fetuses and litters showed retarded fetal development and increased incidence of common spontaneous malformations and visceral deviations. At 35 mg/kg bw/day, females showed transiently reduced food consumption, and their fetuses displayed reduced body weight and retarded ossification as well as increased external, skeletal, and visceral malformation. At 5 mg/kg bw/day, a 10% increase in external, skeletal, and visceral malformation per litter and an increased osseous finding on a per fetus basis was not statistically significant or toxicological relevant.

The maternal and developmental toxicity NOEL/NOAEL was established at 5 mg/kg bw/day based on reduced food consumption in dams, reduced fetal body weight, retarded ossification, and increased external, skeletal, and visceral malformation observed at 35 mg/kg bw/day.

i. Oral Prenatal Developmental Toxicity Study in Non-Rodents (Study 1)

Title: Prenatal Developmental Toxicity Study in the Himalayan Rabbits. (Study No. T1067873)

Report Date: January 30, 2008

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL32 and OECD Test Guideline No. 414. Four groups of 22 copulatory plug positive female Himalayan rabbits per treatment group were administered vehicle only (0.9% aqueous sodium chloride solution) or pradofloxacin dissolved in vehicle at 3, 7 or 16 mg/kg bw/day via daily gavage from post coitus days 6 until 28. Each rabbit was observed once daily for clinical signs of toxicity and twice daily for viability/mortality, appearance and behavior and excretory alterations from days 0 to 29 post coitus. Clinical signs, food and water consumption, and body weight were recorded. On post coitus day 29, females were sacrificed, and the fetuses removed by C-section and sacrificed. The reproductive organs were examined grossly and weighed; early and late resorption analyzed; and total implantations, number of corpora lutea, and appearance of the placentas recorded. Gravid uterine and mean body weights were recorded, and mean body weight changes calculated. Tissues from all maternal animals were prepared for possible histopathological examination. The fetuses were counted, weighed, and sexed, and examined for external, visceral, and skeletal malformations and developmental variations.

Results and Conclusions: Oral prenatal administration of pradofloxacin in rabbits from days 6 to 28 post coitus induced systemic maternal toxicity and development effects at all doses. Administration of pradofloxacin at 16 mg/kg bw/day resulted in three late term abortions following a period of exhibiting clinical signs such as cold ears, reddish discolored eyelids, anorexia, and body weight loss. These abortions resulted in a lower gestation rate. At

necropsy, the females that aborted exhibited either light discoloration of the liver, enlarged gall bladder, and enlarged cecum or distinct liver lobulation. Alopecia was noted in many females. Food and water consumption reduction and a corresponding reduction in the amount of feces, discolored urine as well as impaired body weight were noted. Fetuses exhibited reduced fetal body weights, increased incidence of common skeletal malformations and retarded ossification as well as increased incidence of common skeletal variations. At 7 mg/kg bw/day, a greater number of females showed alopecia as well as decreased food and water consumption and a corresponding reduction of feces and discolored urine. Decreased gestation rate was reported due to the finding of two abortions. The weight of the fetuses was also decreased, and retarded ossification was observed. Treatment at 3 mg/kg bw/day induced one abortion that reduced the gestation rate and at necropsy, two females including the female that aborted exhibited enlarged gall bladder or enlarged cecum. Fetuses produced at 3 mg/kg bw/day showed reduced fetal body weight and the degree of ossification of the frontal bone was reduced.

A maternal toxicity NOEL/NOEAL could not be established because of toxic effects at all doses tested. A developmental toxicity NOEL/NOAEL could not be established due to reduced fetus weight and retarded ossification of the frontal bone at the lowest dose tested. A LOEL/LOAEL of 3 mg/kg bw/day, the lowest dose tested, was established based on maternal and developmental effects noted at all doses tested.

j. Oral Prenatal Developmental Toxicity Study in Rabbits (Follow-up Study 2)

Title: Prenatal Developmental Toxicity Study in the Himalayan Rabbits. (Study No. T2073363)

Report Date: January 21, 2008

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL32 and OECD Test Guideline No. 414. Four groups of 22 copulatory plug positive female Himalayan rabbits per treatment group were administered vehicle (0.9% aqueous sodium chloride solution) or pradofloxacin dissolved in vehicle at 1, 4 or 16 mg/kg bw/day via daily gavage from post coitus day 6 until day 28, in 5 mL/kg bw dose volume. Additional female groups (3 inseminated females per treatment group) were included in a satellite TK group. Each rabbit was observed once daily for clinical signs of toxicity and twice daily for viability/mortality, appearance and behavior and excretory alterations from days 0 to 29 post coitus. Clinical signs, food and water consumption, and body weight were recorded. On post coitus day 29, females were sacrificed, and the fetuses removed by C-section and sacrificed. The reproductive organs were examined grossly and weighed. Early and late resorption were analyzed. Total implantations, number of corpora luteum, and appearance of the placentas were recorded. Gravid uterine weights and mean body weights were recorded, and mean body weight changes were calculated. Reproductive organs and other organs from all maternal animals were

prepared for possible histopathological examination. The fetuses were counted, weighed, and sexed, and examined for external, visceral, and skeletal malformations and developmental variations. For the TK group, blood samples for measurement of pradofloxacin concentration were taken from all females on days 6 and 28 post coitus at pre-dose, 1, 2, 4, 7 and 24 hours after the 1st dose and after the 23rd test article administration, respectively.

Results and Conclusions: Oral prenatal administration of pradofloxacin in pregnant rabbits from days 6 to 28 post coitus caused systemic exposure that achieved more than dose-proportionality at the medium and high dose, and some systemic exposure at the low dose level.

Administration of pradofloxacin at 16 mg/kg bw/day resulted in late term abortion of four females following severe clinical signs of maternal toxicity such as drastic loss of appetite, weight loss, reddish fecal and urinary excretion. These abortions resulted in a 14.3% reduction in gestation rate. At necropsy, the females showed alteration of the stomach, large intestine, liver, gall bladder and/or kidney. Alopecia was also noted in many females. Although there was a 16% increase of post-implantation loss, the numbers of live fetuses as well as total litter size were not statistically significantly affected at first litter check at birth. Food and water consumption, absolute maternal body weight, and body weight gain were reduced. The amount of feces produced was decreased and discolored urine was observed. Fetuses exhibited a 16% reduction in fetal body weights. In addition, the percent litters with malformations were increased. Some of the major malformations observed included reduced size of lens, multiple malformations of heart and major vessels and isolated cardiac ventricular septal defect. At 4 mg/kg bw/day, two rabbits aborted their fetuses during the final trimester after they had shown several clinical signs of maternal toxicity. At necropsy, these females showed alteration of the stomach, large intestine, liver, gall bladder and/or kidney. Many females showed alopecia. Food and water consumption were decreased with a corresponding reduction of feces and discolored urine. The gestation rate was decreased by 10%. The percent litters with malformations were increased. At 1 mg/kg bw/day, one female was found dead on day 28 after a long period of anorexia, reduced fecal output, decreased, and discolored urine and other clinical signs. The group gestation rate was not affected. Food consumption was reduced from days 6 to 9 post coitus only. At necropsy, enlarged stomach, gaseous contents in the large intestine, pale and mottled liver and dark areas on the gall bladder were revealed. Fetuses showed greater than 40% increase in the number of malformations per litter and per fetus.

A NOEL/NOAEL of 1 mg/kg bw/day for maternal toxicity was established based on induction of late term abortion, reduced gestation rate, alopecia, alteration of the stomach, large intestine, liver, gall bladder and/or kidney at the next higher dose level. A NOEL/NOAEL for developmental toxicity could not be established based on the increased number of fetuses with malformations at all dose levels. A LOEL/LOAEL of 1 mg/kg bw/day (the lowest dose tested) was established for developmental toxicity.

k. Two-Generation Oral Reproductive Toxicity Study in Rats

Title: Two-generation Reproductive Toxicity Study in Wistar Rats by Administration in Diet. (Study No. T7073331)

Report Date: March 08, 2008

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL22 and OECD Test Guideline No. 416 (2001). It was designed to evaluate potential toxic effects of pradofloxacin on the reproductive processes in rats through two breeding cycles in two consecutive generations. Four randomized groups of juvenile Wistar rats (the F0 generation; 25 rats/sex/group) were administered through diet pradofloxacin at dietary concentrations of 0, 120, 600, or 3,000 ppm for 10 weeks before mating, and the treatment continued through the mating period, gestation, parturition, lactation, until termination following the weaning of the offspring. Upon parturition, the pups (the F1 generation) were evaluated, and the litter were culled on postnatal days (PNDs) 4 to 8, sex-balanced if possible, and nursed to weaning on PND 28. One male and one female from each F1 litter were randomly selected to receive 10-week treatment before being committed to the same breeding process to produce the F2 offspring. Upon weaning of the F2 pups on PND 28, the F1 parents and the F2 pups were sacrificed for evaluation. Body weights and food consumptions of study animals were monitored throughout the study. Clinical examination of the parents and offspring were carried out at least once weekly. Mortality, morbidity, estrous cycles, sperm parameters, implantation, pregnancy, parturition, lactation, litter composition, postnatal survival and growth, and sexual development of the offspring were evaluated. At necropsy, organ weights were collected, and macroscopic and microscopic evaluations of major organs and tissues were performed for the parental and offspring animals.

Results and Conclusions: The calculated test article intakes during premating period were 10, 52, and 284 mg/kg bw/day for the 120, 600, and 3,000 ppm F0 male groups, respectively, and 11, 55, and 333 mg/kg bw/day F0 female groups, respectively. The corresponding intakes of the test article were 12, 60, 336 mg/kg bw/day in the F1 males, and 14, 73, and 375 mg/kg bw/day in the F1 females. The test article intakes for the F0 dams were 8, 40, and 260 mg/kg bw/day during the last week of pregnancy and 12, 65, and 391 mg/kg bw/day during the first four days after parturition. The F1 dam intakes of the test article were 8, 41, and 205 mg/kg bw/day during the last week of pregnancy and 15, 75, and 382 mg/kg bw/day during the first four days after parturition. The high dose of 3,000 ppm in the two-generation study produced several identifiable toxic effects. Modest decreases in body weight gain, as compared with their respective controls, were seen in F0 males and females and in F1 females at the premating stage; those body weight effects were accompanied by an increase in food intake. Among the litter parameters, reduced average litter size in the F1 generation was evident. Reduced body weight and some organ weights were also found in

the F1 pups at weaning. Evaluations at necropsy revealed that organ weights of the liver, kidney, prostate, epididymis, and seminal vesicles of the F0 males in the 3,000 ppm group were lower than those of the controls; a similar trend of organ weight reductions were also seen in the F1 males (less marked than in the F0 males). Several F0 and F1 rats in the 3,000 ppm group were seen to have produced soft feces; anatomical evaluations found dilated cecum with mucosal accumulation in some animals at the 3,000 ppm dose level. At the lower dose levels, cecal dilation was seen in the 600 ppm group (1/25 and 7/25 for the F0 males and females, and 0/25 and 1/25 for the F1 males and females, respectively) and in the 120 ppm group (3/25 for the F0 females). Histopathological examinations revealed that some adult males and females showed slight fibrosis around basophilic tubules in the kidneys and reduced height of the follicular epithelium in the thyroid gland, all in the 3,000 ppm group. There were no treatment-related effects in sperm parameters and estrous cyclicity of the parental animals. There was no effect on the age of preputial separation in the males, but vaginal opening was delayed in the females at the 3,000 ppm dose level.

The NOEL/NOAEL for the parental effects could not be established due to cecal distention seen at 120 ppm, the lowest dose of the study; the NOEL/NOAEL for reproduction-specific effects was 600 ppm, equal to 40 mg/kg bw/day, based on reduced litter size of the F1 generation.

l. Genetic Toxicity Studies

Pradofloxacin is a typical fluoroquinolone with extensive genotoxicity investigations. Fluoroquinolone-induced genotoxicity is known due to the topoisomerase inhibition. The clastogenicity of topoisomerase inhibitors is likely caused by the transient stabilization of the topoisomerase enzyme with DNA, leading to the formation of a stabilized cleavage complex. This complex may subsequently result in the creation of a DNA strand break. This indirect mechanism is the basis for the concept of threshold for this class of drugs. Results from the genotoxicity studies along with the mechanistic studies on pradofloxacin, as presented in Table IV.1a and 1b, demonstrated that the genotoxic effects induced by pradofloxacin are threshold-based, which is consistent with genotoxicity findings in fluoroquinolone class of drugs. The threshold limit values (NOEL for pradofloxacin-caused topoisomerase II inhibition) were 20 µg/mL in the *in vitro* studies (mechanistic and chromosomal aberration studies) and 160 mg/kg in the *in vivo* mouse micronucleus tests.

It was concluded that pradofloxacin residues in edible tissues of treated cattle would be considerably below the NOEL of topoisomerase II inhibition and, thus, would unlikely cause genetic damages to the human consumers.

Table IV.1a. Summary of Pradofloxacin Genotoxicity Studies

Study Type	Study Number	Results
Bacterial Reverse Mutation Assay (Ames Test)	T 0074766	Positive
In Vitro Mammalian Cell Gene Mutation Test (V79 HPRT)	T 3068874	Positive
In Vitro Mammalian Chromosome Aberrations Test (V79)	T 0068871	Positive
In Vivo Mammalian Erythrocyte Micronucleus Test (Mouse Bone Marrow)	T 6061126	Positive
In Vivo Mammalian Erythrocyte Micronucleus Test (Mouse Bone Marrow)	11125	Positive

Table IV.1b. Summary of Pradofloxacin Genotoxicity Threshold Studies

Studies	Threshold
In Vitro Mammalian Chromosome Aberrations Test (V79), study number: T 0068871	20 µg/mL
In Vivo Mammalian Erythrocyte Micronucleus Test (Mouse Bone Marrow), study number: T 6061126	160 mg/kg
Topoisomerase IIa Inhibition by Fluoroquinolones in V79 Cells	20 µg/mL

(1) Bacterial Reverse Mutation Assay (Ames Test)

Title: BAY 14-1877: Salmonella/Microsome Test. (Study No. T 0074766)

Report Date: July 18, 2005

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL23 and OECD Test Guidance 471. The objective was to evaluate the potential mutagenic activity of pradofloxacin. Tester strains employed were *S. typhimurium* TA1535, TA1537, TA98, TA100, and TA102. The assay was conducted both with and without an Aroclor 1254-induced rat (Sprague-Dawley) liver S9 using four plates per condition. The negative control consisted of a solvent deionized water and the positive controls were 4-nitro-1,2-phenylene diamine (without S9 (-S9)), sodium azide (-S9), nitrofurantoin (-S9), Mitomycin C (-S9), and 2-aminoanthracene (with S9 (+S9)), as appropriate to the strains. Test doses used in the initial test ranged from 0.16 to 50 µg/plate, which were selected based on an initial

dose range-finding study whose results were also considered for mutagenicity induction. Following the initial test, a second test was conducted at a lower dose range.

Results and Conclusion: In the initial test with a dose range of 0.16 to 50 µg/plate, the test article was toxic to all the strains at all concentrations, except for TA102. The test article was mutagenic to TA102 at 0.16 µg/plate and toxic at higher doses. A repeat test was conducted at a lower dose range, 0.0005 to 0.4 µg/plate, with and without S9. For TA1535, TA1537, TA98, and TA100 strains, pradofloxacin was bacteriostatic at doses 0.01 µg/plate and above and no mutagenic effect was detected. For TA102, there was no indication of bacteriostatic effect at doses of up to 0.1 µg/plate. Pradofloxacin was mutagenic to TA102 at dose range from 0.01 to 0.2 µg/plate in the presence and absence of S9 mix.

Pradofloxacin was mutagenic in *S. typhimurium* TA102 under the conditions of the test.

(2) *In Vitro* Mammalian Cell Gene Mutation Test

Title: BAY 14-1877: V79-HPRT-Test *In Vitro* for the Detection of Induced Forward Mutations**.** (Study No. T 3068874)

Report Date: August 22, 2000

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL23 and OECD Test Guidance No. 476. The objective was to evaluate the potential of pradofloxacin to induce forward mutations at the HPRT locus in Chinese hamster lung cells (V79). The assay was performed both with and without an Aroclor 1254-induced rat (Sprague-Dawley) liver S9. The positive controls were ethyl methane sulfonate (-S9) and 7,12-dimethylbenzanthracene (+S9). The negative controls included an untreated (medium) control (±S9) and a solvent (vehicle) control (deionized water, ±S9). Pradofloxacin was dissolved in deionized water to make 500 mg/mL as a suspension. Doses used in the initial cytotoxicity test ranged from 15 µg/mL to 120 µg/mL (±S9). The results from the cytotoxicity assay were considered to select seven concentrations of pradofloxacin ranging from 12 to 72 µg/mL in the mutation assay without S9. In the presence of S9, six concentrations of BAY 14-1877 were tested, ranging from 12 to 64 µg/mL.

Results and Conclusion: In both trials (-S9), pradofloxacin demonstrated a dose-related decrease in the relative population growth. Mutation frequency increased at 48 µg/mL and above as compared with the vehicle controls. In both trials (+S9), pradofloxacin showed a dose-related decrease in the relative population growth. Mutation frequency was increased at 32 µg/mL and above as compared with the vehicle controls.

BAY 14-1877 was considered mutagenic in the V79 forward mutation test.

(3) *In Vitro* Mammalian Chromosome Aberrations Test

Title: Bay 14-1877: *In Vitro* Chromosome Aberration Test with Chinese Hamster V79 Cells**.** (Study No. T 0068871)

Report Date: April 4, 2000

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL23 and OECD Test Guidance No. 473. The objective was to evaluate pradofloxacin using V79 cells for potential induction of structural chromosome aberrations. The assay was performed both with and without an Aroclor 1254-induced rat (Sprague-Dawley) liver S9. Dimethylsulfoxide was used as the solvent control and mitomycin C (-S9) and cyclophosphamide (+S9) were used as positive controls in the assay. The cells treated with culture medium alone constituted the negative control. Cytotoxicity was assessed at doses ranging from 15 to 105 µg/mL. For the cytotoxicity study, the survival index and mitotic index were decreased at 105 µg/mL as compared with the negative control. Disintegration of nuclei and chromosome were observed starting at 60 µg/mL and 75 µg/mL, respectively. Based on the results of cytotoxicity test, two trials were performed. V79 cells were exposed to pradofloxacin (±S9) for 4 hours and then harvested at 18 hours (10, 20, 40, 60, and 80 µg/mL) and 30 hours (40, 60, and 80 µg/mL).

Results and Conclusion: For the pradofloxacin chromosome aberration study, no precipitate was observed at any of the doses used. Under all the testing conditions, the survival index was slightly decreased at 80 µg/mL. Significant increases of numbers of metaphases with aberrations were detected at 18- and 30-hour harvest time. Positive responses (±S9) were observed at the dose of 40 µg/mL and above. A dose-related increase of chromosome aberrations was also observed for the pradofloxacin treated cells. Even though the treatment with the positive controls failed to induce aberrations in this study, the positive effects with pradofloxacin demonstrated the sensitivity of the test system.

Pradofloxacin was considered clastogenic in the mammalian cells *in vitro* under the conditions of the test.

(4) *In Vivo* Mammalian Erythrocyte Micronucleus Test (study I)

Title: Micronucleus Test on the Mouse. (Study No. T 6061126)

Report Date: March 24, 1997

Study Location: Wuppertal, North Rhine-Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL23 and OECD Test Guidance No. 474. The objective was to investigate pradofloxacin in male and female mice (Hsd/Win:NMRI) for a possible clastogenic effect on bone marrow erythrocytes. Cyclophosphamide was used as the positive control and pradofloxacin was prepared in deionized water. Both male and female mice (6-12 weeks old, 5 animals/group) were dosed by oral gavage with pradofloxacin at a single dose of 16, 160, and 1600 mg/kg BW. Positive and negative controls were administered concurrently and met the testing criteria. After treatment, bone marrow was harvested at 16, 24, and 48 hours from pradofloxacin treated animals and 24 hours from the negative and positive control treated animals. For each animal, the micronuclei (MN) were counted in 1,000 polychromatic erythrocytes (PCE). The frequency of PCE versus normochromatic erythrocytes (NCE) was established by scoring a total of 1,000 erythrocytes on the slide (PCE+NCE). The number of NCE with MN was also counted.

Results and Conclusion: There was no mortality associated with any treatment and treated animals showed no toxic symptoms. The ratio of PCE to NCE was decreased at 48 hours and the MN PCE frequencies were increased by 13 folds at 1600 mg/kg BW treated (pradofloxacin) groups as compared with the control. Pradofloxacin was considered as positive in this micronucleus test in mice at the 1600 mg/kg BW dose, but not at and below the 160 mg/kg bw dose, as the increase in micronucleus count at 160 mg/kg bw dose was insignificant (1.84-fold of the control value).

(5) *In Vivo* Mammalian Erythrocyte Micronucleus Test (study 2)

Title: Micronucleus Test Using Male and Female Mice. (Study No. 11125)

Report Date: May 3, 2000

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL23 and OECD Test Guidance No. 474. The objective was to investigate pradofloxacin in male and female mice (Hsd/Win:NMRI) for a possible clastogenic effect on bone marrow erythrocytes. Cyclophosphamide was used as the positive control and pradofloxacin was prepared in 0.5% aqueous Cremophor. Both male and female mice (6-12 weeks old, 5 animals/group) were treated with pradofloxacin at a single dose of 320, 640, and 1280 mg/kg bw by oral gavage. Positive and negative controls were run concurrently and met the testing criteria. After treatment, bone marrow was harvested at 16, 24, and 48 hours from pradofloxacin treated animals and 24 hours from the negative and positive control treated animals. For each animal, the micronuclei were counted in 1,000 polychromatic erythrocytes (PCE). The frequency of PCEs versus mature (normochromatic) erythrocytes (NCEs) was established by scoring a total of 1,000 erythrocytes on the slide (PCE+NCE). The number of NCEs with micronuclei (MN) was also counted.

Results and Conclusion: There was no mortality associated with any treatment. Animals that received the 1280 mg/kg bw dose showed apathy for one hour after the administration before recovering to a normal level of activity. No clinical symptoms were recorded for animals treated with 320 and 640 mg/kg bw pradofloxacin. The ratio of PCE to NCE was decreased in pradofloxacin treated groups with a dose-dependent manner as compared with control at 48 hours. The MN PCE frequencies were increased starting at 320 mg/kg bw as compared with the control and a dose-related increase of MN PCE was also observed.

Pradofloxacin was considered clastogenic in the micronucleus test in mice.

(6) Other Genotoxicity Studies

Bay 14-1877: *In Vitro* Chromosome Aberration Test with Chinese Hamster V79 Cells to Define a No Effect Level

This GLP study was conducted according to VICH GL23 and OECD Test Guidance No. 473. The objective was to define a no-effect level for clastogenic *in vitro* effects of pradofloxacin using V79 cells. The assay was performed both with and without an Aroclor 1254-induced rat (Sprague-Dawley) liver S9. Dimethylsulfoxide was used as the solvent control and mitomycin C (-S9) and cyclophosphamide (+S9) were used as positive controls. V79 cells were exposed to pradofloxacin (±S9) for 4 hours at concentrations of 12, 16, and 20 µg/mL. No increases in the numbers of metaphases with aberrations were observed in all the treated cultures in presence and absence of S9.

It was concluded that pradofloxacin was negative below 20 µg/mL in the *in vitro* chromosomal aberration test with V79 cells.

Topoisomerase IIa Inhibition by Fluoroquinolones in V79 Cells

The objective of this study is to compare the topoisomerase inhibition potencies of several fluoroquinolones. Seven fluoroquinolones were assayed in V79 cells for the effect of stabilizing the topoisomerase II-DNA complex using the TopoGEN *in vivo* link kit. In the first phase, the effects of clinafloxacin, gatifloxacin and lomefloxacin were evaluated over a dose range and at equimolar concentrations, with comparison to ETOP, a non-fluoroquinolone reference and known topoisomerase II poison. In the second phase, three fluoroquinolone drugs (enrofloxacin, marbofloxacin and orbifloxacin) were evaluated in comparison to pradofloxacin. V79 cells were dosed with the test compounds for 4 hours in all studies. This study showed that the tested fluoroquinolones varied in their potencies in inhibition of topoisomerase II in intact V79 cells. The NOEL was 15 µg/mL for clinafloxacin, 20 µg/mL for gatifloxacin, and 100 µg/mL for lomefloxacin, in comparison to 0.01 µg/mL for ETOP; the LOEL was 20 µg/mL for clinafloxacin, 40 µg/mL for gatifloxacin, 200 µg/mL for

lomefloxacin, and 0.1 µg/mL for ETOP. At the comparator concentrations (0.175 mM), clinafloxacin was the most potent. The NOEL and LOEL for enrofloxacin were not determined. The potency in stabilizing the topoisomerase II-DNA complex was similar for pradofloxacin, clinafloxacin, and gatifloxacin. The NOEL was 1200 µg/mL for marbofloxacin, 800 µg/mL for orbifloxacin, and 20 µg/mL for pradofloxacin; the LOEL was 1,500 µg/mL for marbofloxacin, 1,000 µg/mL for orbifloxacin, and 30 µg/mL for pradofloxacin.

It was concluded that the tested fluoroquinolones showed different potencies in the inhibition of topoisomerase II in intact V79 cells. The NOEL of pradofloxacin in this study was 20 µg/mL in V79 cells.

m. Oral Carcinogenicity Study in Rats

Title: Oncogenicity Study in Wistar Rats - 24-months Administration of BAY 14-1877 in the Diet. (Study No. T9074477)

Report Date: May 15, 2007

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL28 and OECD Test Guideline No. 451. The carcinogenic potential of pradofloxacin was assessed in Wistar rats administrated the compound in the diet for 24 months. Males and females (50 rats/sex/dose) were exposed to pradofloxacin at concentrations of 0, 50, 150, or 500 ppm (equal to 0, 2.4, 7.3, or 24.1 mg/kg bw/day for males, and 0, 3.7, 10.1, or 35.8 mg/kg bw/day for females, respectively). All animals were weighed individually every week. Food consumption was recorded every week. All animals were observed daily for morbidity or mortality. Blood samples for hematology and clinical chemistry evaluations were collected at the end of 24 months treatment. Necropsy was conducted at terminal sacrifice at the end of 24 months treatment followed by determination of organ weights and histopathology.

Results and Conclusion: No treatment related effects were noted in survival rates, body weights, food consumption, hematology, clinical chemistry, and pathology analyses. Because a minimal toxic effect was not demonstrated, this study was considered inadequate for establishing a conclusion on the carcinogenicity potential in rats.

n. Oral Carcinogenicity Study in Mice

Title: Oncogenicity study in CD-1 Mice - 21-months Administration of BAY 14-1877 in the Diet. (Study No. T4076407E)

Report Date: December 11, 2008

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to VICH GL28 and OECD Test Guideline No. 451. The carcinogenic potential of pradofloxacin was assessed in CD-1 mice administrated the compound in the diet for about 21 months. Fifty (50) mice/sex/dose were exposed to pradofloxacin at dietary concentrations of 0, 150, 500, 2000, or 7000 ppm (equal to 0, 18, 64, 257, or 1000 mg/kg bw/day for males, and 0, 28, 85, 352, or 1300 mg/kg bw/day for females, respectively). All animals were weighed individually every week. Food consumption was recorded every week. All animals were observed daily for morbidity or mortality. Blood samples for hematology and clinical chemistry evaluations were collected at the end of the 21 months. Necropsy was conducted at terminal sacrifice at the end of the 21 months followed by determination of organ weights and histopathology.

Results and Conclusion: The high-dose group of 7,000 ppm showed reduced body weight, decreased serum/plasma protein and bilirubin levels, dilated cecum, reduced weight of the testis and epididymis and corresponding testicular atrophy, gall bladder dilation and mucosal hyperplasia. The animals in the high dose group were sacrificed at the end of month 19 due to increased mortality. Treatment-related effects on mortality indicated that the high dose exceeded the maximum tolerated dose. In general, male mice started to show treatment-related effects at lower doses compared with female mice. Poor general condition was observed in males at 2,000 ppm. Food consumption and body weight were not affected by the treatment at all doses. Plasma protein and bilirubin concentrations were reduced, and urea concentration was increased in males at 2,000 ppm and above.

Non-neoplastic changes found in liver, mesenteric lymph nodes, epididymides, and seminal vesicles at 7,000 ppm were not associated with increase in proliferative lesions (either hyperplasia or neoplasia). Slight gall bladder pathological changes (dilation, mucosal hyperplasia, adenoma, concretions) observed at 2,000 ppm and above were considered as consequences of gall bladder concretions, not neoplastic lesions.

It was concluded that pradofloxacin was not carcinogenic under the condition of the study in mice.

Summary of the genotoxicity and carcinogenicity:

Pradofloxacin is unlikely to pose carcinogenic concerns to human consumers due to its genotoxicity threshold mechanism and the lack of carcinogenicity evidence in the cancer study in mice and other repeat-dose studies in various species.

o. Photoirritation/photoimmunogenicity study

Title: Study of Photoreactive Potential in Mice. (Study No. T 70698)

Report Date: September 10, 2001

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to OECD Test Guideline No. 406 regarding testing for immunological reactivity of a chemical. This study added the variable UVA light to the test to evaluate photo activation of a chemical. SPF-bred female NMRI mice (6/group) were dosed with pradofloxacin (0, 3, 30, 90 mg/kg bw with UVA radiation, and 90 mg/kg bw without UVA radiation; 10 mL/kg bw in phosphate buffered saline) and a positive control (Bay V 1749, sparfloxacin at 200 mg/kg bw 10 mL/kg bw in propylene glycol 400) for three days, followed by a dose of 20 Joules UVA/cm^2 30 minutes after treatment. The immunological stimulation was measured by auricular lymph node size and cell number, ear swelling (edema), and weight of ear punch biopsy (8 mm). The Integrated Model for the Differentiation of Skin Reactions (IMDS) as published at Toxicology and Applied Pharmacology 153(1), 83-94 (1998) was used to evaluate the data.

Results and Conclusion: The dosing of mice with 90 mg/kg bw pradofloxacin (no UVA light) led to an increase in the auricular lymph node cell count but not auricular lymph node weight, ear punch biopsy weight or ear thickness (swelling). The inclusion of UVA light at 30 and 90 mg/kg BW led to an increase in the auricular lymph node cell number. This increase (5.66) was significantly higher than that with pradofloxacin alone (2.90) indicating phototoxicity. This result demonstrated that pradofloxacin has a photoreactive potential.

A NOEL/NOAEL for phototoxicity was established at 3 mg/kg bw based on increased auricular lymph node cell counts observed at 30 mg/kg bw.

p. Immunotoxicity study in Rats

Title: Study for Subacute Oral Toxicity in Rats. (Study No. T3067316)

Report Date: Nov 24, 1999

Study Location: Wuppertal, North Rhine–Westphalia, Germany

Study Design: This GLP study was conducted according to, in some extent, OECD Test Guideline No. 407 and Method B 7 Directive 67/548/EEC of June 27, 1967. Pradofloxacin was administered in diets to male Wistar rats (5/group) for 34 days and female Wistar rats (5/group) for 35 days at 0, 300, 1000 or 3000 ppm (equal to 0, 28.4, 105.8 or 312.1 mg/kg bw/day for males and 0, 32.3, 119.7 or 415.5 mg/kg bw/day for females, respectively). At day 29, five days before necropsy, rats were injected with sheep red blood cells (sRBC) to prepare for the plaque forming cell assay (PFCA). sRBC were diluted to 5 x 10^8 per mL balanced salt solution and intravenously injected into each rat (100 µL) to induce a humoral immune response. Four aliquots of suspensions from crushed spleens of individual rats were tested. sRBC-specific IgM and IgG plaques were determined in duplicate after incubation with guinea pig complement. The number of plaques per 10^6

spleen cells was calculated and IgG induced plaques were determined after incubating with a goat anti-rat serum.

Results and Conclusion: No statistically significant differences were seen comparing the means of each group. No apparent treatment effects were seen at up to the highest dose 3,000 ppm. The immunotoxic potential of pradofloxacin at these doses should be assessed along with hematology or organ weights in the subchronic and chronic studies.

q. Other Studies

The following safety pharmacology studies with pradofloxacin indicated no evidence of effects on neurology, gastrointestinal tract, lipid metabolism, and blood pharmacology, but exhibited potential effects on heart and kidney:

- *Cardiovascular effect:* In the *in vitro* test on stably transfected HEK293 Cells, pradofloxacin was determined to be a low-potency blocker of the hERG K^+ current at a dose range from 10 to 1,000 µM and only extremely high concentrations (> 1 mM) possessed the potential to delay the repolarization of cardiac action potentials. In addition, pradofloxacin in artificially respired dogs at 10 and 30 µM showed effects on cardiovascular function, respiratory function, ECG, blood gases, or electrolytes.

- *Neurological effect:* In the *in vitro* study using rat hippocampus tissue slices, pradofloxacin (2 µmol/L) showed slight excitatory potential, which was comparable to that of moxifloxacin. In a single dose study with oral or intravenous administration to male Wistar rats, pradofloxacin (at doses up to 30 mg/kg bw) had no effects on convulsive threshold dose of pentylenetetrazole, nocifensive responsiveness to heat, and duration of hexobarbital-induced anesthesia.

- *Gastrointestinal effect:* Pradofloxacin had no effect on gastrointestinal motility at the highest dose tested (30 mg/kg bw). Pradofloxacin did not induce contractions or relaxation of the ileum segments, nor exerted any effects on ileum contractions stimulated by acetylcholine, histamine, serotonin, or barium chloride.

- *Renal function, lipid metabolism, and blood pharmacology*: Pradofloxacin had no effect on blood pharmacology and lipid metabolism of rats at doses from 3 to 30 mg/bw. Pradofloxacin increased urine volume at the doses of 3 and 30 mg/kg bw (diuretic effect) and electrolyte excretion at 30 mg/kg bw (kaliuretic effect).

2. Point of Departure for the Toxicological Acceptable Daily Intake (ADI) Determination

Studies considered for determination of the point of departure for chronic exposure to total residues of pradofloxacin are summarized in Table IV.2. Based on the available toxicology studies, the LOEL/LOAEL of 2.5 mg/kg bw/day from the 52-week chronic oral toxicity study in rats (Study No. T 74621) and the

NOEL/NOAEL of 2.0 mg/kg bw/day from the 13-week subchronic oral toxicity study in Beagle dogs (Study No. PH 31940) were selected to be the most appropriate for the determination of the toxicological ADI for chronic exposure of total residues of pradofloxacin to human consumers.

Table IV.2. Summary of NOEL/NOAEL or LOEL/LOAEL in toxicology studies for pradofloxacin

Study Type	Study Number	NOEL/NOAEL (mg/kg bw/day) (or LOEL/LOAEL (mg/kg bw/day))
Murine dermal photo-activation and immunological assay	T70698	3
Subchronic Oral Toxicity Study in Rats (mainly on liver enzyme induction)	PH29308	1.1 (LOEL/LOAEL)
Subchronic Oral Toxicity Study in Rats (with 4-week recovery)	PH31595 and PH31596	21 (LOEL/LOAEL)
Subchronic Oral Toxicity Study in dogs	PH31940	2
Chronic Oral Toxicity Study in Rats	T74621	2.5 (LOEL/LOAEL)
Developmental Toxicity Study in Rats	T8062974	Maternal: 5 Fetal: 5
Developmental Toxicity Study in Rabbits	T1067873	Maternal: 3 Fetal: 3 (LOEL/LOAEL)
Developmental Toxicity Study in Rabbits	T2073363	Maternal: 1 Fetal: 1 (LOEL/LOAEL)
Two-Generation Reproductive Study in Rats	PH33955	Offspring: 40 Parental: 8 (LOEL/LOAEL)

3. Toxicological ADI

The toxicological ADI for total residue of pradofloxacin is calculated using the following formula. The LOEL/LOAEL of 2.5 mg/kg bw/day from the 52-week oral toxicity study in rats and the NOEL/NOAEL of 2.0 mg/kg bw/day from the 13-week subchronic oral toxicity study in dogs were selected. A safety factor of 1,000 accounts for a 10-fold factor for the estimation of the NOEL/NOAEL from the LOEL/LOAEL for the dog study (or subchronic to chronic exposure for the rat study), a 10-fold factor for animal-to-human variability, and a 10-fold factor for human to-human variability.

$$\text{Toxicological ADI} = \frac{\text{NOEL/NOAEL}}{\text{Safety Factor}} = \frac{2\ \text{mg/kg bw/day}}{1000}$$

$$= 0.002\ \text{mg/kg bw/day} = 2\ \mu\text{g/kg bw/day}$$

The toxicological ADI for total residue of pradofloxacin is established at 2 µg/kg bw/day.

4. Microbiological ADI

   a. Determination of the need for establishing a microbiological ADI

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

      The answer to this question is "**yes,**" supported with antimicrobial susceptibility data from Study # 204910. Bacterial isolates were anaerobically derived from 12 normal subjects with no diarrhea, and no use of antimicrobial drugs, within three months prior to participation in the study. Study subjects were from the U.S. population. A total of 73 human intestinal flora isolates representing seven bacterial groups were recovered and used in the susceptibility study. The isolates used for the submitted minimum inhibitory concentration (MIC) study were all freshly recovered, and the number of species in each group (except for *Escherichia coli*) was evenly distributed, which better represents each group. The susceptibility study is described below.

      Title: Susceptibility Testing of Bacteria Isolated from Human Feces**.** (Study No. 204910)

      Study Dates: January 23 to February 12, 2017

      Study Location: Kalamazoo, MI

      Study Design: The objective of this study was to conduct susceptibility testing to determine the MIC of pradofloxacin against common members of the intestinal microflora from healthy human subjects. The MIC of pradofloxacin against bacterial isolates was determined by dilution methods in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. American Type Culture Collection (ATCC) organisms used for quality control purpose in the study included *Bacteroides fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, *Eubacterium lentum* ATCC 43055, *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Streptococcus pneumoniae* ATCC 49619. Broth microdilution or agar dilution methodologies were used dependent on bacterial groups. A double-dilution scheme was applied to the test article and a final test concentration range for pradofloxacin used was 0.002 to 128 µg/mL.

      Results and Conclusions: Pradofloxacin demonstrated the most potent activity against *E. coli* (MIC_{50}/MIC_{90}: 0.015/0.5) followed by *Bifidobacterium* spp. and *Lactobacillus* spp. (0.5/2), *Enterococcus* spp. (0.25/8), and *Clostridium* and *Bacteroides* spp. (4/8 and 1/8, respectively). Table IV.3 lists the MIC_{50}, MIC_{90} and MIC ranges for the tested groups.

Table IV.3. Susceptibility of pradofloxacin against representative bacterial groups from human subjects

Bacterial group/# isolate	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC Range (µg/mL)
Escherichia coli	0.015	0.5	0.015 - 4
Enterococcus species	0.25	8	0.06 - 8
Lactobacillus species	0.5	2	0.12 - 2
Bifidobacterium species	0.5	2	0.12 - 8
Clostridium species	4	8	0.5 - 16
Bacteroides fragilis and other species	1	8	0.25 -16
E. lentum	1	Not applicable	0.5 - 128

In conclusion, pradofloxacin is active against human intestinal bacterial flora. Based on the assessed MIC_{50} values, the MIC_{calc} determined for subsequent use in the determination of the microbiological ADI was 0.175 µg/mL.

(2) Step 2: Do pradofloxacin residues enter the human colon?

Based on analysis of similar advanced fluroquinolones using published information, oral bioavailability for pradofloxacin is estimated at 90%, leaving at least 10% ingested pradofloxacin residues entering the human colon; therefore, the answer is '**yes**".

(3) Step 3: Do pradofloxacin residues entering the human colon remain microbiologically active?

The answer is "**yes**". The sponsor's data from an *in vitro* fecal binding study using 25% fecal slurries demonstrated that 75% of pradofloxacin binds to human feces; thus, part of the pradofloxacin residues within the colon are considered biologically active.

Details of the fecal binding study are described below.

Title: Effect of Fecal Binding on the Antibacterial Activity of Pradofloxacin**.** (Study No. 204421)

Study Dates: December 6, 2016, to February 7, 2017

Study Location: Kalamazoo, MI

Study Design: The objective of the study was to determine the effects of fecal binding on the antibacterial activity of pradofloxacin. Fecal specimens were freshly collected from three volunteers with no history of antimicrobial use three months prior to the study. The final fecal suspension used in the experiments was 25% w/v prepared with sterile

growth medium, and growth medium alone without fecal material was the control group. Test pradofloxacin concentrations were prepared from stock solutions to obtain final testing concentrations of 0, 0.05, 0.1, 0.2, 0.5, 1, 2, and 5 µg/mL in both the control and 25% fecal suspension series, respectively. Following incubation, aliquots were removed at 0, 0.5, 1, 2, 6, and 12 hours from the control and test groups at each tested pradofloxacin concentration, respectively, and were centrifuged to pellet fecal solids. The resulting supernatant served as the test sample and was then evaluated for *in vitro* inhibitory activity against indicator *E. coli* ATCC 25922 strain.

The supernatant from the mixtures from each test was inoculated with *E. 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 with non-inoculated control cells of each fecal/drug mixture. An estimate of bound pradofloxacin was performed applying the approach for assessing the binding of an antimicrobial to serum, which is calculated as follows:

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

A quality control broth microdilution MIC range of 0.008 to 0.03 µg/mL for pradofloxacin for *E. coli* ATCC 25922 was based on CLSI standards.

Results and Conclusions: Based on the residual *in vitro* activity of pradofloxacin against *E. coli* ATCC 25922 after incubation in the presence of 25% w/v human feces, at least 50% of the pradofloxacin was immediately bound. Additional binding of pradofloxacin was also observed to occur in a time dependent and specimen dependent fashion. The slowest and lowest amount of pradofloxacin estimated to be bound by the 25% suspension of human feces was observed with one of the three specimens where 75% of the pradofloxacin was bound after 6 and 12 hours of incubation. The fastest and highest amount of pradofloxacin estimated to be bound to feces was observed with another specimen, where 75% of the pradofloxacin was bound after 0.5 hours of incubation, and 90% of the pradofloxacin was bound after 6 and 12 hours of incubation. The third specimen also exhibited rapid binding of pradofloxacin to feces with an estimated 75% bound after 0.5 and 1 hours of incubation.

In conclusion, 75% fecal binding was estimated for pradofloxacin, resulting in only 25% of ingested pradofloxacin biologically active in the colon.

(4) Step 4: Is there a scientific justification to eliminate testing of either one or both endpoints of concern: colonization barrier disruption and/or increases in populations of resistant intestinal bacteria?

Yes, CVM determined that there was justification to eliminate testing for the endpoint: *increases in populations of resistant intestinal bacteria*. This conclusion was made because of the following factors:

(a) Pradofloxacin is essentially like enrofloxacin with respect to activity, spectrum, and mechanisms of action and resistance, as both belong to the fluoroquinolone class; thus, there should be a consistency in the approach in considering Step 4 pertaining to this endpoint.

(b) According to FDA-funded research performed in chemostats and HFA-rodents, the *Bacteroides fragilis* group was the most likely group to pose a public health concern with respect to fluoroquinolones [Carman RJ, Simon MA, Petzold III HE, Wimmer RF, Batra MR, Fernández AH, Miller MA, Bartholomew M (2005). Antibiotics in the human food chain: Establishing no effect levels of tetracycline, neomycin, and erythromycin using a chemostat model of the human colonic microflora. Regulatory Toxicology and Pharmacology 43,168-180].

(c) Another FDA-funded research project also concluded that the *B. fragilis* group was recommended as a suitable index bacterial group for use in assessing this endpoint [Perrin-Guyomard A, Poul JM, Corpet DE, Sanders P, Fernández AH, Bartholomew M (2005). Impact of residual and therapeutic doses of ciprofloxacin in the human-flora associated mice model. Regulatory Toxicology and Pharmacology 42, 151-160].

(d) In the case of enrofloxacin, a survey was conducted to determine the baseline MICs of *Bacteroides fragilis* group isolated from the intestinal tracts of healthy human subjects, and it was found that there are existing populations in healthy fecal donors with MICs as high as 128 µg/mL against ciprofloxacin.

(e) Because of the presence of the existing FQ-resistant subpopulation and its wide variation in healthy human subjects associated with the index bacterial group, it is difficult to determine a no observable adverse effect concentration/effect level (NOAEC or NOAEL) for use in setting the microbiological ADI for this endpoint.

Therefore, the microbiological ADI should be determined and calculated based on the “colonization barrier disruption” endpoint.

b. Determination of the microbiological ADI

(1) Determination of the fraction of oral dose available for microorganisms

Based on information provided in the stepwise assessment above, the fraction is a consideration of the function of projected oral bioavailability of pradofloxacin (90%) and its fecal-bound portion (75%); thus, the fraction available in the colon for potential interaction with human intestinal flora is: 0.1 x 0.25 = 0.025.

(2) Determination of the microbiological ADI using MIC_{calc}

Because the endpoint of concern is disruption of the colonization barrier, the microbiological ADI is derived from MIC data using the formula below:

$$\text{Microbiological ADI (µg/kg bw/day)} = \frac{MIC_{calc} \times \text{volume of colon content}}{\text{fraction of oral dose available to microorganisms X human body weight}}$$

Where: MIC_{calc} is 0.175 µg/mL, volume of colon content is 500 mL/day, fraction of oral dose available to microorganisms is 0.025, and human body weight is 60 kg; therefore, the final calculation of the microbiological ADI is 58 µg/kg bw/day.

C. Establishment of the Final ADI

Because the toxicological ADI of 2 µg/kg bw/day determined from the 52-week oral toxicity study in rats and the 13-week subchronic oral toxicity study in dogs is considered more appropriate to protect human health than the calculated microbiological ADI of 58 µg/kg bw/day, the toxicological ADI (2 µg/kg bw/day) is established as the final ADI for total residue of pradofloxacin.

D. Safe Concentrations for Total Residues in Edible Tissues and Injection Sites

1. The calculation of the tissue safe concentrations is based on the "General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals" (FDA/CVM, Guidance for Industry #3, May 2022). The safe concentration for total residues of pradofloxacin in edible tissues is calculated using the following formula:

$$\text{Safe Concentration (SC)} = \frac{\text{ADI} \times \text{Human Body Weight}}{\text{Food Consumption Value}}$$

For example, the safe concentration for total residues of pradofloxacin in muscle is calculated as:

$$\text{SC (muscle)} = \frac{2\ \mu g/kg\ bw/day \times 60\ kg}{300\ g/day} = 0.4\ \mu g/g = 0.4\ ppm$$

2. For the injection site, the safe concentration is calculated using ten times (10X) of the muscle safe concentration.

   Safe Concentration Injection Site = SC (Muscle) × 10 = 4.0 µg/g = 4.0 ppm

3. Safe Concentrations for total residue of pradofloxacin in edible tissues of cattle and swine using the Food Consumption Values.

Table IV.4. Summary Table of Safe Concentrations for Total Residues

Edible Tissue	Amount Consumed Per Day	Safe Concentration
Muscle	300 g	0.4 ppm
Liver	100 g	1.2 ppm
Kidney	50 g	2.4 ppm
Skin/Fat	50 g	2.4 ppm
Injection Site	300 g	4.0 ppm

E. Residue Chemistry

1. Summary of Residue Chemistry Studies

   a. Total Residue and Metabolism Studies

   Cattle

   Title: Total Radioactive Residue Depletion and Metabolism of [2-^{14}C] Pradofloxacin in Beef Cattle Tissues Following a Single Subcutaneous Injection. (Study No. 202129)

   Study Dates: September 2015 to September 2016

   Study Locations: Las Cruces, NM (in-life phase) and Plainsboro, NJ (analytical phase)

   Study Design:

   Objective: This GLP-study was conducted to determine the total radioactive residue (TRR) concentrations and the nature and quantity of TRR of pradofloxacin and its metabolites in the edible tissues, urine, and feces of cattle treated with pradofloxacin.

   Study Animals: Twelve crossbred beef cattle (6 steers and 6 heifers), weighing 237.5 to 283 kg prior to dosing

   Dose Administration: Animals were individually housed in metabolism cages and received a single subcutaneous injection of 10 mg [^{14}C]-pradofloxacin/kg BW.

   Sampling: Daily excretion of urine and feces was collected separately via collection devices suspended under each metabolism cage. Animals were

slaughtered at 10, 20, 36, and 48 hours post-dose and kidney, liver, loin muscle, perirenal fat and injection site tissues were collected.

Analysis: Total radioactivity in tissues and feces was measured by combustion followed by liquid scintillation counting (LSC). Total radioactivity in urine was measured by direct LSC. Concentrations of pradofloxacin in cattle liver, kidney, loin muscle, perirenal fat and injection site were determined using high-performance liquid chromatography-mass spectrometry (HPLC-MS). The metabolic profile was determined using reverse-phased HPLC with radio-detection.

Results and Conclusions: Of the non-injection site tissues, kidney was the tissue in which total radiolabeled residues and parent pradofloxacin equivalents depleted most slowly (Tables IV.5 and IV.6, respectively). This indicates that when total residue concentrations in kidney are less than their safe concentration, the total residues in the other non-injection site edible tissues would also be below their respective safe concentrations.

The data (Table IV.5) indicate that injection site total residue concentrations were highly variable and depleted to less than their safe concentration at the latest timepoint compared with other edible tissues (*i.e.,* 48 hours). This indicates that injection site residues would be the determining factor in establishing the human food safety of edible tissues in cattle. To assess the safety of injection site residues, the concentration of parent pradofloxacin was determined at the time point when total injection site residues were less than their safe concentration (*i.e.,* 48 hours) and was established as 100 parts per billion (ppb) (Table IV.6).

Parent pradofloxacin was the only major residue detected in all edible tissues and a known relationship exists between pradofloxacin and total residues in the edible tissues (Table IV.7). The total percent dose excreted over 48 hours in cattle urine and feces samples ranged from ~35% to ~81% (Table IV.8).

Table IV.5. Mean (± Std Dev) Concentrations (ppm) of Total Radiolabeled Residues (TRR) in Edible Tissues of Beef Cattle Treated with 10 mg [^{14}C]-pradofloxacin/kg BW.

Hours Post-Dose	TRR Kidney	TRR Liver	TRR Loin Muscle	TRR Perirenal Fat	TRR Injection Site Core	TRR Injection Site Surrounding
10	7.87 ± 3.97	2.66 ± 0.98	1.59 ± 0.40	0.11 ± 0.02	315.53 ± 414.89	40.29 ± 55.0
20	2.73 ± 1.79	0.92 ± 0.25	0.41 ± 0.20	0.04 ± 0.001	355.28 ± 293.86	4.28 ± 3.75
36	0.81 ± 0.04	0.60 ± 0.12	0.05 ± 0.01	0.05 ± 0.02	4.17 ± 1.41	0.39 ± 0.17
48	0.69 ± 0.23	0.47 ± 0.04	0.02 ± 0.01	0.01 ± 0.001	0.88 ± 0.84	0.40 ± 0.63

*Limit of quantitation (LOQ) – 0.001 to 0.003 ppm

Table IV.6. Mean (± Std Dev) Concentrations (ppm) of Parent Pradofloxacin Equivalents in Edible Tissues of Beef Cattle Treated with 10 mg [^{14}C]-pradofloxacin/kg BW.

Hours Post-Dose	Kidney	Liver	Loin Muscle	Perirenal Fat	Injection Site Core	Injection Site Surrounding
10	6.06 ± 3.29	1.76 ± 0.90	1.44 ± 0.31	0.08 ± 0.02	278.02 ± 376.75	Not measured
20	1.95 ± 1.43	0.47 ± 0.21	0.34 ± 0.19	0.02	306.79 ± 263.13	Not measured
36	0.19 ± 0.09	0.07 ± 0.02	0.045	Not measured	0.54 ± 0.23	Not measured
48	0.10 ± 0.06	0.09 ± 0.04	Not measured	Not measured	0.09 ± 0.01	0.165

*LOQ – 0.001 ppm

Table IV.7. Ratio Between Parent Pradofloxacin Concentrations (the marker residue) and Total Residue Concentrations in Edible Tissues of Beef Cattle Treated with 10 mg [^{14}C]-Pradofloxacin/kg BW.

Hours Post-Dose	Kidney	Liver	Loin Muscle	Perirenal Fat	Injection Site Core	Injection Site Surrounding
10	0.76 ± 0.04	0.64 ± 0.10	0.91 ± 0.03	0.71 ± 0.13	0.79 ± 0.16	Not applicable
20	0.68 ± 0.13	0.49 ± 0.11	0.81 ± 0.08	0.391	0.71 ± 0.27	Not applicable
36	0.24 ± 0.10	0.12 ± 0.01	0.726	Not applicable	0.15 ± 0.10	Not applicable
48	0.14 ± 0.05	0.20 ± 0.11	Not applicable	Not applicable	0.08 ± 0.10	0.15

Table IV.8. Percent Dose Present in Urine and Feces Samples from Cattle 48 Hours Post-Dose.

Animal Number	Time Interval (hr)	% Dose Urine	% Dose Feces	% Dose Total
5609F	0-24	60.96	0.75	61.71
5609F	24-48	1.73	3.67	5.40
5614F	0-24	7.58	22.25	29.83
5614F	24-48	1.05	4.29	5.34
5621M	0-24	63.88	11.11	74.99
5621M	24-48	1.19	4.36	5.55

Swine

Title: Total Radioactive Residue Depletion and Metabolism of [2-^{14}C] Pradofloxacin in Swine Tissues Following a Single Intramuscular Injection. (Study No. 202669)

Study Dates: January 2016 to December 2016

Study Locations: Las Cruces, NM (in-life phase) and Plainsboro, NJ (analytical phase)

Study Design:

Objective: This GLP-study was conducted to determine the TRR concentrations and nature and quantity of TRR of pradofloxacin and its metabolites in the edible tissues, urine, and feces of swine treated with pradofloxacin.

Study Animals: Twelve crossbred swine (6 barrows and 6 gilts), weighing 51.5 to 72 kg prior to dosing

Dose Administration: Animals were individually housed in metabolism cages and received a single intramuscular injection of 7.5 mg [^{14}C]-pradofloxacin/kg BW.

Sampling: Daily excretion of urine and feces was collected separately via collection devices suspended under each metabolism cage. Animals were slaughtered at 10, 20, 36, and 48 hours post-dose and kidney, liver, loin muscle, skin with fat and injection site tissues were collected.

Analysis: Total radioactivity in tissues and feces was measured by combustion followed by LSC. Total radioactivity in urine was measured by direct LSC. Concentrations of pradofloxacin in swine liver, kidney, loin muscle, skin with fat and injection site were determined using HPLC-MS. The metabolic profile was determined using reverse-phased HPLC with radio-detection.

Results and Conclusions: Of the non-injection site tissues, kidney was the tissue in which total radiolabeled residues depleted most slowly (Table IV.9). This indicates that when total residue concentrations in kidney are less than their safe concentration, the total residues in the other non-injection site edible tissues would also be below their respective safe concentrations.

To assess the safety of injection site residues, the concentration of parent pradofloxacin was determined at the time point when total injection site residues were less than their safe concentration of 4 ppm (*i.e.,* 20 hours). This concentration was determined to be 400 ppb (Table IV.10).

Because swine injection site total residues are below their safe concentration at the time point when total residues in swine kidney fall below their safe concentration (*i.e.,* 36 hours), the depletion of parent pradofloxacin from the

target tissue (kidney) would be the determining factor in establishing the human food safety of edible tissues in swine.

Parent pradofloxacin was the only major residue detected in all edible tissues and a known relationship exists between pradofloxacin and total residues in the edible tissues (Table IV.11). The total percent dose excreted over 48 hours in swine urine and feces samples ranged from ~17% to ~58% (Table IV.12).

Table IV.9. Mean (± Std Dev) Concentrations (ppm) of Total Radiolabeled Residues (TRR) in Edible Tissues of Swine Treated with 7.5 mg [^{14}C]-pradofloxacin/kg BW.

Hours Post-Dose	TRR Kidney	TRR Liver	TRR Loin Muscle	TRR Skin with Fat	TRR Injection Site (Core + Surrounding)
10	7.14 ± 0.94	2.16 ± 0.05	1.67 ± 0.24	0.54 ± 0.36	10.71 ± 8.57
20	3.86 ± 3.37	1.26 ± 0.69	0.78 ± 0.65	1.00 ±0.78	2.25 ± 1.23
36	0.57 ± 0.05	0.45 ± 0.08	0.04 ± 0.002	0.39 ± 0.28	1.45 ± 1.57
48	0.46 ± 0.03	0.40 ± 0.04	0.02 ± 0.003	0.21 ± 0.19	2.00 ± 1.03

*LOQ – 0.001 to 0.003 ppm

Table IV.10. Mean (± Std Dev) Concentrations (ppm) of Pradofloxacin Equivalents in Edible Tissues of Swine Treated with 7.5 mg [^{14}C]-pradofloxacin/kg BW.

Hours Post-Dose	Kidney	Liver	Loin Muscle	Skin with Fat	Injection Site (Core + Surrounding)
10	5.69 ± 0.78	1.54 ± 0.09	1.50 ± 0.22	0.38 ± 0.26	3.65 ± 2.71
20	2.60 ± 2.62	0.69 ± 0.52	0.70 ± 0.59	0.78 ± 0.62	0.42 ± 0.28
36	0.11 ± 0.02	0.04 ± 0.01	Not measured	0.26 ± 0.20	0.13 ± 0.10
48	0.04 ± 0.01	0.02 ± 0.004	Not measured	0.18 ± 0.11	0.23 ± 0.21

*LOQ – 0.005 ppm

Table IV.11. Marker:Total Ratios

Hours Post-Dose	Kidney	Liver	Loin Muscle	Skin with Fat	Injection Site (Core + Surrounding)
10	0.80 ± 0.01	0.71 ± 0.03	0.90 ± 0.03	0.72 ± 0.05	0.34 ± 0.08
20	0.62 ± 0.11	0.51 ± 0.10	0.89 ± 0.01	0.75 ± 0.07	0.20 ± 0.08
36	0.19 ± 0.03	0.08 ± 0.02	NA	0.58 ± 0.19	0.14 ± 0.16
48	0.09 ± 0.01	0.06 ± 0.01	NA	0.38 ± 0.33	0.17 ± 0.21

Table IV.12. Percent Dose Present in Urine and Feces Samples from Swine 48 Hours Post-Dose.

Animal Number	Time Interval (hr)	% Dose Urine	% Dose Feces	% Dose Total
5635F	0-24	46.63	6.60	53.23
5635F	24-48	2.58	2.01	4.59
5632F	0-24	16.64	20.95	37.59
5632F	24-48	2.01	17.65	19.66
5639M	0-24	11.24	3.65	14.89
5639M	24-48	1.17	0.58	1.75

b. Comparative Metabolism Study

Title: Comparative *In Vitro* Metabolism of [2-^{14}C] Pradofloxacin in Hepatocytes and Liver Microsomes from Rats, Dogs, Beef Cattle, Swine and Humans. (Study No. 204021)

Study Dates: February 2016 to September 2016

Study Location: Plainsboro, NJ

Study Design:

Objective: This GLP-study was conducted to determine the metabolite profiles of [2-^{14}C] pradofloxacin in liver microsomes and hepatocytes from Wistar rats, Beagle dogs, beef cattle, swine, and humans and to characterize the major metabolites of [2-^{14}C] pradofloxacin in the samples by LC-MS.

Test Systems: Male and female rats, beagle dogs, beef cattle and swine plated hepatocytes, and mixed gender human plated hepatocytes and liver microsomes were used.

Dose Administration, Sampling and Analysis: [2-^{14}C] Pradofloxacin at 1 and 10 µM was separately incubated in duplicate with rat, dog, beef cattle, swine, and human-plated hepatocytes for 24 hours and with rat, dog, beef cattle, swine, and human liver microsomes for 0 and 2 hours. After incubation, the incubation mixture from each well was extracted. Radioactivity was determined by LSC. Radioprofiling was determined by HPLC with radio-detection.

Results and Conclusions: The major metabolite, parent pradofloxacin, in the target animal species was detected in all hepatocytes and liver microsomes, indicating comparative metabolism between the target animal species (cattle and swine) and toxicological relevant species.

c. Studies to Establish Withdrawal Periods

Tissue Residue Depletion Study

Cattle

Title: Pradofloxacin Depletion in Cattle Tissues Following a Single Subcutaneous Administration with Pradofloxacin Trihydrate Injection (22.73% w/v). (Study No. ELA220960)

Study Dates: June 2022 to May 2023

Study Locations: Parma, ID (in-life phase) and Indianapolis, IN (analytical phase)

Study Design:

Objective: This GLP-study was conducted to evaluate the depletion of pradofloxacin injection in cattle tissues after a single subcutaneous injection.

Study Animals: Thirty crossbred Angus beef cattle (15 males and 15 females), weighing 294 to 416 kg prior to dosing

Drug Administration: Animals received a single subcutaneous injection of 10 mg pradofloxacin trihydrate/kg BW.

Sampling and Analysis: Animals were slaughtered at 12, 24, 48, 72, and 96 hours post-dose and kidney, loin muscle, and injection site tissues were collected. Parent pradofloxacin concentrations were determined in cattle tissues using an LC-MS/MS method.

Results and Conclusions: The mean concentrations of parent pradofloxacin in the edible tissues of cattle are provided in Table IV.13. Residues of parent pradofloxacin at the injection site depleted to less than the previously determined safety number, 100 ppb, at 96 hours withdrawal.

Based on the relationship between parent pradofloxacin and total pradofloxacin residues (Table IV.7), concentrations of total pradofloxacin residues in kidney tissues are less than their safe concentration at 96 hours. Because kidney is the target tissue and will be used for monitoring to establish the human food safety of edible tissues in cattle, it was necessary to determine the maximum concentration of parent pradofloxacin expected to occur in cattle kidney at the timepoint when total residue concentrations in all edible tissues deplete to less than their respective safe concentrations (*i.e.,* 96 hours). The upper tolerance limit for the 99th percentile with 95% confidence (99/95 UTL) for concentrations of pradofloxacin in cattle kidney at 96 hours post-dose was calculated to be 30 ppb.

Table IV.13. Mean (± Std Dev) Concentrations (ppb) of Pradofloxacin in Edible Tissues of Beef Cattle Treated Subcutaneously with 10 mg pradofloxacin/kg BW.

Hours Post-Dose	Kidney	Loin Muscle	Injection Site Core	Injection Site Surrounding
12	3634 ± 1247.4	731.8 ± 214.4	87005.8 ± 67859.0	8684.5 ± 7213.0
24	396.2 ± 241.2	79.7 ± 41.4	9367.2 ± 8898.1	1155.4 ± 898.3
48	36.9 ± 15.9	7.7 ± 2.0	819.7 ± 1614.9	79.6 ± 121.8
72	15.1 ± 6.9	4.7 ± 1.5	216.0 ± 407.7	24.2 ± 44.1
96	8.7 ± 1.4	BLOQ	4.93	BLOQ

*BLOQ = Below LOQ (< 4.27 ppb)

Swine

Title: Pradofloxacin Depletion in Swine Tissues Following a Single Intramuscular Administration with Pradofloxacin Trihydrate Injection (22.73% w/v). (Study No. ELA221070)

Study Dates: July 2022 to February 2023

Study Locations: Parma, ID (in-life phase) and Indianapolis, IN (analytical phase)

Study Design:

Objective: This GLP-study was conducted to evaluate the depletion of pradofloxacin injection in swine tissues after a single intramuscular injection.

Study Animals: Thirty-two crossbred swine (16 barrows and 16 gilts), weighing 52 to 73 kg prior to dosing

Drug Administration: Animals received a single intramuscular injection of 7.5 mg pradofloxacin trihydrate/kg BW.

Sampling and Analysis: Animals were slaughtered at 12, 24, 48, 72, and 96 hours post-dose and kidney, loin muscle, and injection site tissues were collected. Pradofloxacin concentrations were determined in swine tissues using an LC-MS/MS method.

Results and Conclusions: The mean concentrations of parent pradofloxacin in the edible tissues of swine are provided in Table IV.14. Kidney residue data were analyzed using a statistical tolerance limit algorithm that determines the 99/95 UTL. The data support assignment of a 48-hour (2-day) withdrawal period.

At 48-hour withdrawal, residues of parent pradofloxacin at the injection site depleted to less than the previously determined safety number, 400 ppb,

indicating that a 48-hour withdrawal period in swine ensures the human food safety of the edible tissues in swine.

Table IV.14. Mean (± Std Dev) Concentrations (ppb) of Pradofloxacin in Edible Tissues of Swine Treated Intramuscularly with 7.5 mg pradofloxacin/kg BW.

Hours Post-Dose	Kidney	Loin Muscle	Injection Site Core	Injection Site Surrounding
12	3040.2 ± 916.1	1031.2 ± 245.9	18724.5 ± 26498.3	876.2 ± 320.9
24	197.3 ± 38.5	62.1 ± 15.4	108.5 ± 156.4	44.5 ± 6.0
48	31.8 ± 8.5	10.9 ± 3.2	9.8 ± 2.5	17.4 ± 9.7
72	29.5 ± 24.9	8.5 ± 6.1	10.1 ± 7.8	9.3 ± 5.0
96	33.8 ± 29.5	9.8 ± 6.7	7.8 ± 4.0	8.2 ± 3.5

*LOQ = 4.33 ppb

2. Target Tissue and Marker Residue

   The target tissue for residue monitoring in cattle and swine is kidney. The marker residue in edible tissues of cattle and swine is pradofloxacin (parent drug).

3. Tolerances

   Cattle

   Based on the results from the total residue and metabolism study (Study Number 202129) and tissue residue depletion study (Study Number ELA220960), CVM established the tolerance for residues of parent pradofloxacin as 30 ppb in cattle kidney by calculating the 99/95 UTL at 96 hours. A tolerance of 30 ppb in cattle kidney ensures that total residues in the edible tissues of cattle, including injection site tissues, do not exceed their respective safe concentrations.

   Swine

   Based on the results from the total residue and metabolism study (Study Number 202669), CVM established the tolerance for residues of pradofloxacin as 1 ppm in swine kidney.

4. Withdrawal Periods

   Cattle

   The data support assignment of a 4-day withdrawal period when used according to label directions in cattle and ensures the human food safety of the edible tissues in cattle.

Swine

The data support assignment of a 2-day withdrawal period when used according to label directions in swine and ensures the human food safety of the edible tissues in swine.

F. Analytical Methods for Residues

1. Description of Analytical Methods

   a. Determinative Procedure

   One gram of homogenized bovine or swine kidney is fortified with the internal standard (pradofloxacin-D_4). After addition of 1 mL water, the mixture is homogenized, followed by the addition of 1 mL of acetonitrile. The mixture is vortexed and centrifuged. After decanting the supernatant into a clean test tube, the tissue pellet is re-extracted twice with 2 mL of a mixture of acetonitrile and 5% aqueous formic acid solution. Each of the supernatants from the two acetonitrile/aqueous formic acid extractions are added to the initial acetonitrile/water extract. The combined extract is centrifuged. After decanting into a clean test tube, the volume of the extract is made up to 7 mL with water. The extract is filtered with a polytetrafluoroethylene (PTFE) syringe filter, followed by LC-MS/MS analysis. The following ion transitions are monitored for quantitation:

   Pradofloxacin: *m/z* 397 → *m/z* 353
   Pradofloxacin-D_4: *m/z* 401 → *m/z* 258

   For confirmation, the following ion transitions are monitored in the positive-ion mode:

   m/z 397 → *m/z* 353 (reference ion)
   m/z 397 → *m/z* 110
   m/z 397 → *m/z* 256

   b. Confirmatory Procedure

   Sample extraction for the confirmatory procedure is identical to the one for the determinative procedure. Pradofloxacin is detected by LC-MS/MS in the positive-ion mode. The following pradofloxacin-specific ion transitions are monitored to obtain ion ratios, signal to noise ratios and retention time reproducibility data that meet the required acceptability criteria:

   m/z 397 → *m/z* 353 (reference ion)
   m/z 397 → *m/z* 110
   m/z 397 → *m/z* 256

2. Availability of the Method

   The validated analytical methods in cattle and swine for analysis of residues of pradofloxacin injection are on file at the Center for Veterinary Medicine,

7500 Standish Place, Rockville, MD 20855. To obtain a copy of the analytical method, please submit a Freedom of Information request to: https://www.accessdata.fda.gov/scripts/foi/FOIRequest/requestinfo.cfm.

V. USER SAFETY

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

User Safety Warnings: Not for use in humans. Keep out of reach of children. Avoid contact with eyes and skin. In case of ocular contact, immediately remove contact lenses and flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water for at least 20 seconds. Consult a physician if irritation persists following ocular or dermal exposures, or in case of accidental ingestion. 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. Do not eat, drink or smoke while handling this product. To obtain a copy of the Safety Data Sheet, contact Elanco at 1-800-428-4441.

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 Pradalex™, when used according to the label, is safe and effective for the conditions of use in the General Information Section above. Additionally, data demonstrate that residues in food products derived from species treated with Pradalex™ 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 order of a licensed veterinarian (Rx marketing status). This decision was based on the following factors: adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this drug product, professional expertise is required to monitor the safe use of this product, and restricting this drug product to use by or on the order of a licensed veterinarian is critical for assuring the safe and appropriate use of this drug product in animals in order to mitigate the potential risk of bacteria developing resistance to this and other antimicrobial drugs.

B. Exclusivity

Pradalex™, as approved in our approval letter, qualifies for THREE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act because the sponsor submitted an original NADA that contains new studies that demonstrate the safety and effectiveness of Pradalex™.

C. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.