Date of Approval Letter: September 5, 2003

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-209

NAXCEL XT STERILE SUSPENSION
(ceftiofur crystalline free acid sterile suspension)

"for treatment of bovine respiratory disease (BRD, shipping fever, pneumonia) associated with *Mannheimia haemolytica*, *Pasteurella multocida* and *Haemophilus somnus*. NAXCEL XT STERILE SUSPENSION is also indicated for the control of respiratory disease in cattle which are at high risk of developing BRD associated with *M. haemolytica*, *P. multocida* and *H. somnus*."

Sponsored by:
PHARMACIA & UPJOHN COMPANY
A wholly owned subsidiary of PFIZER INC

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1. GENERAL INFORMATION:

a. File Number:	NADA 141-209
b. Sponsor:	Pharmacia & Upjohn Company 7000 Portage Road Kalamazoo, MI 49001-0199 Drug Labeler Code: 000009
c. Established Name:	ceftiofur crystalline free acid
d. Proprietary Name:	NAXCEL XT STERILE SUSPENSION
e. Dosage Form:	sterile oil suspension for injection
f. How Supplied:	100 mL glass vial
g. How Dispensed:	Rx
h. Amount of Active Ingredients:	200 mg ceftiofur equivalents (CE) per mL
i. Route of Administration:	subcutaneous injection in the middle third of the posterior aspect of the ear
j. Species/Class:	cattle, beef and non-lactating dairy
k. Recommended Dosage:	single injection of 6.6 mg CE/kg (3.0 mg CE/lb) body weight (1.5 mL sterile suspension per 100 lb body weight)
l. Pharmacological Category:	antimicrobial
m. Indications:	NAXCEL XT STERILE SUSPENSION is indicated for treatment of BRD, (shipping fever, pneumonia) associated with *Mannheimia haemolytica*, *P. multocida* and *H. somnus*. NAXCEL XT STERILE SUSPENSION is also indicated for the control of respiratory disease in cattle which are at high risk of developing BRD associated with *Mannheimia. haemolytica*, *P. multocida* and *H. somnus*.

2. EFFECTIVENESS:

a. Dosage Characterization:

"Pivotal Dose Determination of Ceftiofur Crystalline Free Acid Sterile Suspension (CCFA-SS) (PNU-64279; 200 mg ceftiofur equivalents/mL) Administered Subcutaneously in the Posterior Ear for the Treatment of Induced Bovine Pneumonic Pasteurellosis". (TR 829-9690-97-003)

The purpose of this study was to evaluate the effectiveness of CCFA-SS administered subcutaneously (SC) in the posterior aspect of the ear for the treatment of pneumonic pasteurellosis in young dairy calves. The study was conducted under model/challenge conditions in order to select one or more doses for further field dose confirmation testing. Study investigators were B. Hibbard, E. J. Robb, S. T. Chester, Jr., K. J. Dame, and W. J. Seaman, Pharmacia & Upjohn, Kalamazoo, MI, and T. N. TerHune and J. N. Davidson, Health Management Services, Tulare, CA. The study was conducted in Tulare, California. Eighty-one recently weaned male Holstein calves (64.0-101.5 [mean 80.3] kg body weight (BW) at assignment) were used in the study. Following a 3-day acclimation period, all calves were challenged intra-tracheally with *Mannheimia haemolytica.* Calves that met the inclusion criteria [≥ 1.26°F (0.7°C) elevation in rectal temperature from that calf's basal mean temperature (mean for Days -2 to 0), and ≥ 10 count/minute increase in respiratory rate from that calf's basal mean respiratory rate (Days -2 to 0), or a depression score of 1 (depression evident)] within 48 hours after challenge were ranked by descending rectal temperature and randomly assigned to one of seven treatment groups on the day they met the inclusion criteria. Animals that did not meet the inclusion criteria within 48 hours of challenge were not assigned to the study. Calves in the CCFA-SS groups were administered CCFA-SS (CCFA-SS ready-to-use formulation; 200 mg CE/mL) at 1.1, 3.3, 4.4, 5.5, 6.6, or 8.8 mg CE/kg BW. Vehicle was administered as a placebo to negative controls at 0.044 mL/kg BW. All treatments were administered as a single subcutaneous injection in the middle third of the posterior aspect of the ear. Calves were clinically evaluated daily by the study veterinarian, who remained blinded to the assigned treatment groups throughout the study. Clinical evaluations continued through nine days after assignment. Calves that died during the study were necropsied by the study veterinarian. Surviving calves were euthanized nine days after treatment administration (Day 10 or 11 depending on day of assignment). The study pathologist, who remained blinded to the assigned treatment groups, scored the lung lesions on Days 10 and 11.

The primary decision variables were mortality due to BRD, rectal temperature 96 hours after treatment, and total calculated lung lesion scores for animals euthanized on Days 10 and 11 only. Data from one animal were removed due to incorrect dosing. Lung lesion data from two other calves were excluded due to missing scores for the intermediate lobe. Data for the primary decision variables are summarized in Table 2.1.

Table 2.1. Primary Decision Variables for Dose Justification Study

Variable		Dosage						
	mg CE/lb →	0	0.5	1.5	2.0	2.5	3.0	4.0
	mg CE/kg →	0	1.1	3.3	4.4	5.5	6.6	8.8
	# calves	11	11	12	12	11	11	12
Cumulative BRD mortality, %		27.3	9.1	0.0	8.3	9.1	0.0	0.0
Mean rectal temp. 96 h after treatment administration, °F		102.9	102.6	101.6	101.1	101.5	101.4	101.0
Mean Day 10 or 11 total calculated lung lesions, %		16.7	31.5	16.8	11.2	13.0	9.8	4.9

Effective doses were calculated for each primary decision variable. Based on nonlinear regression analysis of plasma samples, the effective doses were determined to be 1.80, 5.35 and 8.51 mg CE/kg BW for mortality due to BRD, rectal temperature 96 hours after treatment administration, and Day 10 or 11 lung lesion scores, respectively. There were no reports of adverse effects in this study.

Based on the decision making process described in the protocol, 5.35 mg CE/kg BW was determined to be the effective dose. The doses chosen for field dose confirmation were 4.4 and 6.6 mg CE/kg BW. These doses bracket the effective dose determined in this study.

b. Substantial Evidence:

Dose confirmation was based on the following studies: 1) a natural infection negative control clinical field study which evaluated single doses of 4.4 and 6.6 mg CE/kg BW administered as a SC injection in the posterior aspect of the ear for the treatment of naturally occurring BRD in feedlot cattle (see 2.b.1); and 2) a control study which evaluated single doses of 4.4 and 6.6 mg CE/kg BW administered as a SC injection in the posterior aspect of the ear for the control of naturally occurring BRD in high risk feedlot cattle under field conditions (see 2.b.2).

The clinical field effectiveness studies were conducted with the original 200 mg CE/mL CCFA-SS formulation. After the completion of these studies, the formulation was adjusted, resulting in the revised 200 mg CE/mL CCFA-SS formulation. To validate use of the original clinical field studies to support the effectiveness of the revised CCFA-SS product, the bioavailability of the new formulation was demonstrated as being equal to or greater than that of the original formulation through the evaluation of product relative bioavailability**.** This pharmacokinetic bridge was based upon a comparison of contemporary pharmacokinetic data (generated on the revised CCFA-SS formulation) and historical data (original formulation) and historical blood level data (generated with

the original CCFA-SS formulation because lots of the original CCFA-SS formulation within the expiry date were no longer available).

Data from these two studies confirmed the effectiveness of a single administration dose of CCFA-SS at 6.6 mg CE/kg BW for the treatment and control of the bacterial component of naturally occurring BRD in feedlot cattle.

1. "Pivotal Negative Control Dose Confirmation of PNU-64279 Ceftiofur Crystalline Free Acid Sterile Suspension (200 mg ceftiofur equivalents/mL) Administered Subcutaneously in the Posterior Ear for the Treatment of Naturally Occurring Bovine Respiratory Disease". (TR 829-9690-97-004)

a) Type of Study: Clinical field study

b) Investigators: B. Hibbard, E. J. Robb, S. T. Chester, Jr., and K. J. Dame, Pharmacia & Upjohn, Kalamazoo, MI; and E. G. Johnson and S. Lincoln, Johnson Research, Parma, ID.

c) Study Design:

1) *Objective:* To evaluate the effectiveness of CCFA-SS administered SC in the posterior aspect of the ear for the treatment of naturally occurring BRD in feedlot cattle.

2) *Experimental Animals:* Beef steer calves (n=344; 504 lb average weight) were purchased and transported to the research feedlot. When calves developed clinical BRD and met the inclusion criteria [Rectal temperature ≥104°F; and abnormal respiratory rate (respiratory index = 1); and mild, moderate, or severe depression (depression index ≥1)], they were ranked by rectal temperature (and body weight if necessary) and randomly assigned to one of three treatment groups of 54 animals each (Day 1). One hundred sixty (160) calves met inclusion criteria and were included in the statistical analysis.

3) *Control Group*: Vehicle, as a placebo, was administered to all negative control steers.

4) *Test Article Administration:* CCFA-SS ready-to-use formulation was used as the test article (200 mg CE/mL). Steers in the CCFA-SS treatment groups received 4.4 or 6.6 mg CE/kg BW. Placebo-treated negative controls were administered vehicle at 0.033 mL/kg BW. All treatments were administered as a single SC injection in the middle third of the posterior aspect of the ear on the day the animal met study inclusion criteria (Day 1).

5) *Measurements and Observations:* Nasal swabs were obtained from each animal assigned to the study prior to treatment administration. Swabs were

cultured for the primary BRD pathogens (*M. haemolytica, P. multocida* and *H. somnus)*. Minimum inhibitory concentrations (MIC) were determined for the BRD pathogens isolated according to National Committee for Clinical Laboratory Standards (NCCLS) methods.

Calves were clinically evaluated on Days 1, 4, 14, and 28; and were observed on all other study days. The investigators, who remained blinded to the assigned treatment groups throughout the study, made all evaluations and observations. Calves exhibiting clinical signs of BRD on Day 4 or later were administered standard feedlot therapy and were counted as treatment failures. The primary decision variable was the Day 14 overall treatment success rate. Calves were considered treatment successes if they had not received standard feedlot therapy by Day 14, and on Day 14 had rectal temperatures <104.0°F; normal respiratory rates (respiratory index = 0); and no or mild depression (depression index ≤1).

d) Results: The Day 14 overall treatment success rates were 54.7%, 69.8% (p=0.0553) and 70.4% (p=0.0479) for negative controls, CCFA-SS at 4.4 mg CE/kg BW and CCFA-SS at 6.6 mg CE/kg BW, respectively.

M. haemolytica was isolated from 45.1%, *P. multocida* was isolated from 26.5% and *H. somnus* was isolated from 6.8% of nasal swabs. Ceftiofur MIC_{90}s were 0.015, 0.004 and 0.004 mcg/mL for *M. haemolytica*, *P. multocida,* and *H. somnus*, respectively.

e) Statistical Analysis: The study was conducted as a randomized complete block design. The primary decision variable was analyzed by a generalized linear mixed effects model including a random effect for block.

f) Conclusions: A single dose of CCFA-SS (200 mg CE/mL) administered SC in the posterior aspect of the ear at 6.6 mg CE/kg BW is an effective treatment for the bacterial component of naturally occurring BRD in feedlot cattle.

g) Adverse Reactions: No adverse reactions were observed in this study.

2. "Conditions of Use Study: Pre-emptive Administration of PNU-64279 Ceftiofur Crystalline Free Acid Sterile Suspension (200 mg ceftiofur equivalents/mL) Injected Subcutaneously in the Posterior Ear at Arrival in High Risk Feedlot Cattle for the Control of BRD". (TR 829-9690-98-001)

a) Type of Study: Clinical field study: nine sites

b) Investigators: W. M. Moseley, B. Hibbard, S. T. Chester, Jr., E. J. Robb, K. J. Dame, and K. A. Ash, Kalamazoo, MI; D. Bechtol, Canyon, TX; G. Weaver, Groom, TX; S. Lewis, Hereford, TX; M. Hanna, Oakland, NE; E. G. Johnson,

Parma, ID; M. Coe, Garden City, KS; M. Hiscocks, Carroll, IA; and K. Rogers, Greeley, CO.

c) Study Design:

1) *Objective:* To evaluate the effectiveness of CCFA-SS administered SC in the posterior aspect of the ear at arrival processing for the control of naturally occurring BRD in feedlot cattle; and to evaluate the acceptability and practicality of this novel administration site and procedure, and the local tolerance of the ear under natural field conditions.

2) *Experimental Animals:* Beef feeder calves (n=3911) were purchased and transported to the feedlots. At arrival processing (Day 1), calves were processed according to standard feedlot procedures, and were randomly assigned to receive a single dose of CCFA-SS at 0.0, 4.4, or 6.6 mg CE/kg BW, or a positive control.

3) *Control Group*: Vehicle or sterile saline for injection was administered to all calves assigned to the negative control group.

4) *Test Article Administration:* All treatments were administered as a single injection at arrival processing (Day 1). CCFA-SS ready-to-use formulation (200 mg CE/mL) was used as the test article. Calves in the CCFA-SS treatment groups were administered 4.4 mg CE/kg BW or 6.6 mg CE/kg BW. Negative controls were administered placebo at 0.033 mL/kg BW. All treatments were administered as a single SC injection in the middle third of the posterior aspect of the ear using a 16-gauge ¾" or 1" sterile needle on an eccentric hub syringe. A positive control was also included in the study but is not reported.

5) *Measurements and Observations:* Pen riders, who were trained by the principal investigators, observed all study cattle daily through Day 29. Study calves with depression and abnormal respiration (after Day 2) were administered standard feedlot therapy and were considered arrival treatment failures. The ease of treatment administration and animal response to treatment were evaluated at arrival processing. Ear tolerance, as measured by lack of swelling or other problems, was evaluated by observation daily from Days 2 to 29 and by palpation at the Day 29 evaluation.

The primary decision variable for effectiveness was the incidence of BRD in the 28-day period following arrival processing for each CCFA-SS treatment group compared to negative controls. Only arrival lots with $\geq$ 20% BRD in the negative controls were included in this analysis (n=3007). This included 750 calves in the 4.4 mg CE/kg BW CCFA-SS treatment group, 755 calves in

the 6.6 mg CE/kg BW CCFA-SS treatment group, and 749 negative controls and 753 positive controls.

Injection site tolerance was also evaluated. All treated cattle were observed daily for signs of intolerance to the SC injections in the posterior aspect of the ear, as evidenced by droopy ears, abscesses, and/or excessive swelling. Once between Days 29 and 34 (i.e., 28 and 33 days after injection), the animals were individually weighed and both ears of each animal were palpated and scored using the following system:

0 - Normal;
1 - Slight thickening to moderate swelling (width < 3/4", blemish < 3" long);
2 - Large amount of swelling (width > 3/4", blemish > 3" long);
3 - Abscesses, pus, or open lesion;
4 - Other (describe).

Of the 3911 animals purchased for the study, only 2883 are included in the analysis for injection site tolerance. The animals from the positive control group were excluded. Animals from the arrival lots with < 20% BRD in the negative controls that were excluded from the effectiveness evaluation are included in the injection site evaluation. This leaves a total of 2883 animals evaluated with 964 calves in the 4.4 mg CE/kg BW CCFA-SS treatment group, 963 calves in the 6.6 mg CE/kg BW CCFA-SS treatment group, and 956 negative controls.

d) Effectiveness Results: The data were pooled across all nine sites and the BRD incidence rates were 40.0%, 26.5% and 28.3% for negative controls, CCFA-SS at 4.4 mg CE/kg BW, and CCFA-SS at 6.6 mg CE/kg BW, respectively. Both CCFA-SS treatment groups had significantly reduced BRD incidence rates compared to negative controls ($p<0.0001$).

Ear Injection Site Results:

1) *Injection Procedures:* Measures of injection procedures are presented in Table 2.2.

Table 2.2 Observations of Injection Procedures and Animal Response from the CCFA-SS Arrival (Conditions of Use) Study (TR 829-9690-98-001)

Treatment group	Animals requiring additional restraint for injection or other problem, %	Required reinjection, %[1]	Animals with post-injection problems, %[2]
Negative control[3]	1.6	7.5	3.9
CCFA-SS, 4.4 mg CE/kg (2.0 mg/lb)	1.8	5.1	3.9
CCFA-SS, 6.6 mg CE/kg (3.0 mg/lb)	2.3	5.6	5.3

[1] Animal moved before injection was complete or other administration problem.
[2] Bleeding, leak back of injected material, or other problems
[3] Negative controls received vehicle or sterile saline administered SC in the posterior aspect of the ear.

2) *Clinical Evaluations:* No animals were removed from the study due to ear irritation.

3) *Injection Tolerance:* Data on tolerance to injection, as measured by a single palpation between Days 29 and 34, are presented in Table 2.3.

Table 2.3 Irritation Scores of Treated Ears (TR 829-9690-98-001)[1]

Treatment	Irritation Scores[2]					
	0	1	2	3	4	Total
Negative Control	857	98	0	1	0	956
4.4 mg/kg (2.0 mg/lb)	327	556	55	26	0	964
6.6 mg/kg (3.0 mg/lb)	270	550	105	35	3	963
Total	1454	1204	160	62	3	2883

[1] CCFA-SS was injected into the left ear at all study sites except Site E.
[2] 0 = normal, 1 = slight swelling, 2 = large amount of swelling, 3 = abscesses, 4 = other.

At the single palpation on Days 29 to 34, 92% of the 4.4 mg/kg CCFA-SS treated group and 85% of the 6.6 mg/kg CCFA-SS treated group had no or slight swelling present at the ear injection site.

e) Statistical Analysis: The study was conducted as a randomized complete block design, pooled across sites. The binary primary decision variable was analyzed by analysis of variance following Freeman-Tukey transformation.

f) Conclusions: Control administration of a single dose of CCFA-SS at 4.4 or 6.6 mg CE/kg BW at arrival processing significantly reduced the incidence of BRD during the 28-day observation period compared to negative controls in high risk feedlot cattle. The administration of CCFA-SS SC in the posterior aspect of the ear was practical and acceptable for use under field conditions. In addition, the route of administration was well tolerated by the cattle.

g) Adverse Reactions: One animal died of anaphylactic shock approximately 10 minutes post-treatment. This animal received four other products at the same time (on-arrival). Epinephrine was administered, but the animal did not respond. A second animal died approximately 30 minutes after injection. This animal fought in the chute and was suspected to have spinal cord damage. The animal bloated, then died.

c. Pharmacokinetic Bridge:

For the BRD claim, the clinical field effectiveness studies were conducted with the original 200 mg CE/mL CCFA-SS formulation. After the completion of these studies, the formulation was adjusted, resulting in the revised 200 mg CE/mL CCFA-SS formulation. To validate use of the original clinical field studies to support the effectiveness of the revised CCFA-SS product, the bioavailability of the new formulation needed to be demonstrated as being equal to or greater than that of the original formulation.

Since current lots of the original CCFA-SS formulation were no longer available, the pharmacokinetic bridge was based upon the comparison of historical data (original CCFA-SS formulation) and contemporary data (revised CCFA-SS formulation). The results of the cross-study comparison are provided in Section 2.c.3, Relative bioavailability assessment.

1. Historical Data: "Pharmacokinetics of Ceftiofur and Desfuroylceftiofur-related Metabolites after Injection of Ceftiofur Crystalline Free Acid Sterile Suspension (200 mg/mL; lot no. 40,676) Administered Subcutaneous in the Neck or in the Posterior Ear of Cattle Monthly for Six Months after Date of Manufacture".

a) Investigators: S. A. Brown, E. J. Robb, P. J. Hamlow, J. K. Callahan, V. L. Hubbard, S. T. Chester, Jr., T. S. Arnold, T. D. Cox, T. F. Flook, and V. R. Lewis; Pharmacia & Upjohn, Kalamazoo, MI.

b) Study design:

1) *Objective:* The purpose of this investigation is to determine the potential change in CCFA-SS bioavailability as the product is aged over a six month period.

2) *Experimental Animals:* Bovine/beef crossbred cattle (n = 96; 48 heifers/48 steers; 215 to 354 kg; 4 animals/gender/route/month) were housed in individual tie stalls.

3) *Dosage Form:* CCFA-SS, 200 mg CE/mL, ready-to-use formulation.

4) *Route of Administration:* CCFA-SS subcutaneous (SC) injections were administered in the neck or in the middle third of the posterior aspect of the ear. Since this investigation was conducted as a parallel study design, only those data from animals administered CCFA-SS in the ear were considered pivotal.

5) *Dosage:* Each animal received a single administration dose of CCFA-SS at 6.6 CE/kg BW.

6) *Sampling:* Blood samples were collected at 0, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 168, and 240 hours following treatment. Plasma was harvested and frozen prior to analysis. A complete set of blood samples were obtained monthly for the six-month study duration.

7) *Assay Method:* The ceftiofur and desfuroylceftiofur-related residues in each plasma sample were determined using the validated HPLC-DCA method. The limit of quantitation (LOQ) for this assay was 0.150 mcg/mL plasma. Each sample was analyzed as a single determination.

8) *Decision Variables:* The area under the plasma concentration-time curve to the LOQ ($AUC_{0\text{-}LOQ}$) and the duration over which plasma total ceftiofur concentrations remained above 0.2 mcg/mL ($t_{>0.2}$) were the pivotal metrics for establishing formulation comparability.

9) *Pharmacokinetic Analysis Method:* Trapezoidal summation was used to estimate the $AUC_{0\text{-}LOQ}$. A one compartment model was used to estimate $t_{>0.2}$.

c) Results: The estimates of $AUC_{0\text{-}LOQ}$ and $t_{>0.2}$ values, generated for each month and averaged over the entire six month study period, are presented in Table 2.4:

Table 2.4 Pharmacokinetic Parameters: Original CCFA-SS Formulation [mean (%CV)].

Parameter	Month						
	1	2	3	4	5	6	Mean
$AUC_{0\text{-}LOQ}$ (mcg•h/mL)	231 (18)	307 (24)	283 (23)	236 (27)	281 (29)	274 (18)	269
$t_{>0.2}$ (h)	152 (23)	180 (27)	146 (13)	147 (26)	175 (25)	142 (18)	157

d) Conclusions: Although differences in mean monthly parameter values could be observed, there were no consistent increases or decreases over time. For this reason, it is concluded that the observed fluctuations in values are attributable to intersubject variability rather than to changes in the physico-chemical properties of the formulation. Variance estimates were also similar across all six monthly testing intervals. Therefore, it is determined that the average monthly values can be used to support the relative bioavailability assessment.

2. Contemporary Data: "Pharmacokinetic Equivalence Study of Ceftiofur in Cattle Treated with Ceftiofur Crystalline Free Acid Sterile Suspension (200 mg ceftiofur equivalents (CE)/mL) by Subcutaneous Injection in the Posterior Ear at a Dose of 6.6 mg CE/kg Bodyweight". (SR a0058859)

a) Investigators: J. A. Robinson, F. M. Kausche, S. A. Brown, S. T. Chester, Jr., J. K. Callahan, T. D. Cox, T. F. Flook, V. L. Hubbard, V. R. Lewis, and M. J. Prough, Pharmacia & Upjohn, Kalamazoo, MI.

b) Study Design:

1) *Objective:* The purpose of this GLP study was to characterize the plasma total ceftiofur concentrations following administration of the revised 200 mg CE/mL formulation.

2) *Experimental Animals:* Angus and Angus crossbred cattle (n = 24; 12 steers, 12 heifers; 270-400 kg) were housed in individual tie stalls.

3) *Dosage Form:* CCFA-SS (revised 200 mg CE/mL formulation), ready-to-use formulation (GLP out-take 10630 from GMP lot 40,755).

4) *Route of Administration:* CCFA-SS injections were administered SC in the middle third of the posterior aspect of the ear.

5) *Dosage:* Each animal received a single dose of CCFA-SS at 6.6 mg CE/kg BW.

6) *Sampling:* Blood samples were collected at 0, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 168, and 240 hours following treatment administration. Plasma was harvested and frozen prior to analysis.

7) *Assay Method:* The ceftiofur and desfuroylceftiofur-related residues in each plasma sample were determined using the validated HPLC-DCA method. The limit of quantitation (LOQ) for this assay was 0.150 mcg/mL plasma. Each sample was analyzed as a single determination.

8) *Decision Variables:* The area under the plasma concentration-time curve to the LOQ ($AUC_{0\text{-}LOQ}$) and the time plasma concentrations remained above 0.2 mcg/mL ($t_{>0.2}$) were the decision variables for this study.

9) *Pharmacokinetic Analysis Method:* Trapezoidal summation was used to estimate the $AUC_{0\text{-}LOQ}$. A one compartment model was used to estimate $t_{>0.2}$.

c) Results:

The arithmetic mean values and corresponding percent coefficients of variation (% CV) associated with the pivotal pharmacokinetic parameters are provided in Table 2.5.

Table 2.5 Pharmacokinetic Parameters: Revised CCFA-SS Formulation

Pharmacokinetic Parameter	Mean	%CV
$AUC_{0\text{-}LOQ}$ observed (mcg•hour/mL)	376	18
$AUC_{0\text{-}LOQ}$ modeled (mcg•hour/mL)	364	19
$t_{>0.2}$ (hour)	183	22

3. **Relative bioavailability assessment:** The least square (LS) means of the $AUC_{0\text{-}LOQ}$ and $t_{>0.2}$ values estimated for the original (n = 48) and revised formulations (n = 24), and the corresponding lower limit of the 90% confidence interval about the ratio of treatment means (expressed relative to the original formulation) are presented in Table 2.6.

2.6 Pharmacokinetic Parameters Relative Bioavailability Comparison (LS means)[1]

Pharmacokinetic Parameter	Mean for the Revised CCFA-SS Formulation (Estimate of μ_{rev})[2]	Mean for the Original CCFA-SS Formulation (Estimate of μ_{orig})[3]	Ratio of Means	Lower limit of the 90% confidence interval
$AUC_{0\text{-}LOQ}$ (mcg•hour/mL)	364	271	1.34	1.24
$t_{>0.2}$ (hour)	183	157	1.17	1.06

[1] LS means were used for this product comparison in an effort to adjust for the imbalance in the number of subjects within each study. Since arithmetic means were used to define the results obtained within the individual investigations (historical and the contemporary datasets), those arithmetic means may differ slightly from the LS means used for the treatment comparison statistically generated during this product relative bioavailability assessment.

[2] Based on n=24 cattle for the revised formulation

[3] Based on n=48 cattle for the original formulation

Conclusions: For the two formulations to be considered clinically equivalent, the lower limit of the two one-sided test procedures (i.e., the 90% confidence interval about the ratio of treatment means) was required to be $\geq$0.80. This criterion was easily met for the two pivotal parameters, $AUC_{0\text{-}LOQ}$ and $t_{>0.2}$. Therefore, a relevant pharmacokinetic bridge is established, and the clinical studies conducted with the original 200 mg CE/mL formulation remain pivotal for the determination of effectiveness of NAXCEL XT STERILE SUSPENSION in the treatment and control of BRD.

d. Microbiology:

MIC Survey: "Results of 1997-98 Resistance Monitoring Program for Premafloxacin with Veterinary Pathogens". (SR a0032820)

This study was conducted to determine the *in vitro* activity of veterinary antimicrobials against veterinary pathogens isolated from food animals across the U.S. The investigators were E. S. Portis, S. A. Salmon, C. A. Case, and J. L. Watts, Pharmacia & Upjohn, Kalamazoo, MI. Isolates (1997-1998) were obtained from eight U.S. veterinary diagnostic labs accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). MICs were determined for eight antimicrobials. Ceftiofur MICs were determined using a commercially available microdilution system according to NCCLS guidelines.

Control quality assurance strains were included in each run and were within acceptable ranges. Ceftiofur MICs for the BRD pathogens *M. haemolytica*, *P. multocida*, and *H. somnus* are presented in Table 2.6.

Table 2.6 Ceftiofur MIC for BRD Pathogens

Pathogen, 1988-1992		MIC_{90} (mcg/mL)	Range[1] (mcg/mL)
Mannheimia haemolytica	n=461	0.06	≤ 0.03 - 0.13
Pasteurella multocida	n=318	0.06	≤ 0.03 - 0.25
Haemophilus somnus	n=109	0.06	≤ 0.03 - 0.13
Pathogen, 1997-1998		MIC_{90} (mcg/mL)	Range* (mcg/mL)
Mannheimia haemolytica	n=110	0.06	≤ 0.03 - 0.25
Pasteurella multocida	n=107	≤ 0.03	≤ 0.03 - 0.25
Haemophilus somnus	n=48	≤ 0.03	≤ 0.03 - 0.25

[1] The ceftiofur range tested in these studies was 0.03-32 mcg/mL.

The results of surveillance study demonstrated that the primary BRD pathogens *M. haemolytica, P. multocida*, and *H. somnus* remain sensitive to ceftiofur ten years after ceftiofur was first approved in the U.S.

3. TARGET ANIMAL SAFETY:

The safety of NAXCEL XT STERILE SUSPENSION is based upon a comparison of the total and peak systemic exposure to ceftiofur and desfuroylceftiofur metabolites following a one-time administration of NAXCEL XT STERILE SUSPENSION (administered as a subcutaneous injection in the middle third of the posterior aspect of the ear) versus the approved dosage of NAXCEL Sterile Powder (ceftiofur sodium) (five sequential intramuscular injections into the neck at a dosage of 2.2 mg CE/kg BW). The safety of ceftiofur sodium has previously been demonstrated in studies discussed in the FOI Summary for NAXCEL Sterile Powder, NADA 140-338, approved January 25, 1988.

To facilitate the comparison between NAXCEL XT STERILE SUSPENSION (revised formulation) and NAXCEL, the NAXCEL AUC values are multiplied by a factor of 5, since NAXCEL XT STERILE SUSPENSION is intended to be comparable to 5 daily injections of NAXCEL. CMAX values are also provided as single dose (observed) data and predicted steady state values (i.e., CMAX * 1.21, where 1.21 is the extent to which the ceftiofur moieties are expected to accumulate at steady state).[1] The relative bioavailability of ceftiofur sodium (observed and predicted values) versus a single dose of NAXCEL XT STERILE SUSPENSION is provided in Table 3.1.

Table 3.1 Ceftiofur Sodium (Na) treatment means for PK estimates

	CMAX	$AUC_{0\text{-}LOQ}$	$T_{1/2}$
Ceftiofur Na – dose 1(observed values)	16.5±2.91	142±25.4	9.5±1.15
Predicted ceftiofur Na – dose 5	19.5	710 – predicted total exposure after 5 sequential daily doses	
NAXCEL XT STERILE SUSPENSION	6.9	364	62.3

Based upon these results, we conclude that a single dose of NAXCEL XT STERILE SUSPENSION provides lower AUC and CMAX values as compared to that obtained from five daily NAXCEL injections. Accordingly, the target animal safety data generated for NAXCEL can be confidently extrapolated to NAXCEL XT STERILE SUSPENSION to verify its safety when used as a one-time administration of 6.6 mg CE/kg BW as a subcutaneous injection in the middle third of the posterior aspect of the ear of cattle.

[1] We can expect the concentration of ceftiofur metabolites to be about 21% higher at steady state as compared to that seen after a single dose based upon the equation:
accumulation = $1/[1-\exp((0.693/t_{1/2})*\text{dosing interval})]$.

a. Injection Site Tolerance Study:

"PNU-64279: Subcutaneous Ear Tissue Injection Site Irritation Study in Cattle". (SR a0063896)

1. Type of Study: Injection site tolerance study, conducted and inspected according to GLP regulations

2. Investigator: W. J. Seaman, Pharmacia & Upjohn, Inc., Kalamazoo, MI

3. Study Design:

   a) *Objective:* To provide injection site tolerance and resolution information following administration of a single SC dose of NAXCEL XT STERILE SUSPENSION in the middle third of the posterior aspect of the ear of beef cattle.

   b) *Experimental Animals:* A total of 6 crossbred beef cattle (3 heifers, 2 steers and 1 bull) were enrolled in this study. Animals were approximately 7 months old. Body weights over the course of the study ranged from 201 to 288 kg.

   c) *Test Article Administration:* CCFA-SS ready-to-use formulation (revised formulation) was used in this study (200 mg CE/mL). All treatments were administered SC in the middle third of the posterior aspect of the ear using a 16-gauge 1" sterile needle on an eccentric hub syringe. Each of the six cattle was injected once in each ear (12 total injections) at a dose level of 6.6 mg CE/kg [animals 2, 4, 5, and 6 were injected on Day 1; animals 1, 3, 4, and 6 were injected on Day 29; and animals 1, 2, 3, and 5 were injected on Day 50. Injections were staggered so that necropsies were performed at 1, 4, and 8 weeks after injection.] All animals were necropsied on Day 57 and an evaluation of injection sites was performed to determine the local irritation potential, and resolution time at the three time points (1 week, 4 weeks, and 8 weeks).

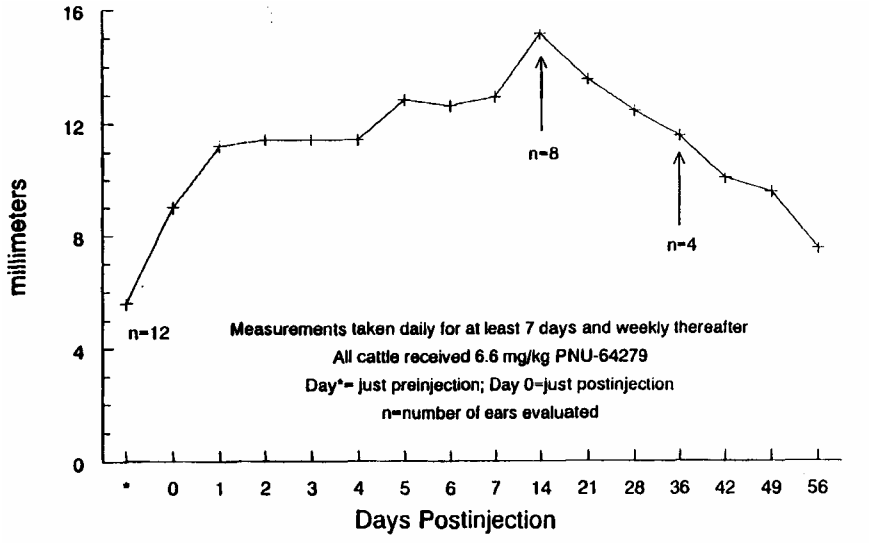
   d) *Measurements and Observations:* Ear carriage observations were recorded just before dosing and daily for one week post-treatment or until the ears returned to normal carriage. Mid-ear thickness was measured with calipers just before dosing, immediately after dosing, and daily until two consecutive readings were equal to or less than the previous reading. Ear thickness was then measured once weekly until necropsy. Ears were evaluated grossly and microscopically at necropsy. Body weights were measured just before dosing for dose volume calculation.

4. Results:

a) Ear Carriage: Ear carriage for 11 ears was normal throughout the study. One animal carried one ear in a downward position for 7 days post-treatment, after which the animal's ear carriage returned to normal.

b) Ear Thickness: Mean ear thickness during the study is presented in Figure 3.1.

Figure 3.1 Daily and Weekly Mean Ear Thickness



c) Gross and Microscopic Observations: At necropsy, all injection sites had an area of tan discoloration in the subcutaneous space. The size of the discolored area decreased over time. There was no evidence of any auricular cartilage reaction. By 8 weeks post-treatment, the subcutaneous tissue had thickened as a result of fibrosis.

5. Conclusions: Subcutaneous administration of NAXCEL XT STERILE SUSPENSION into the posterior aspect of the ear of cattle at a dose of 6.6 mg CE/kg BW had minimal effect on ear carriage in this study. Mean ear thickness increased between post-treatment Days 1 and 14. After Day 14, ear thickness decreased. At necropsy, granulomatous inflammation occurred as expected following administration of an oil-based formulation. Although these injection sites did not completely resolve by 8 weeks post-treatment, this effect was not considered clinically significant, since cattle ears are inedible in the US (9 CFR 301.2) and are removed from the carcass during normal slaughterhouse procedures.

b. Acute Toxicity Studies:

Two additional studies were conducted because in approximately 6000 tested animals, nine animals died following injection of NAXCEL XT STERILE SUSPENSION. Two deaths are referenced in Section 2.b.2. The other seven deaths occurred with this product in unpublished studies. All deaths occurred within 30 minutes of the time of injection. The exact cause was confirmed in three animals. These three deaths resulted from inadvertent intra-arterial injection of CCFA-SS into one of the major auricular (ear) arteries. Intra-arterial injection at this location resulted in direct administration of the oil-based formulation into the arterial blood supply of the brain resulting in embolism and death. The consequences of purposeful intra-arterial or intravenous injection of NAXCEL XT STERILE SUSPENSION were investigated in feeder cattle.

1. "PNU-64279: Acute Intravenous Toxicity Study Using Ceftiofur Crystalline Free Acid Sterile Suspension in Cattle". (SR a0094894)

a) Investigator: W. Seaman, Pharmacia Animal Health Research Farm, Richland, MI

b) Study Design:

1) *Objective:* To determine the potential of accidental intravenous administration of CCFA-SS to cause acute toxicity or death in cattle.

2) *Experimental Animals:* Six calves (3 heifers and 3 steers), weighing 196.5 to 223.0 kg.

3) *Test Article Administration:* All cattle were administered a single injection of CCFA-SS at 6.6 mg CE/kg body weight into the jugular vein.

4) *Measurements and Observations:* Physical examinations were conducted on the day of intravenous administration. Heart rate and auscultation, respiration and signs of neurobehavioral toxicity were monitored just prior to dosing, for the 15 minutes following dosing, and for several hours after dosing. In addition, animals were observed for general condition, behavior, evidence of irritation or thrombus formation at the injection site, and signs of systemic toxicity once daily through Day 10.

c) Results: One steer had more audible respiration (with no change in rate) between 2 and 5 minutes postinjection, which returned to normal by 12 minutes post-injection. One steer and one heifer had approximately 43% and 33% increases, respectively, in their postinjection respiratory rates when compared to pre-dose rates. Respiratory rates in all animals were noted to be normal at later observations. No animal showed any signs of distress, pain, or discomfort during or after the intravenous injection.

d) Conclusions: No severe adverse effects were noted after intravenous administration of CCFA-SS into the jugular vein at 6.6 mg/kg body weight.

2. "Acute Intra-arterial Toxicology Study Using Ceftiofur Crystalline Free Acid Sterile Suspension (CCFA-SS) in Cattle". (P&T Number 2002-0008)

a) Investigator: W. Seaman, Pharmacia Animal Health Research Farm, Richland, MI

b) Study Design:

1) *Objective:* To determine the potential adverse effects of intra-arterial administration of a 6.6 mg CE/kg dose of CCFA-SS in the middle auricular artery of feeder cattle.

2) *Experimental Animals:* Two female feeder cattle, weighing 225 kg each, were used in the study.

3) *Test Article Administration:* A dose of 6.6 mg CCFA-SS per kg bodyweight was injected into the middle auricular artery of the left pinna. The test article contained 0.2% Sudan Black B dye to aid in visualization of the injected material. The injections were made using a butterfly catheter with a 19-gauge needle; the dose was injected into the artery as quickly as it could be expressed (15-20 seconds).

4) *Measurements and Observations:* Pertinent observations included clinical signs following administration, gross evaluation of the head (if needed), and histopathology using frozen sections.

c) Results: Immediately following the test article administration, both heifers went down in the chute. The animals were released from the head-catch and removed from the chute via a side-opening release. One heifer vocalized with bellowing. Both heifers were laterally recumbent and showed alternating periods of no activity and thrashing or paddling of the legs. Respiration became irregular, heart rate increased (>200 beats/minute), capillary refill time was delayed, the lips and tongue became cyanotic, and palpebral and corneal reflexes diminished. Both heifers died approximately eight minutes after initiation of clinical signs.

At necropsy, variable amounts of darkly stained cerebral cortex were visible in both animals. Gross transverse sectioning of the brains revealed darkened gray matter under the areas of darkened cerebral cortex surfaces. One heifer had two grossly visible foci of hemorrhage in the cerebellum.

d) Conclusions: The clinical signs and gross necropsy observations in both heifers support the hypothesis that inadvertent or purposeful administration of CCFA-SS into the auricular artery of the pinna of the ear can cause delivery of the formulation to the brain, resulting in emboli formation, and acute death.

4. HUMAN SAFETY:

Ceftiofur sodium, ceftiofur hydrochloride, and CCFA-SS all have ceftiofur as the active component and all three are rapidly metabolized to desfuroylceftiofur in both the target species and laboratory animals.

a. Microbial Food Safety:

Microbial food safety information for CCFA-SS was assessed by a qualitative risk methodology and the sponsor has satisfied the requirements of the microbial food safety component of the human food safety technical section. The Agency has determined that the use of CCFA-SS under the proposed conditions of use [single injection of 3.0 mg CE/lb (6.6 mg CE/kg) BW (1.5 mL NAXCEL XT STERILE SUSPENSION per 100 lb body weight), for treatment and control of bovine respiratory disease (BRD) in cattle] should not pose a risk to public health with respect to generation or dissemination of resistant bacteria that would impact the effectiveness of similar antimicrobial drugs used in human medicine.

b. Toxicity:

The toxicity testing of ceftiofur is summarized in the FOI Summary for NAXCEL (ceftiofur sodium) Sterile Powder (NADA 140-338) and in the FOI Summary for the original approval of EXCENEL (ceftiofur hydrochloride) Sterile Suspension (NADA 140-890) for use in swine. Tolerances for cattle are summarized in the FOI Summary for EXCENEL for use in cattle. The current FOI summarizes the additional studies required to assess the residue decline of NAXCEL XT STERILE SUSPENSION.

1. "Comparative Oral Bioavailability Study of Ceftiofur Sodium and Ceftiofur Crystalline Free Acid in Sprague-Dawley Rats". Fate GD. Upjohn Technical Report No. 829-7926-95-001, 13 December 1995.

a) Purpose: The objective of this GLP study was to assess the relative oral bioavailability of CCFA and ceftiofur sodium. Bioavailability was assessed by measuring the total ceftiofur residues in plasma, urine, feces, kidney, and liver after oral administration of either ^{14}C-CCFA or ^{14}C-ceftiofur sodium in rats when administered at an oral dose of 100 mg CE/kg BW.

b) Study Director: G. D. Fate, Animal Health Drug Metabolism, Unit 7926, Pharmacia & Upjohn, Kalamazoo, MI

c) General Design: A randomized complete block design was used to evaluate the plasma residues over time, urine and fecal excretion of total residues, and quantitation of total ceftiofur-related residues in kidney and liver 72 hours after drug administration.

1) *Test Animals:* Sprague-Dawley rats [CRL:CD(BD)]; 22 males and 22 females

2) *Dosage Form and Route of Administration:* Ceftiofur sodium and ceftiofur crystalline free acid, both labeled with ^{14}C at the same location in the aminothiazol ring, were administered orally dissolved or suspended, respectively, in sterile water.

3) *Dosage:* All rats were administered 100 mg ceftiofur equivalents/kg body weight by oral gavage, with only one oral dose per test animal.

4) *Pertinent Parameters Measured:* Primary decision variables were the plasma concentration-time curve from zero to 72 hours (AUC_{0-72}), excretion of total ceftiofur residues in urine and feces through 72 hours, and total residue levels in liver and kidney at 72 hours post-treatment.

d) Results:

Table 4.1 Pharmacokinetic and Tissue Concentration Data from Rats Orally Dosed with Ceftiofur Sodium (Na) or Ceftiofur Crystalline Free Acid (100 mg CE/kg Body Weight)

Value	CCFA (males)	CCFA (females)	Ceftiofur Na (males)	Ceftiofur Na (females)
C_{max} (μg/mL)	3.43 ± 1.31	1.86 ± 0.51	4.78 ± 1.76	2.19 ± 0.60
T_{max} (hours)	3.8	3.5	3.2	2.9
Kidney (μg/g)	0.47 ± 0.12	0.50 ± 0.11	0.18 ± 0.02	0.27 ± 0.04
Liver (μg/g)	0.13 ± 0.02	0.15 ± 0.09	0.02 ± 0.01	0.06 ± 0.01
Urine excretion (μg)	542 ± 178	407 ± 93	549 ± 55	389 ± 43
AUC_{0-72} (μg•h/mL)	44.44 ± 17.34	23.62 ± 5.09	49.53 ± 11.54	23.53 ± 3.89

e) Conclusions: Given that the mean excretion into urine is quite consistent across ceftiofur sodium and ceftiofur crystalline free acid, the AUC data supports equal bioavailability and the kidney residue data are consistent with slower absorption of ceftiofur crystalline free acid than ceftiofur sodium. The data from this study support the conclusion that ceftiofur crystalline free acid and ceftiofur sodium are equally bioavailable when administered orally. The demonstration of equal oral bioavailability demonstrates that the results of toxicology testing conducted for ceftiofur sodium may be applied to the evaluation of ceftiofur crystalline free acid.

2. Determination of an acceptable single-dose intake (ASDI) level for residues at the injection site – Hypersensitivity consideration

Studies conducted in the guinea pig model of ceftiofur hypersensitivity and cross hypersensitivity between penicillin G and ceftiofur were previously submitted to NADA 140-338 (technical report number 7220-88-026). The results of these studies were reassessed to provide the basis of an acceptable single-dose intake for residues of ceftiofur crystalline free acid at the injection site.

a) Title: Passive Cutaneous Anaphylaxis Study in Guinea Pigs. Technical Report No. 7263/87/077

b) Study Director: T. A. Jackson, Animal Health Drug Metabolism, Unit 7926, Pharmacia & Upjohn, Kalamazoo, MI

c) Identification of Substance and Dosage Form: Ceftiofur (U-64,279E) was tested in four forms:

1) Parent compound (bulk drug)
2) Parent compound conjugated to hen egg albumin as the carrier protein
3) Metabolite II
4) Extract of residue from kidney and injection site muscle of treated animals

In addition to the four forms of ceftiofur, two additional conjugates with hen egg albumin were tested:

1) Compound A - structurally similar to Metabolite VII
2) Compound B - common to parent compound and all metabolites

d) Species and Strain: Hartley Albino Guinea Pigs

e) Number of Animals Per Sex Per Treatment Group: Females only, variable number per group (2 - 12), depending on challenge material and level

f) Route of Drug Administration: IV and Oral

g) Drug Levels Tested and Duration of Dosing: Single challenge doses were given to animals passively sensitized with antibody to benzylpenicillin G and/or ceftiofur. The challenge levels tested were based on anticipated maximum human exposure level (approximated 0.083 mg/kg), varied with the form of the drug and were usually given in 10-fold increments. Routinely, oral challenges were at levels 10X greater than those given IV:

Parent compound (bulk drug) IV: 0.076, 0.76, 7.6 mg/kg
Oral: 0.76, 7.6, 76 mg/kg

Ceftiofur conjugate (ceftiofur to hen egg albumin) IV: 0.01, 0.10, 1.0 mg/kg
Oral: 0.10, 1.0, 10.0 mg/kg

Metabolite II IV: 0.076, 0.76, 7.6, 76, 760, 7600 µg/kg
Oral: 0.76, 7.6, 76, 760, 7600 µg/kg

Compound A conjugate (to hen egg albumin) IV: 0.0083, 0.083, 0.83 mg/kg (actual content of Compound A not determined)

Compound B conjugate (to hen egg albumin) IV: 0.83 mg/kg (actual content of Compound B not determined)

Extract of drug residue from injection-site muscle and kidney Oral: 830 µg/kg

h) Parameters Tested: Passive Cutaneous Anaphylaxis (PCA) following IV and/or oral challenge. Briefly, skin sites on guinea pigs were passively sensitized with antibody of desired specificity and five days later the sensitized animals were given Evans blue dye and challenged by either the IV or oral route with appropriate test material. Sensitized skin sites were subsequently examined for evidence of PCA reactions as indicated by the leakage of Evans blue dye from the vascular system into the skin.

i) Significant Findings: Regardless of dose, there were no positive reactions of passive cutaneous anaphylaxis (PCA) when guinea pigs sensitized with penicillin antibody were challenged by either route with any form of ceftiofur.

Guinea pigs sensitized with ceftiofur antibody did not respond to challenges with the parent compound by either route. However, positive PCA reactions occurred following challenge with protein conjugate by both routes. While an IV challenge of 0.01 mg/kg produced positive reactions, oral exposure resulted in PCA activity only at a challenge level of 10 mg/kg.

Metabolite II caused PCA reactions over a wide range of dose levels when given by either the IV or oral route to guinea pigs sensitized with ceftiofur antibody. While as little as 0.076 µg/kg produced positive responses following IV challenge, levels of at least 76 µg/kg were required to induce positive PCA activity as a sequel to oral exposure.

Guinea pigs sensitized with ceftiofur antibody then challenged IV with conjugates of Compound A or Compound B responded to the challenge. These positive responses confirm that the model is able to recognize all metabolites in the extract of kidney or muscle.

Guinea pigs sensitized with ceftiofur antibody and challenged orally at a level of 0.83 mg/kg with acetic acid extract of drug (ceftiofur) residue from kidney and injection-site muscle were uniformly negative.

No Observed Effect Level - 0.83 mg/kg. The relevant NOEL is for oral challenge of sensitized animals with the acetic acid extract of ceftiofur residue from kidney and injection site muscle.

j) Statistical Analysis: Not applicable.

k) Conclusions: Antibody to benzylpenicillin G did not react with any form of ceftiofur tested, regardless of route or dose. Data from the IV challenges indicate that ceftiofur antibody used for this study was capable of detecting the drug as a conjugated hapten in challenge material given at a level as low as 0.01 mg/kg body weight (lowest level tested). Positive PCA reactions also occurred with IV challenges of Metabolite II at levels as low as 0.076 µg/kg body weight (lowest level tested). The route was of considerable importance for both the protein conjugate and Metabolite II because of the differences in the level of challenge necessary to elicit a positive PCA reaction. With both materials, the oral challenge level necessary to produce a positive response was approximately 1,000X the minimum level required for a PCA reaction following

IV challenge. This suggests that the gastrointestinal tract may play an important role in modulating the effect of potential hypersensitive reactions with ceftiofur.

Further, the data suggest that ceftiofur does not exist in residue from injection site muscle or kidney in a form or concentration such that PCA reactions occur following oral exposure of animals sensitized with ceftiofur antibody and challenged with extract of the residue. The NOEL derived from oral challenges of injection site muscle extract (IME) is 0.83 mg/kg BW. Of all the challenge materials tested in this study, IME is considered the most appropriate one to use for evaluating acute exposure. A safety factor of 1 was used in the calculation of an ASDI for residues of ceftiofur at the injection site (see Section d, below).

3. Microbial Safety

The potential for residues of CCFA to affect the microflora of the human gut was evaluated in accordance with Guidance for Industry #52. The Agency has concluded that the amount of microbiologically active residues of CCFA that reach the colon would most likely not cause adverse effects on the human intestinal microflora of the consumer.

c. Determination of No Observed Effect Level (NOEL):

The lowest No Observed Effect Level (NOEL) for chronic exposure was determined from the 90-day feeding studies in both dogs and rats: 30 mg/kg body weight (BW).

The No Observed Effect Level (NOEL) for acute exposure was determined from challenges of injection site muscle extract (IME): 0.830 mg/kg BW.

d. Determination of an Acceptable Daily Intake (ADI):

The ADI for chronic exposures is 0.03 mg/kg BW/day. Twenty-seven percent of the ADI is allocated for milk and the remaining 73% is allocated for tissues resulting in the following ADI assignments:

0.008 mg/kg BW/day for milk and
0.022 mg/kg BW/day for edible tissues.

The NOEL from the IME challenge materials is considered the most appropriate NOEL to use for the basis of the acceptable single-dose intake (ASDI). A safety factor of 1 was used in the calculation of an ASDI for residues of ceftiofur at the injection site:

ASDI = 0.830 mg/kg BW/1

= 0.830 mg/kg BW

e. Calculation of the Safe Concentrations:

The calculation of the safe concentrations for total residues in edible tissues resulting from chronic exposure is summarized in the FOI Summaries for NADA 140-338 and 140-890.

The calculation of the safe concentration for total residues in injection site tissues resulting from acute exposure is:

Safe Concentration in the injection site = (0.830 mg/kg BW X 60 kg)/0.300 kg*
= 166 mg/kg or 166 ppm

*The consumption factor for muscle is used in this calculation.

Because residues determined using the HPLC-DCA assay correlated nearly 1:1 with the radiolabeled residues determined by liquid scintillation counting for short withdrawal periods, the ASDI and the injection site tolerance for residues determined using the HPLC-DCA assay are numerically equivalent for withdrawal times less than 48 hours.

f. Total Residue and Metabolism:

The total residue and metabolism in the target species and comparative metabolism in the toxicological species for ceftiofur are summarized in the FOI Summaries under NADAs 140-338 and NADA 140-890.

The marker residue in edible tissues, including milk, is the sum of ceftiofur and desfuroylceftiofur-related metabolites, measured by HPLC as the stable derivative desfuroylceftiofur acetamide (DCA). The target tissue for residue monitoring is kidney.

g. Tolerance for the Marker Residue:

Tolerances for DCA were established for bovine tissues under NADA 140-890 and are based on the relationship between total residues measured by radioactive monitoring and DCA in edible tissues, and the Safe Concentrations calculated above.

Approved Safe Concentrations and Tolerances for ceftiofur in edible tissues determined are presented in Table 4.2.

Table 4.2 Consumption Factors, Ceftiofur Safe Concentrations, and Tolerances for Edible Tissues

Tissue	Daily Consumption (g)	Safe Concentration (mg/kg)	Tolerance (mg/kg)
Muscle (non-injection)	300	4.40	1.0
Liver	100	13.2	2.0
Kidney	50	26.4	8.0
Fat	50	26.4	NE[1]
Milk	1500 (1.5 L)	0.320	0.100

[1] NE – Not Established

h. Establishment of a Withdrawal Period:

Tissue Residue Depletion Study: "Residue Decline of Ceftiofur in the Injection Site of Cattle Administered a Sterile Suspension of Ceftiofur Free Acid (PNU-64279) at 200 mg/mL by Subcutaneous Injection in the Posterior Ear at a Dose of 6.6 mg Ceftiofur Equivalents/kg Bodyweight". Hornish RE. Pharmacia & Upjohn Study Report a0067497, 17 March 2000.

1. Purpose: This study was designed to measure concentrations of ceftiofur and desfuroylceftiofur-related metabolites, measured as desfuroylceftiofur, the marker residue, using the official regulatory method, in kidney and edible tissue adjacent to the inedible tissues of the ear (termed "injection sites") at 12, 24, 48, 72, 96, 120, and 240 hours after the subcutaneous injection of ceftiofur crystalline free acid at the highest approved dosage in the posterior aspect of the ear in cattle.

2. Study Director: R. E. Hornish, Animal Health Drug Metabolism, Unit 7926, Pharmacia & Upjohn, Kalamazoo, MI

3. Study Location: The animal phase of this study took place at Animal Health Drug Metabolism Laboratories, Pharmacia & Upjohn Research Farm, Richland, MI.

4. Study Design:

   a) *Test Animals:* Forty-two Angus crossbred cattle (21 steers, 21 heifers; 180-273 kg BW at time of dosing) were blocked by weight and sex, then randomly assigned into seven necropsy groups of six cattle each, with two additional heifers (within the same body weight range) serving as nontreated controls. Slaughter times were 12, 24, 48, 72, 96, 120, and 240 hours after drug administration. Inedible ear tissues were removed at slaughter according to routine slaughterhouse practices. Both kidneys were also collected at necropsy for residue analysis.

b) *Dosage Form and Route of Administration:* CCFA-SS (200 mg/mL) was administered subcutaneously into the middle third of the posterior ear of the animal using a 16G, 1 in. needle attached to an eccentric hub syringe. After the needle was fully inserted and drug administered, direct pressure was applied to the insertion point during injection and before releasing the ear to minimize backflow of injected material.

c) *Dosage*: Each animal received one injection of 3.0 mg CE/lb (6.6 mg CE/kg) BW.

d) *Pertinent Parameters Measured*: Concentrations of ceftiofur and desfuroylceftiofur-related metabolites were determined as the marker residue (DCA) by HPLC-UV assay in kidney and edible tissues (termed "injection site") immediately adjacent to the inedible auricular tissues removed at the time of slaughter at each slaughter point. The total weight of edible injection site and underlying muscle removed was approximately 500 grams.

5. Results: No clinical signs of irritation (except for local transient swelling at injection sites) were observed during the course of the study. Concentrations of desfuroylceftiofur-related metabolites (HPLC-DCA) in injection site samples and kidney were determined. Concentrations of ceftiofur-related residues (HPLC-DCA) are presented in Table 4.3.

Table 4.3 Ceftiofur-related Residues (HPLC-DCA) in Kidney and Injection Site Samples after Administration of NAXCEL XT STERILE SUSPENSION (Ceftiofur Crystalline Free Acid Sterile Suspension) Administered Subcutaneously in the Posterior Ear of Cattle at a Dose of 6.6 mg CE/kg BW.

Withdrawal Time (hr)	HPLC-DCA (ppm)	
	Kidney	Injection site
12	2.91±0.63	3.36±3.29
24	2.97±0.41	6.81±6.28
48	2.15±0.58	18.96±11.15
72	1.64±0.12	0.47±0.17
96	1.16±0.20	0.63±0.38
120	0.84±0.15	1.78±2.21
240	0.21±0.04	0.40±0.34

See Section 3.a for discussion of local effects of injection of CCFA-SS in the ears of cattle.

6. Conclusions: Kidney residue data were evaluated using a statistical upper tolerance limit algorithm for the 99th percentile kidney residue data with 95 percent confidence.

Injection site residues, while not assessed statistically, do not exceed the ASDI value in any samples collected for the first 48 hours following administration, when residues are rising, and are significantly less than the ASDI at sampling times beyond 48 hours.

7. Withdrawal Time: A withdrawal period of zero days is established for the use of ceftiofur crystalline free acid sterile suspension when administered to cattle at a dose of 6.6 mg ceftiofur equivalents/kg BW as a subcutaneous injection in the middle third of the posterior aspect of the ear.

i. Regulatory Method for Residues:

The regulatory method for determination of DCA in bovine kidney, muscle, and milk; and swine kidney and muscle, is the HPLC-DCA assay which successfully completed a sponsor-monitored multilaboratory method trial. The method is on file with the Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855.

j. User Safety:

Studies to evaluate the safety of ceftiofur to users are discussed in detail in the FOI Summary for NADA 140-338 (NAXCEL), approved January 25, 1988.

5. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that NAXCEL XT STERILE SUSPENSION is safe and effective for the treatment of BRD (shipping fever, pneumonia) associated with *Mannheimia haemolytica*, *P. multocida*, and *H. somnus*. NAXCEL XT STERILE SUSPENSION is also indicated for the control of respiratory disease in cattle which are at high risk of developing BRD associated with *Mannheimia haemolytica*, *P. multocida*, and *H. somnus*.

Labeling restricts this drug to use by or on order of a licensed veterinarian. This decision was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product to treat bovine respiratory disease and (b) restricting this drug to use by or on order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues.

An Acceptable Daily Intake (ADI) of 0.03 mg/kg/day has been established for ceftiofur. An Acceptable Single-Dose Intake (ASDI) of 0.83 mg/kg body weight has been established for ceftiofur at the injection site. A tolerance of 8 ppm for residues of desfuroylceftiofur (marker residue) in kidney (target tissue) of cattle has been established. A withdrawal period of zero days is required for this use of ceftiofur crystalline free acid sterile suspension (CCFA-SS) in cattle. Tolerances of 1 ppm and 2 ppm desfuroylceftiofur have been established in muscle and liver of cattle, respectively. A tolerance of 166 ppm desfuroylceftiofur has been established for the injection site of CCFA-SS in cattle."

Under section 512(c)(2)(F)(ii) 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 application contains investigations conducted or sponsored by the applicant that demonstrate animal safety and substantial evidence of effectiveness.

NAXCEL XT STERILE SUSPENSION is under the following U.S. patent number:

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

6. ATTACHMENTS:

Facsimile Labeling is attached as indicated below.

A. Vial Label (actual size, 100 mL)

B. Package Insert

C. Shipper Carton (actual size, 100 mL)