Date of Approval: December 16, 2009

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-209

EXCEDE Sterile Suspension

Ceftiofur Crystalline Free Acid
Horses

For the treatment of lower respiratory tract infections in horses caused by susceptible strains of *Streptococcus equi* ssp. *zooepidemicus.*

Sponsored by:

Pharmacia & Upjohn Co.,
a Division of Pfizer, Inc.

TABLE OF CONTENTS

I. GENERAL INFORMATION:

A. File Number:	NADA 141-209
B. Sponsor:	Pharmacia and Upjohn Co., a Division of Pfizer, Inc. 235 East 42d Street New York, NY 10017 Drug Labeler Code: 000009
C. Proprietary Name(s):	EXCEDE Sterile Suspension
D. Established Name(s):	Ceftiofur Crystalline Free Acid
E. Pharmacological Category:	Antimicrobial
F. Dosage Form(s):	Sterile oil suspension for injection
G. Amount of Active Ingredient(s):	200 mg ceftiofur equivalents (CE) per mL
H. How Supplied:	100 mL vial
I. How Dispensed:	Rx
J. Dosage(s):	Two intramuscular injections, 4 days apart, at a dose of 3.0 mg/lb (6.6 mg/kg) body weight (BW). Therapeutic concentrations are maintained for 6 days after the second injection (or a total of 10 days from the beginning of treatment) against *Streptococcus equi* ssp. *zooepidemicus*.
K. Route(s) of Administration:	Intramuscular injection
L. Species/Class(es):	Horses
M. Indication(s):	For the treatment of lower respiratory tract infections in horses caused by susceptible strains of *Streptococcus equi* ssp. *zooepidemicus.*

N. Effect(s) of Supplement: This supplement provides for a new indication, for the treatment of lower respiratory tract infections in horses caused by susceptible strains of *Streptococcus equi* ssp. *zooepidemicus.*

II. EFFECTIVENESS:

A. Dosage Characterization:

The beta-lactam class of antibiotics, which includes ceftiofur, is effective primarily on infections located in the extracellular spaces of the body. For this class of antibiotics, plasma drug concentrations are more closely related to effectiveness than are tissue homogenate concentrations. In tissue homogenates, the active drug concentration at the infection site is diluted by the intracellular fluid volumes released during the homogenization process. Thus, historically from literature, the beta-lactam pharmacokinetic value most correlated with effectiveness is the time plasma concentrations exceed the minimal inhibitory concentration.

Substantial historical information with the use of ceftiofur sodium (NAXCEL) in species for which it is approved (i.e., bovine, poultry, swine, horse, sheep, and goat) indicates that, at effective doses, plasma concentrations of ceftiofur and desfuroylceftiofur metabolites remain above the minimal inhibitory concentration (MIC) of the target pathogens for the interval between injections. In the horse, at the approved dosage regimens, concentrations remain at or above 0.2 µg ceftiofur equivalents (CE)/mL plasma for the entire interval between doses. In a pharmacokinetic study in horses given ceftiofur sodium, plasma concentrations of ceftiofur and desfuroylceftiofur-related metabolites (using the same analytical methods as have been used in other species) remained above the threshold of 0.2 µg/mL for nearly the entire 24-hour dosing interval, relatively consistent with other species. This ceftiofur concentration is higher than the MIC required to inhibit 90% of the tested isolates associated with equine respiratory disease ($MIC_{90} \leq 0.06$ and 0.12 µg ceftiofur equivalents/mL, respectively). The well established relationship of dose/ blood concentration/ effectiveness for ceftiofur sodium was used to bridge between the effective cattle dose of ceftiofur sodium to the horse. It provided dosage justification for the dose tested in the field effectiveness study and supported the original approval of ceftiofur sodium in the horse for the treatment of respiratory disease (N 140-338).

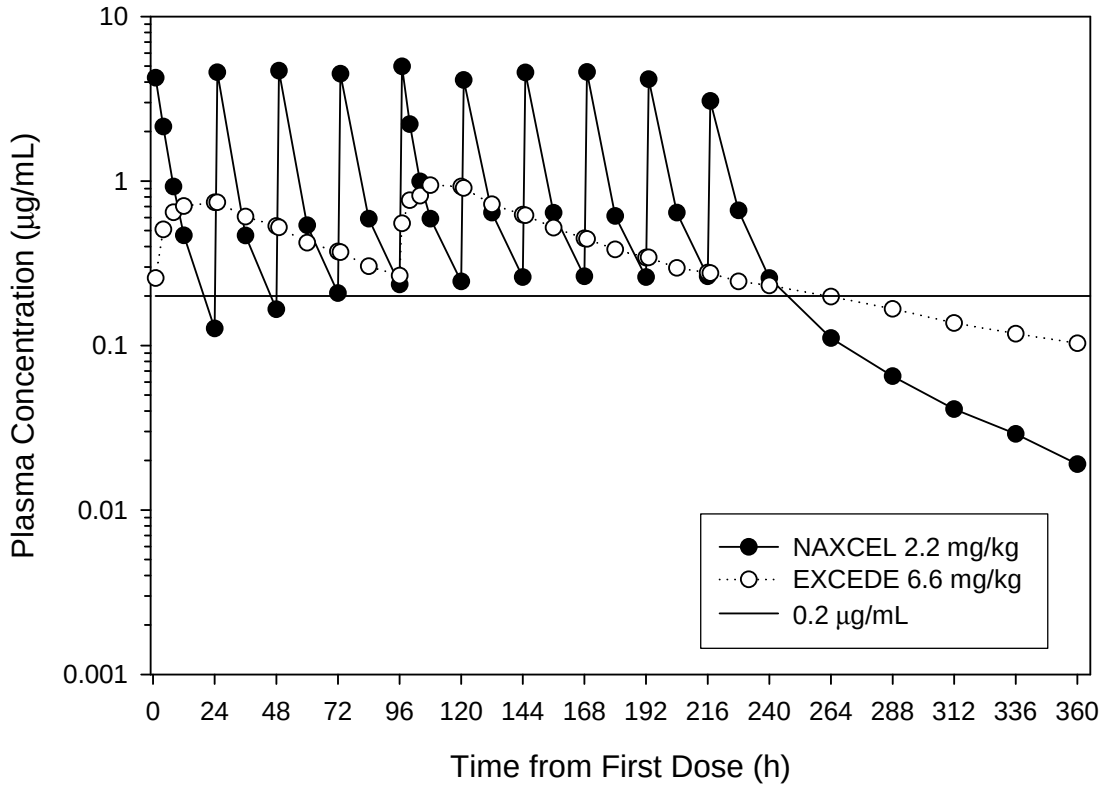
In addition to the historical ceftiofur data, the following pharmacokinetic data justified testing a dose of 6.6 mg/kg BW EXCEDE Sterile Suspension in the field effectiveness study. These data demonstrate that the proposed dose is one that results in blood concentrations of ceftiofur and its desfuroylceftiofur metabolites exceeding the MIC_{90} of the target pathogen (*Streptococcus equi* subsp. *zooepidemicus*) throughout the proposed dosing regimen.

1. **Pharmacokinetic Data:** Pharmacokinetics of Ceftiofur in the Equine Following Two IM Injections of EXCEDE® Sterile Suspension in the Neck 96 Hours Apart at Doses of 3.3, 6.6 or 13.2 mg CE/kg BW vs. 10 IM Injections of NAXCEL® Sterile Powder in the Neck 24 Hours Apart at 2.2 mg CE/kg BW. Study Report 1552N-60-06-209.

   This GLP pharmacokinetic (PK) and injection site safety study was designed to characterize PK properties of EXCEDE Sterile Suspension (ceftiofur crystalline free acid sterile suspension) and to compare exposure of ceftiofur and desfuroylceftiofur-related metabolites following administration of two 6.6 mg CE/kg IM injections of EXCEDE Sterile Suspension 96 hours apart vs. ten 2.2 mg CE/kg IM injections of NAXCEL Sterile Powder (ceftiofur sodium, NADA 140-338) once daily. In addition, the study evaluated the time the plasma concentrations of ceftiofur and desfuroylceftiofur-related metabolites remained above 0.2 µg/mL. For details of this pivotal PK study, see the Target Animal Safety section.

   In summary, the results of this study demonstrated EXCEDE's ability to maintain serum drug concentrations at or above a therapeutic plasma concentration target of 0.2 µg/mL for the entire 96 hour (4-day) dosing interval up to a mean value of 10.9 days following the first dose (see Figure 1).

Figure 1. Average plasma concentration of ceftiofur and desfuroylceftiofur related metabolites in horses following the intramuscular administration of either EXCEDE Sterile Suspension at a dose of 3.0 mg CE/lb (6.6 mg CE/kg) administered twice at a 96 hour interval or NAXCEL Sterile Powder at a dose of 1.0 mg CE/lb (2.2 mg CE/kg BW) once daily for 10 consecutive days.



B. Substantial Evidence:

1. Dose Confirmation Study

a. Study Title and Number: Field Dose Confirmation and Safety of EXCEDE Sterile Suspension (200 mg/mL) Administered Twice at a Five Day Interval at 6.6 mg/kg BW in Horses for the Treatment of Lower Respiratory Disease Associated with *Streptococcus zooepidemicus* or Other Relevant Bacterial Pathogens. Study Report 1153C 60-06-208.

b. Type of Study: Multi-center field effectiveness (GCP) study.

c. Study Objective: The study objective was to confirm the effectiveness and field safety of EXCEDE Sterile Suspension (ceftiofur crystalline free acid sterile suspension, 200 mg/mL) administered twice, approximately 96 hours apart at the dose of 6.6 mg/kg body weight, in the treatment of horses with

naturally acquired lower respiratory tract infections involving *Streptococcus equi* subsp. *zooepidemicus* or other relevant bacterial pathogens.

d. Investigators:

Table 1. Investigators

Location	**Investigator Name**
Boone, Iowa	Scott McClure, DVM, PhD, DACVS
Butler, Missouri	Kris Hennessy, DVM, PhD, DACVM
Mexico, Missouri	Kent Haden, DVM
Parma, Idaho	Matthew Edmonds, DVM, PhD
Preston, Idaho	Breck D. Hunsaker, DVM, PhD
Rockwood, Tennessee	Craig R. Reinemeyer, DVM, PhD
Sallisaw, Oklahoma	Gary W. White, DVM
Saskatoon, Saskatchewan	Claire Card, DVM, PhD, DACT
St. Claude, Manitoba	Raymond LeHeiget, DVM

e. General Design:

i. Test Animals: 374 horses were randomly assigned to receive either 6.6 mg/kg EXCEDE Sterile Suspension or a saline placebo at the equivalent injection volume, twice, approximately 96 hours apart. Ultimately 373 cases fully met the clinical enrollment criteria. Clinical cases included frame sizes ranging from miniature horse and pony to draft horse. Horse breeds included in the study reflect the general population of pure breed and mixed breed horses in North America. Female, intact male, and castrate male (gelding) horses were distributed similarly between treatments. Study animals ranged in age from 4 months to 20 years.

ii. Control and Treatment Groups:

Table 2. Treatment Groups

Treatment group	**Dose mg/kg**	**Regimen**	**Number of Horses Enrolled (evaluable)**
Sterile Saline	0 mg/kg	Days 0, 4	95 (57)
EXCEDE Sterile Suspension	6.6 mg/kg	Days 0, 4	278 (136)

iii. Inclusion Criteria:

To be eligible for inclusion in the trial, horses were required to demonstrate clinical evidence of a lower respiratory tract bacterial infection. The inclusion criteria are defined below. For a listing of the scoring system see Table 3 Clinical Outcomes.

Depression ≥ 1 (slightly lethargic, eyes dull, head/ears down when alone)

Dyspnea ≥ 1 (aggravated breathing; mild abnormal lung sounds)

Cough ≥ 2 (frequent cough-strained; more than 1 episode per minute)

Nasal discharge ≥ 2 (muco-purulent; translucent)

Temperature > 100.6° F (38.1° C)

A mid-cervical trans-tracheal wash was performed on every study horse prior to treatment. All horses meeting the clinical criteria were enrolled in the study and treated; however, only horses culturing ≥100 colonies of a respiratory pathogen on the primary isolation plate from the transtracheal wash sample were included in the final effectiveness analysis.

iv. Dosage Form:

EXCEDE- Final market formulation 200 mg/mL EXCEDE Sterile Suspension (ceftiofur crystalline free acid)

Control- Sterile Saline

v. Route of Administration: Intramuscular injection

vi. Study Duration: 25 days

vii. Variables Measured:

Animals were evaluated for 25 days after the first dose. The veterinarian examined horses for clinical signs of fever, depression, dyspnea, nasal discharge, and cough on days 0, 4, 9, 15 and 25. Hematology and clinical chemistry samples were obtained prior to treatment and on days 15 and 25. Horses were evaluated twice daily for abnormal health observations, including injection site reactions, inappetance and fecal consistency. Transtracheal wash samples were obtained from all horses prior to treatment and evaluated for cytology and bacterial culture. A second transtracheal wash was performed on horses removed from the study for rescue treatment prior to day 25.

viii. Statistical Analysis:

A horse successfully completed the study if it was a treatment success on days 15 and 25. Horses that met the rescue criteria at any time during the study were evaluated as treatment failures. Clinical outcomes are defined in Table 3.

Table 3. Clinical Outcomes

Day	Temp (in °C)		Depression		Dyspnea		Cough		Nasal discharge	Outcome
0	>38.1	and	≥ 1	and	≥ 1	and	≥ 2	and	≥ 2	Enroll
1-4	Increase >0.5	and /or	3	and	Any	and	Any	and	Any	Rescue/ Failure
5-14	>38.1**	and /or	Increase	and /or	Increase	and /or	Increase	and /or	Increase	Rescue/ Failure
15	≤ 38.1 *	and	0	and	≤ 1	and	≤ 1	and	≤ 1	Initial success^
16-24	>38.1**	and /or	≥ 1	and /or	≥ 1	and /or	≥ 2	and /or	≥ 2	Rescue/ Failure
25	≤ 38.1 *	and	0	and	0	and	≤ 1	and	≤ 1	Success

^ Any horse not meeting the success criteria on day 15 was a failure. Only horses declared successes on days 15 and 25 were evaluated as a success in the effectiveness analysis.
*Temperature ≤ 38.1°C or decreased by 0.5°C from the day 0 temperature
**Temperature >38.1°C and decreased by less than 0.5°C from the day 0 temperature

Depression Score:
0 = normal, alert
1 = slightly lethargic, eyes dull, head/ears down when alone; responds readily to other horses and handlers, moves normally
2 = moderately lethargic, head hanging, moves slowly, but responds to handling
3 = severely lethargic, does not respond to handling/inactive/moribund

Dyspnea Score:
0 = normal breathing, no abnormal lung sounds
1 = aggravated breathing, mild abnormal lung sounds
2 = shallow rapid breathing, moderate abnormal lung sounds e.g. crackling
3 = labored breathing; neck extended; marked abdominal lift; marked abnormal lung sounds e.g. audible wheeze

Cough Score:
0 = no cough
1 = occasional cough—not strained; less than 1 episode per minute
2 = frequent cough—strained; more than 1 episode per minute

Nasal Discharge Score:
0 = normal, no discharge
1 = serous; clear
2 = muco-purulent; translucent
3 = purulent; opaque; malodorous

The determination of effectiveness was based on the number of horses that successfully completed the study 25 days after the first dose.

Clinical success was analyzed with a generalized linear mixed model with a logit link and binomial error distribution. The model includes fixed effect of treatment and random effects of site, treatment by site interaction and residual error. The placebo group was compared to the EXCEDE group and test results reported and declared significant if the p-value is less than 0.05.

f. Results:

A total of 373 horses fully met the clinical enrollment criteria (278 EXCEDE and 95 placebo). One hundred eighty horses were excluded from the effectiveness analysis. The most common reason for exclusion was failure to meet the microbiological enrollment criteria (>100 colonies of a respiratory pathogen on the primary isolation plate from the pre-treatment transtracheal wash). Other reasons for exclusion included missing data, culture of a different pathogen on the post-treatment transtracheal wash (only performed on failures), and insufficient number of evaluable cases from a site.

The effectiveness analysis was based on 193 horses. The database included 57 placebo-treated horses and 136 EXCEDE-treated horses. Horses that were withdrawn from the study for rescue treatment prior to day 25 were included in the effectiveness analysis as treatment failures. All animals, including those not evaluated for effectiveness, were included in the safety evaluation.

On day 15, 100/136 (73.53%) EXCEDE-treated horses and 22/57 (38.6%) placebo-treated horses met the criteria for initial clinical success. Horses were evaluated through day 25 to assess for signs of relapse. On day 25, 94/136 (69.12%) EXCEDE-treated horses and 18/57 (31.58%) placebo treated horses met the criteria for final clinical success. On day 25, the percent success in the EXCEDE-treated group (69.12%) was statistically significantly better (p=0.0215) than the percent success in the placebo group (31.58%). The following table summarizes the clinical success rates obtained 15 and 25 days after the initiation of treatment.

Table 4. Number and Percentage of Horses Successfully Treated

Effectiveness parameter	**EXCEDE**	**Saline Control**	**P-value**
Clinical success day 15	73.53%	38.6%	N/A
Clinical success day 25	69.12%	31.58%	0.0215

i. Adverse Reactions: Injection site swelling (edema) was reported in 10 of 278 (3.6%) EXCEDE-treated horses and 1 of 95 (1%) of the placebo-treated horses. Of the 10 EXCEDE-treated horses with injection site swelling, 8 horses had swellings of 4 cm or less in diameter, one horse had a 10 cm diameter swelling and one horse had injection site reactions to both injections measuring 25 x 12 cm each. Injection site reactions in EXCEDE-treated horses resolved over 1 to 20 days.

   At least one episode of diarrhea, loose, soft, or cowpie stools were observed in 25 of 278 (9%) of the EXCEDE-treated horses and 7 of 95 (7%) of the placebo-treated horses. The duration of episodes in EXCEDE-treated horses ranged from a single observation of loose stool to observations lasting 6 days. All cases were self-limiting and resolved with minimal (two horses received a single dose of loperamide) or no treatment.

   Ten horses, 5/278 (1.7%) EXCEDE and 5/95 (5.3%) placebo, died or were euthanized during the study. Seven horses (4 EXCEDE and 3 placebo) died of progression of severe respiratory disease. These horses had necropsy findings of severe diffuse suppurative or fibrinosuppurative bronchopneumonia, fibrinosuppurative pleuritis, and/or severe necrotizing bronchopneumonia. Necropsy of the other EXCEDE-treated horse revealed cranial mesenteric arteritis and *Clostridium perfringens* colitis in addition to bronchopneumonia. This horse had clinical signs of gastrointestinal disease prior to enrollment. One placebo-treated horse was euthanized after a reaction to a procaine penicillin injection, and necropsy of the other placebo treated horse revealed a combination of cranial mesenteric arteritis, severe parasitism, and bronchopneumonia.

ii. Clinical Pathology: There were no notable differences in mean values for all laboratory tests between EXCEDE-treated and placebo-treated horses. Many individual animals had abnormalities relating to respiratory disease at enrollment and throughout the study. No treatment related abnormalities or trends were identified.

iii. Microbiology:

   EXCEDE is a cephalosporin antibiotic. Like other β-lactam antimicrobials, EXCEDE exerts its inhibitory effect by interfering with bacterial cell wall synthesis. This interference is primarily due to its covalently binding to the penicillin-binding proteins (PBPs) (i.e., transpeptidase and carboxypeptidase), which are essential for synthesis of the bacterial cell wall.

   Identification of bacterial pathogens was made to the species level, based on morphology, Gram stain, growth characteristics, standard individual biochemical testing and/or commercially available identification test kits. Minimum inhibitory concentration (MIC) testing was conducted in

accordance with applicable Clinical and Laboratory Standards Institute (CLSI) standards. EXCEDE MICs for the pre-treatment bacterial pathogens isolated from enrolled horses are summarized in Table 5. Fourteen of forty-two EXCEDE-treated failures received a post-treatment transtracheal wash. The *S. equi* subsp. *zooepidemicus* isolates obtained from these horses had a ceftiofur MIC_{50} of 0.25 mg/L and MIC_{90} of 0.5 mg/L, with a range of 0.03 – 0.5 mg/L.

Table 5: Activity of EXCEDE Against Pathogens Isolated from Horses Treated with EXCEDE in Field Studies in the U.S. During 2007-2008

Disease	Pathogen	Treatment Outcome	# of Isolates	Time of Sample Collection	MIC_{50} µg/mL	MIC_{90} µg/mL	MIC Range µg/mL
Lower Respiratory Tract Infection	*Streptococcus equi subsp. zooepidemicus*	Success	93*	Pre-Treatment	0.06	0.12	0.03 - 0.5
		Failure	42	Pre-Treatment	0.06	0.25	0.03 - 0.5

***** One horse cultured *Staphylococcus aureus* (successfully treated) and is not represented in the table.

g. Conclusions: EXCEDE Sterile Suspension administered to horses at the dose of 6.6 mg/kg body weight twice, 96 hours apart, was shown to be effective in the treatment of naturally acquired lower respiratory tract infections involving susceptible strains of *Streptococcus equi* subsp. *zooepidemicus*.

III. TARGET ANIMAL SAFETY:

A. Pharmacokinetic and Injection Site Tolerance Study:

1. Study Title and Number**:** Pharmacokinetics of Ceftiofur in the Equine Following Two IM Injections of EXCEDE Sterile Suspension (ceftiofur crystalline free acid sterile suspension) in the Neck 96 Hours Apart at Doses of 3.3, 6.6 or 13.2 mg/kg vs. 10 IM Injections of NAXCEL Sterile Powder (ceftiofur sodium) in the Neck 24 Hours Apart at 2.2 mg/kg. Study Report 1552N-60-06-209.
2. Type of Study: GLP pharmacokinetic study.
3. Study Objective: The objective of the study was to obtain PK data following two IM injections of EXCEDE Sterile Suspension 96 hours apart at doses of 3.3, 6.6 or 13.2 mg/kg and to compare the exposure of the 6.6 mg/kg dose group to 10 injections of NAXCEL Sterile Powder administered IM daily 24 hours apart at 2.2 mg/kg. In addition, the objective was to evaluate injection site reactions for all treatment groups and assess the histopathology of the injection site tissues of the horses receiving EXCEDE Sterile Suspension at 6.6 mg/kg.

4. Study Director: Steven P. Lesman, BA, MBA, Study Director
Pfizer Animal Health Veterinary Medicine Research and Development
Richland, MI

5. General Design:

   a. Test Animals: Forty-eight healthy, adult, male and female, grade horses were selected from a pool of 54 horses and randomly assigned to four treatment groups.

   b. Test Article:

      EXCEDE- Final market formulation, 200 mg/mL EXCEDE Sterile Suspension (ceftiofur crystalline free acid)

      NAXCEL- Approved formulation of NAXCEL Sterile Powder (NADA 140-338 ceftiofur sodium)

   c. Route of Administration: Intramuscular

   d. Dosage: Study animals were divided into the following four groups of 12 horses each:

      T01 Reconstituted NAXCEL Sterile Powder administered at 24-hour intervals for 10 consecutive days at 2.2 mg/kg.

      T02 EXCEDE at 3.3 mg/kg (0.5X) administered twice, 96 hours apart

      T03 EXCEDE at 6.6 mg/kg (1X) administered twice, 96 hours apart

      T04 EXCEDE at 13.2 mg/kg (2X) administered twice, 96 hours apart

Table 6. Treatment Groups

Group	Treatment	Number of Horses
T01	2.2 mg/kg NAXCEL	12 (7 male, 5 female)
T02	3.3 mg/kg EXCEDE (0.5X)	12 (6 male, 6 female)
T03	6.6 mg/kg EXCEDE (1X)	12 (6 male, 6 female)
T04	13.2 mg/kg EXCEDE (2X)	12 (7 male, 5 female)

e. Duration of study: All horses were evaluated for 15 days. Horses with abnormal clinical findings were kept on study until those findings resolved or until study day 25, whichever came first.

f. Measurements and observations:

In all treatment groups: clinical observations, physical exams, injection site evaluations, and plasma drug concentrations. Blood samples for drug concentration analysis were collected at 0, 1, 4, 8, 12, 24, 25, 36, 48, 49, 60, 72, 73, 84, 96, 97, 100, 104, 108, 120, 121, 132, 144, 145, 156, 168, 169, 180, 192, 193, 204, 216, 217, 228, 240, 264, 288, 312, 336, and 360 hours post-treatment.

In the 6.6 mg/kg treatment group: pathology and histopathology of injection sites.

g. Drug Assay: The ceftiofur and desfuroylceftiofur-related residues in each plasma sample were determined using the validated LC-MS/MS method. In this assay, dithioerythritol is used to convert ceftiofur and all desfuroylceftiofur-related metabolites containing an intact β-lactam ring to desfuroylceftiofur, which is then stabilized by derivatization to desfuroylceftiofur acetamide (DCA). The limit of quantitation (LOQ) for this assay was 0.010 µg/mL plasma.

h. Statistical analysis: Clinical assessment and general health observations were summarized as frequency tables for each day and treatment. Frequency distributions were calculated for the presence of signs for injection site evaluations. Summary statistics were provided for the post-mortem injection site volumes computed for the 6.6 mg/kg EXCEDE group. Foot pain incidence was defined as foot pain days, the number of study days for which the horse was recorded as evidencing foot pain as a percentage of all days the horse was on study, and was summarized. Duration of foot pain was analyzed with a mixed model with treatment as a fixed effect and block as a random effect.

Relative bioavailability assessment for ceftiofur and its metabolites was based upon comparisons of product values of the area under the concentration versus

time curve (AUC) from hour zero to the last quantifiable concentration (AUC_{0-t}) and peak concentration (C_{max}). To determine means and confidence intervals for the DCA plasma concentration data, the log transformed concentrations were modeled using a mixed linear model. The model included the fixed effects of sex, treatment, sample time and all interactions and the random effects of block (sex), between and within animal error. In addition, the ratio of NAXCEL to 6.6 mg/kg EXCEDE groups for C_{max} and AUC_{0-inf} was estimated by taking the difference between the least squares means. The difference and confidence interval were back-transformed to provide the ratio estimate for each PK parameter. The exposure of the 6.6 mg/kg EXCEDE group was considered equivalent to or less than that of the NAXCEL group if the upper limit of the back-transformed confidence interval of the ratio estimate was less than 1.25.

6. Results:

   a. General health: Once daily physical exams and twice daily viability observations were performed. Most abnormalities noted during the study involved the integumentary or musculoskeletal systems and were the result of injection site reactions. Few gastrointestinal abnormalities were observed. One, 6.6 mg/kg EXCEDE-treated horse had soft stool on days 3 and 4, and one 13.2 mg/kg EXCEDE-treated horse had a mild episode of colic on day 5 (1 day after the second injection). The horse was lying down and generally uncomfortable with decreased GI motility, and recovered without treatment.

   Several horses in all treatment groups developed clinical signs of foot pain. The investigator described the horses as stiff in front when turned in tight circles, and in most cases as having increased digital pulses and heat in their feet. No other diagnostics were performed. Two of these horses were euthanized during the study due to laminitis (one NAXCEL and one 13.6 mg/kg EXCEDE-treated horse). Clinical signs of foot pain affected more horses, for more days, in all EXCEDE-treated groups than in the NAXCEL treated group. Table 7 describes the number of horses affected in each group, the percent of total study days that horses were affected, and the range of days that individual horses were affected.

Table 7. Clinical Signs of Foot Pain

Treatment Group	Horses affected/ Horses treated	% of study days with foot pain	Duration of abnormal signs
2.2 mg/kg NAXCEL	3/12	4.1*	1-5*
3.3 mg/kg EXCEDE	9/12	32.6	1-22
6.6 mg/kg EXCEDE	9/12	21.2	1-23
13.2 mg/kg EXCEDE	11/12	22.4^	1-20^

*One horse euthanized due to laminitis on day 2
^One horse euthanized due to laminitis on day 6

The relationship between ceftiofur and the development of foot pain in this study cannot be definitively determined. The study housing (multi-horse pens on concrete slabs) and diet (free choice alfalfa/grass mix and once a day pellets) may have contributed to the development of foot pain. Seven horses of the 54 horses in the initial pool demonstrated clinical signs of foot pain prior to treatment. Four of these horses were removed from the study prior to treatment. The other three horses were enrolled in the study despite being described as stiff in front on day -4. The investigator diagnosed eight horses with mild laminitis on day 1 (one day after the first injection). Horses developing a stiff gait on subsequent study days were not diagnosed, and were described as stiff in front with increased digital pulses and heat in their feet. Horses treated with EXCEDE Sterile Suspension had a greater incidence of foot pain for a longer duration than horses treated with NAXCEL Sterile Powder. It is likely that the severity and prevalence of the injection site reactions may have contributed to the increased incidence of reported foot pain in EXCEDE-treated groups.

b. Injection site evaluations: All injection sites were observed daily on an individual animal basis. Injection site inflammation was evaluated qualitatively by visual observation and palpation, and quantitatively by measuring lesion dimensions. Qualitative evaluation criteria were documented as present or absent, and consisted of erythema, heat, sensitivity, firmness, necrosis, and drainage. Swelling was scored quantitatively by measuring the shortest and longest superficial dimensions and by estimating the elevation of the swelling.

   There were no observations of erythema, heat, sensitivity, necrosis, or drainage at the injection site. At the label dose (6.6 mg/kg), adverse events noted after treatment administration were generally limited to stiffness/lameness, neck soreness, swelling, and edema at the injection site. All EXCEDE-treated horses had edema with the maximum incidence occurring on days 6-9 (2-5 days after the second injection). Areas of edema in the 6.6 mg/kg EXCEDE-treated group ranged from 9x10 cm to 30x36 cm. No NAXCEL-treated horses had edema at the injection site on any study day. Two NAXCEL-treated horses, one 3.3 mg/kg, two 6.6 mg/kg, and seven 13.2 mg/kg EXCEDE-treated horses had stiff necks. Most injection site reactions resolved within 15 days; however, two horses in the 6.6 mg/kg EXCEDE-treated group had small, firm injection site reactions (0.5 x 0.5 x 0.1 cm and 1 x 2 x 0.2 cm) that did not completely resolve by the end of the study (day 25).

c. Injection site pathology and histopathology: Animals receiving the 6.6 mg/kg dose of EXCEDE Sterile Suspension were euthanized and necropsied following the last blood sampling time (360 hours) or once adverse events were resolved, up to Study Day 25 (3 weeks following the second injection of EXCEDE), whichever was later. Individual injection sites on each animal were identified for gross examination in situ by the pathologist. The pathologist also conducted histopathology evaluations of injection sites.

Intramuscular injection of EXCEDE Sterile Suspension at a dose of 6.6 mg/kg resulted in local irritation at the injection site indicated at necropsy by tan or tan-and-red mottled discoloration in muscle and/or fascia. Most areas of altered tissue oozed white or tan creamy material from the cut surface that was consistent with test article. Post-mortem injection sites measured from 12.6 to 219.9 cm^3 for the first injection site and 0.9 to 130.8cm^3 for the second injection site. Histopathologic evaluation of the injection sites revealed treatment-related, variable, chronic eosinophilic inflammation characterized by fibrosis/fibroplasia (mild to moderate), granulomas (moderate to marked), infiltrates of eosinophils (minimal to moderate), and perivascular lymphoid infiltrates (mild to moderate). Many of the sites also had minimal to mild hemorrhage.

d. Plasma drug concentrations:

Based on the administration of ceftiofur in horses as either two IM injections of EXCEDE Sterile Suspension at a dose of 6.6 mg ceftiofur equivalents (CE)/kg BW administered twice 96 hrs apart or NAXCEL Sterile Powder administered IM at a dose of 2.2 mg CE/kg once daily for 10 consecutive days, the following average pharmacokinetic parameters were estimated:

Table 8. Pharmacokinetic Parameters

PK Parameter	**EXCEDE at 6.6 mg CE/kg BW administered twice 96 hrs apart (Mean ± SD; n = 12)**		**NAXCEL at 2.2 mg CE/kg BW once daily for 10 days (Mean ± SD; n = 11)**	
$AUC_{0-\infty}$ (µg•hr/mL)	157 (19.1)		353 (44.9)	
$t_{>0.2}$ (hr)	262 (29.0)		ND	
	Dose 1	Dose 2	Dose 1	Dose 10
T_{max} (hr)	21.6 (5.8)	15.6 (6.3)	1.0	2.0 (3.3)
C_{max} (µg/mL)	0.78 (0.19)	1.0 (0.24)	4.31 ± 0.78	3.99 (1.23)

Ceftiofur exposure in horses was statistically lower when administered as EXCEDE Sterile Suspension at 6.6 mg /kg twice 96 hours (4 days) apart than when administered as NAXCEL Sterile Powder at 2.2 mg/kg dosed once daily for 10 days. Based on the $AUC_{0-\infty}$ comparison, the extent of exposure to desfuroylceftiofur acetamide from two IM doses of EXCEDE Sterile Suspension administered 96 hours apart was about 45% of the 10 daily IM doses of NAXCEL at the label dose of 2.2 mg/kg. The C_{max} ratio of EXCEDE Sterile Suspension and NAXCEL Sterile Powder was approximately 0.19.

7. Conclusions: Based on the AUC and C_{max} data, horses receiving two IM injections of EXCEDE Sterile Suspension at a dose of 6.6 mg/kg administered 96 hours apart were exposed to significantly less ceftiofur and desfuroylceftiofur metabolites than horses receiving NAXCEL Sterile Powder at 2.2 mg/kg dosed once daily for 10 days. These data allow a pharmacokinetic bridge between NAXCEL Sterile Powder and EXCEDE Sterile Suspension, and justify the use of

the systemic target animal safety data from NAXCEL Sterile Powder previously approved under NADA 140-338 for use with EXCEDE Sterile Suspension in the horse.

In this study, administration of EXCEDE Sterile Suspension caused injection site reactions resulting in edema and stiffness at the injection site. Injection site reactions, as well as study housing and diet, may have contributed to the higher incidence of foot pain in EXCEDE-treated horses as compared to horses treated with NAXCEL.

B. Safety and Injection Site Tolerance Study

1. Study Title and Number: Gastrointestinal Safety of Ceftiofur Crystalline Free Acid (CCFA) in the Equine Following Six IM Injections of EXCEDE in the Neck 96 Hours Apart at Doses 6.6, 13.2, and 19.8 mg CE/kg BW. Study Report 1452N-60-07-213.
2. Type of Study: GLP laboratory study.
3. Study Objective: The primary objective of this study was to demonstrate the gastrointestinal safety of EXCEDE Sterile Suspension (200 mg/mL) administered six times (3X maximum proposed duration) to the horse, 96 hours apart at 6.6 (1X), 13.2 (2X), and 19.8 (3X) mg/kg. A secondary objective of this study was to qualitatively and quantitatively evaluate injection site tolerance in the 6.6 mg/kg (1X) treatment group. Additionally, plasma samples were collected for determination of CCFA concentrations at all dose levels.
4. Study Director: Devendra Kumar, BVSc, MS, PhD

   Pfizer Animal Health
   Veterinary Medicine Research and Development Research Farm
   Richland, MI
5. General Design:
   a. Test Animals: Thirty-two healthy, adult, male and female, grade horses were randomly assigned to four treatment groups.
   b. Control: Saline.
   c. Dosage Form: Final market formulation, 200 mg/mL EXCEDE Sterile Suspension.
   d. Route of Administration: Intramuscular.
   e. Dosage: Horses were administered six intramuscular injections of either saline, 6.6 mg/kg, 13.2 mg/kg, or 19.8 mg/kg EXCEDE Sterile Suspension at 4 day intervals.

Table 9. Treatment Groups

Group	Treatment	Dosing Regimen	Number of Horses
T01	Saline (0.033 mL/kg)	Six IM Injections 96(±1) hours apart in the neck	8 (4 male/4 female)
T02	6.6 mg/kg (1X) (0.033 mL/kg) EXCEDE	Six IM Injections 96(±1) hours apart in the neck	8 (4 male/4 female)
T03	13.2 mg/kg (2X) (0.066 mL/kg) EXCEDE	Six IM Injections 96(±1) hours apart in the neck	8 (4 male/4 female)
T04	19.8 mg/kg (3X) (0.099 mL/kg) EXCEDE	Six IM Injections 96(±1) hours apart in the neck	8 (4 male/4 female)

f. Duration of study: All horses were evaluated for 35 days (11 days after the last injection). Horses showing any abnormality on or after day 35 (other than injection site reactions) were maintained on the study until resolution of the abnormality.

g. Variables measured: Clinical observations, physical exams, GI assessments, injection site evaluations, clinical pathology, and plasma drug concentrations were evaluated in all horses. Pathology and histopathology of injection sites were also evaluated in the 6.6 mg/kg treatment group.

h. Statistical analysis methodology: Clinical observations were summarized. Clinical pathology and plasma concentration data were analyzed using repeated measures mixed linear models. Fixed effects in the model were: treatment, sex, time and all their interactions. The random effect was block nested in sex. The baseline value was included in the model as a covariate. The significance level used was 0.10.

6. Results:

a. General health observations and clinical assessments: Once daily Clinical Assessments, and twice daily General Health Observations were conducted until 4 days after the last injection (Day 24). Thereafter, once weekly Clinical Assessments and once daily General Health Observations were conducted until Day 35. There were no mortalities during the study and no adverse events that required any therapeutic intervention. There was no incidence of abnormal temperature, respiration rate, or pulse rate in any animal throughout the study.

The musculoskeletal system had numerous abnormal findings related to neck swelling and injection site reactions. Four horses developed hind limb swelling during the study. In one horse the swelling increased throughout the study, and was up to the level of the stifle by day 21. This horse may have suffered a puncture wound during the study, and was short-strided and painful

to palpation. Three other horses developed mild transient hind limb swelling consistent with stall confinement.

b. GI Assessments: If any animal exhibited an abnormality of the GI system during the Clinical Assessment, then the veterinarian also conducted a targeted GI assessment of that animal.

   There were no treatment related clinical observations or laboratory findings suggesting abnormal gastrointestinal function associated with the test article. One horse had one observation of loose stool in the 6.6 mg/kg group and two horses each had one observation of loose stool in the 19.8 mg/kg group.

c. Injection site evaluations: Once daily qualitative and quantitative evaluations of the injection site were conducted in the 1X treatment group, whereas injection sites in other treatment groups (0X, 2X, and 3X) were observed only qualitatively. Injection sites in all treatment groups were observed until 4 days after the last injection (Day 24).

   The most common reaction to treatment administration was local reaction at the injection sites, including edema, stiffness of the neck, and firmness and swelling at the injection site. All horses in all EXCEDE-treated groups reacted to at least one injection and all 6.6 mg/kg EXCEDE-treated horses reacted to the second injection. Swelling volumes at the injection sites of horses treated at the label dose were greatest on Day 6 (two days after the second injection). Maximum injection site measurements for the second injection ranged from 8 x 8 x 0.3 cm to 16 x 33 x 1.5 cm. Injection site reactions developed within 0-2 days of injection and resolved within 1-18 days. Three out of eight horses in the 6.6 mg/kg EXCEDE-treated group had mild to moderate pain or sensitivity at the injection site, and one horse was described as having extreme pain on palpation. In several horses, swelling at the injection site resolved then recurred 1-5 days later. Table 10 below demonstrates injection site volumes and clinical signs for the three days following the second injection of EXCEDE Sterile Suspension in horses treated at the label dose.

Table 10. 1X Injection Site Volumes (cm^3) and Clinical Signs for Second Injection Site

Horse	Volume Day 5	Volume Day 6	Volume Day 7	Clinical Signs
570	62.8	56.5	1.68	None
571	176	85.8	0	None
592	83.8	425	364	Severe swelling Mild-moderate pain
599	188	37.7	8.8	Moderate-severe swelling Mild-moderate pain
618	6.28	0	10.1	None
624	-	22.6	0	None
630	4.58	415	286	Extreme swelling Extreme pain on palpation
635	56.5	0	28.3	Moderate swelling Mild sensitivity

Injection site pathology and histopathology: Horses treated with the label dose of EXCEDE were euthanized on day 35 (11 days after the last injection) and injection site pathology and histopathology were performed.

Injection sites in 1X horses showed mild to moderate local irritation indicated by tan, white, brown or various combinations of tan, brown, white and red mottled discoloration in muscle and/or fascia. Most areas of altered tissue oozed white or tan liquid (fluid) from the cut surface that was consistent with test article. Histopathologic evaluation of the injection sites revealed treatment-related chronic eosinophilic inflammation characterized by fibrosis/fibroplasia, granulomas, infiltrates of eosinophils, and perivascular infiltrates of lymphocytes. Many of the sites also had hemorrhage and edema.

d. Clinical pathology: CBC, chemistry, and coagulation panels were evaluated pre-treatment and on days 7 and 24.

   All EXCEDE-treated groups had statistically significant increases in fibrinogen on day 7 (3 days after the second injection) as compared to the control group. Statistically significant increases in WBC and decreases in lymphocytes on day 7 were identified in the 19.8 mg/kg EXCEDE-treated horses. 6.6 mg/kg and 13.2 mg/kg EXCEDE-treated horses showed similar trends in WBC and lymphocytes. These alterations are likely due to acute inflammation and stress caused by the injection site reactions.

e. Plasma drug concentrations: For determination of plasma concentrations of ceftiofur and desfuroylceftiofur related metabolites, samples were collected on the day of treatment immediately before the drug administration and approximately 8 hours after administration. On all other days, samples were

collected at approximately the same time of the day (± 1 hour). An additional blood sample was collected at the end of the in-life phase (Day 35). Desfuroylceftiofur acetamide plasma concentrations increased in a dose related manner, and it was determined that the exposure was nearly equal to the 1X, 2X, and 3X ratios of the proposed 6.6 mg/kg dose.

7. Conclusions: An adequate margin of safety was demonstrated for EXCEDE Sterile Suspension when administered under the conditions of this study. EXCEDE Sterile Suspension did not cause any clinical or laboratory evidence of abnormal gastrointestinal function. Injection of the label dose of EXCEDE Sterile Suspension caused swelling, firmness, and sensitivity at the injection site. Treatment with EXCEDE Sterile Suspension resulted in an inflammatory leukogram.

IV. HUMAN FOOD SAFETY:

This indication for EXCEDE Sterile Suspension is intended for use in horses, which are non-food animals. Because this indication for this new animal drug is not intended for use in food producing animals, CVM did not require data pertaining to drug residues in food (i.e., human food safety) for approval of this supplemental NADA. Information regarding human food safety can be found in the FOI Summary for the original approval of this drug (NAXCEL XT Sterile Suspension, NADA 141-209) dated September 5, 2003.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to EXCEDE Sterile Suspension:

Not for use in humans. For use in animals only. Keep this and all drugs out of reach of children. Consult a physician in case of accidental human exposure.

Penicillins and cephalosporins can cause allergic reactions in sensitized individuals. Topical exposure to such antimicrobials, including ceftiofur, may elicit mild to severe allergic reactions in some individuals. Repeated or prolonged exposure may lead to sensitization. Avoid direct contact of the product with the skin, eyes, mouth and clothing. Sensitization of the skin may be avoided by wearing protective gloves.

Persons with a known sensitivity to penicillin or cephalosporins should avoid exposure to this product.

In the case of accidental eye exposure, flush with water for 15 minutes. In case of accidental skin exposure, wash with soap and water. Remove contaminated clothing. If allergic reaction occurs (e.g. skin rash, hives, difficult breathing) seek medical attention.

The material safety data sheet (MSDS) contains more detailed occupational safety information. To obtain a material safety data sheet, please call 1-800-733-5500. To report any adverse event please call 1-800-366-5288.

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 EXCEDE, when used according to the label, is safe and effective for the treatment of lower respiratory tract infections in horses caused by susceptible strains of *Streptococcus equi* ssp. *zooepidemicus.*

A. Marketing Status:

This drug is restricted to use by or on the order of a licensed veterinarian because professional expertise is needed in the diagnosis and treatment of lower respiratory tract infections in horses, and for monitoring for possible adverse reactions of the drug.

B. Exclusivity:

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

C. Supplemental Applications:

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

D. Patent Information:

EXCEDE Sterile Suspension is under the following U.S. patent numbers:

U.S. Patent Number	Date of Expiration
5,721,359	February 24, 2015

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

VII. ATTACHMENTS:

Facsimile Labeling:

A. EXCEDE Sterile Suspension – Vial Label

B. EXCEDE Sterile Suspension – Package Inserts (horses and cattle)

C. EXCEDE Sterile Suspension – Carton

D. EXCEDE Sterile Suspension – Shipper Label

09