FREEDOM OF INFORMATION SUMMARY

Supplement to NADA 140-890

EXCENEL® Sterile Suspension
(ceftiofur hydrochloride injection)

"For the treatment of bovine respiratory disease (BRD) associated with *Pasteurella multocida, P. haemolytica,* and *Haemophilus somnus* and for the treatment of acute bovine interdigital necrobacillosis (foot rot) associated with *Fusobacterium necrophorum* and *Bacteroides melaninogenicus.*"

SPONSORED BY:

PHARMACIA & UPJOHN COMPANY

Date of Approval: July 26, 1998

I. GENERAL INFORMATION

NADA Number:	140-890
Sponsor:	Pharmacia & Upjohn Company 7000 Portage Rd. Kalamazoo, MI 49001
Established Name:	ceftiofur hydrochloride
Trade Name:	EXCENEL® Sterile Suspension*
Marketing Status:	This is a prescription product and will include the caution statement as follows: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.
Supplement Effect:	Provides for the use of an intramuscular or subcutaneous route of administration for EXCENEL® Sterile Suspension in cattle.

II. INDICATIONS FOR USE

EXCENEL® Sterile Suspension is indicated for the treatment of bovine respiratory disease (BRD) associated with *Pasteurella multocida, P. haemolytica,* and *Haemophilus somnus* and for the treatment of acute bovine interdigital necrobacillosis (foot rot) associated with *Fusobacterium necrophorum* and *Bacteroides melaninogenicus.*

*EXCENEL® Sterile Suspension contains the hydrochloride salt of ceftiofur, which is the active component of the approved product, NAXCEL® Sterile Powder (ceftiofur sodium). EXCENEL® Sterile Suspension is approved for use in swine. The dose range and clinical indications approved for NAXCEL® Sterile Powder in cattle are approved for EXCENEL® Sterile Suspension. Ceftiofur administered as either the sodium or the hydrochloride salt is rapidly metabolized to desfuroylceftiofur, the principal active moiety. The hydrochloride salt formulation is a terminally irradiated sterile suspension. The sodium salt formulation requires reconstitution prior to use.

Only data unique to the hydrochloride salt formulation or data which addresses residues in edible tissues, bioavailability, clinical efficacy, and tissue tolerance are included in this summary. Other relevant data are included in the EXCENEL® Sterile Suspension Freedom of Information (FOI) summary (NADA 140-890 for swine respiratory disease, April 1996), and in the NAXCEL® Sterile Powder FOI summary (NADA 140-338, approved in August 1992) for swine respiratory disease and in the NAXCEL® Sterile Powder FOI summary for bovine respiratory disease (NADA 140-338, initial approval in January 1988, with supplemental FOIs in January 1990, June 1992, and August 1993).

III. PRODUCT INFORMATION

A. *Dosage Form:* EXCENEL® Sterile Suspension is available in 100 mL glass vials. Each mL contains ceftiofur hydrochloride equivalent to 50 mg ceftiofur.

EXCENEL® Sterile Suspension should be stored at controlled room temperature (20 to 25° C or 68 to 77° F; see USP). Protect from freezing. Shake well before using.

B. *Route of Administration:* EXCENEL® Sterile Suspension should be administered by intramuscular (IM) or subcutaneous (SC) injection.

C. *Recommended Dosage:* EXCENEL® Sterile Suspension should be administered to cattle at a dose of 0.5 to 1.0 mg ceftiofur equivalents (CE)/lb (1.1 to 2.2 mg CE/kg) body weight (BW). Treatment regimes include: 1) Daily administration repeated at 24-h intervals for a total of three consecutive days. Additional treatments may be administered on days four and five to animals which do not show a satisfactory response (not recovered) after the initial three treatments, and 2) for BRD only, administer IM or SC 1.0 mg CE/lb (2.2 mg/kg) BW every other day on Days 1 and 3 (48-h interval). Do not inject more than 15 mL per IM injection site.

IV. EFFECTIVENESS

A. Dose Determination

Ceftiofur, administered as either the hydrochloride or sodium salt, is rapidly metabolized to desfuroylceftiofur, the primary metabolite and the principal active moiety for both salts. Ceftiofur sodium (NAXCEL® Sterile Powder, NADA 140-338) is approved for the treatment of BRD and acute bovine interdigital necrobacillosis (foot rot, pododermatitis). Based on the similar metabolism of the two salts, the dose for ceftiofur hydrochloride was based on a pharmacokinetic comparison of the two salts. The following studies characterized the concentration of ceftiofur and desfuroylceftiofur-related metabolites in plasma when ceftiofur hydrochloride was administered IM or SC at 1.0 mg CE/lb (2.2 mg CE/kg) BW compared to ceftiofur sodium administered IM at 1.0 mg CE/lb (2.2 mg CE/kg) BW. The results of these studies demonstrated that the administration of ceftiofur hydrochloride IM or SC to cattle provides similar plasma absorption and distribution profiles as the administration of an equivalent dose of ceftiofur sodium administered IM.

1. Plasma Disposition and Pharmacokinetics Study #1 (TR 788-7926-95-007).

Pharmacokinetic comparison of ceftiofur hydrochloride sterile suspension and ceftiofur sodium sterile powder administered once to cattle intramuscularly at a dose of 2.2 mg ceftiofur free acid equivalents/kg body weight. S.A. Brown, S.T. Chester, P.J. Hamlow, A.K. Speedy, V.L. Hubbard, J.K. Callahan, B.J. Hanson, T.S. Arnold, T.D. Cox, T.F. Flook, R.L. Janose, V.R. Lewis, and D.L. Kiefer.

a. Purpose: The purpose of this study was to compare the pharmacokinetics of ceftiofur and desfuroylceftiofur metabolites following a single IM injection of ceftiofur as either ceftiofur sodium or ceftiofur hydrochloride at 1.0 mg CE/lb (2.2 mg CE/kg) BW.

b. Investigators: S.A. Brown, S.T. Chester, P.J. Hamlow, A.K. Speedy, V.L. Hubbard, J.K. Callahan, B.J. Hanson, T.S. Arnold, T.D. Cox, T.F. Flook, R.L. Janose, V.R. Lewis, and D.L. Kiefer. Pharmacia & Upjohn Company, Kalamazoo, MI 49001.

c. General Design:

1) Experimental Animals: Fourteen crossbred beef cattle (7 heifers and 7 steers), weighing 267 to 292 kg BW at initial dose.

2) Dosage Form: Ceftiofur hydrochloride sterile suspension (50 mg CE/mL), ready to use formulation, clinical supplies lot #40583. Ceftiofur sodium sterile powder reconstituted with sterile water. This formulation (50 mg CE/mL) of ceftiofur sodium is the approved product NAXCEL® Sterile Powder for Injection (Lot #849KR).

3) Experimental Design: Cattle were randomly assigned to two groups of seven animals (Groups 1 and 2).

- Each animal received a single injection in each period.
- Group 1 received treatments in the following order: ceftiofur hydrochloride, ceftiofur sodium (samples not analyzed), ceftiofur sodium, ceftiofur sodium.
- Group 2 received treatments in the following order: ceftiofur sodium, ceftiofur hydrochloride (samples not analyzed), ceftiofur hydrochloride, ceftiofur hydrochloride.
- There was a 14-day washout period between periods.

4) Sampling: Blood samples were collected before dosing and at 20 and 40 minutes and 1, 1.5, 2, 3, 4, 8, 12, 16, 24, 36, 48, 60, 72, and 96 hours after treatment administration.

5) Assay Method: HPLC of ceftiofur and desfuroylceftiofur-related metabolites. LOQ is 0.15 mg CE/mL plasma. Each sample was analyzed as a single determination.

6) Pharmacokinetic Analysis Method: The main pharmacokinetic parameters were estimated using noncompartmental analysis. AUC values were calculated using the trapezoidal rule.

d. Results:

The 90% confidence interval of the difference in the $AUC_{0\text{-}LOQ}$ between ceftiofur hydrochloride and ceftiofur sodium was completely contained in the ± 20% of the mean $AUC_{0\text{-}LOQ}$ for ceftiofur sodium.

The upper end of the one-sided 95% confidence interval of the difference in C_{max} for ceftiofur hydrochloride and ceftiofur sodium was not greater than 10% of the mean C_{max} for ceftiofur sodium.

The lower end of the one-sided 95% confidence interval of the difference in $t_{>0.2}$ for ceftiofur hydrochloride and ceftiofur sodium was not less than 10% of the mean $t_{>0.2}$ for ceftiofur sodium.

The pharmacokinetic parameters (mean ± standard deviation) following IM administration of ceftiofur hydrochloride or ceftiofur sodium at a dose of 1.0 mg CE/lb (2.2 mg CE/kg) BW are presented in Table 4.1 and Figure 4.1.

Table 4.1. Pharmacokinetic parameters following IM administration of ceftiofur hydrochloride or ceftiofur sodium to cattle at 1.0 mg CE/lb (2.2 mg CE/kg) BW

Parameter	Ceftiofur HCl		Ceftiofur Na	
	Period 1	Combined	Period 1	Combined
C_{max} (µg/mL)	9.79 ± 1.20	11.0 ± 1.69	15.3 ± 3.99	16.5 ± 2.91
t_{max} (h)	1-4 (range)	1-4 (range)	0.33-2 (range)	0.33-2 (range)
$t_{>0.2}$ (h)	58.0 ± 6.45	60.5 ± 6.27	49.9 ± 5.36	50.9 ± 4.81
$AUC_{0\text{-}LOQ}$ (µg· h/mL)	134 ± 23.5	160 ± 30.7	125 ± 23.1	142 ± 25.4
$t_{1/2}$ (h)	13.3 ± 3.84	12.0 ± 2.63	9.68 ± 1.29	9.50 ± 1.15
$C_{24\,h}$ (µg/mL)				
Period 1	1.47 ± 0.380		1.16 ± 0.210	
Period 2	1.96 ± 0.300		1.35 ± 0.380	
Period 3	2.44 ± 0.641		1.34 ± 0.268	
$C_{48\,h}$ (µg/mL)				
Period 1	0.340 ± 0.110		0.250 ± 0.0700	
Period 2	0.400 ± 0.0900		0.270 ± 0.0900	
Period 3	0.502 ± 0.109		0.287 ± 0.0578	

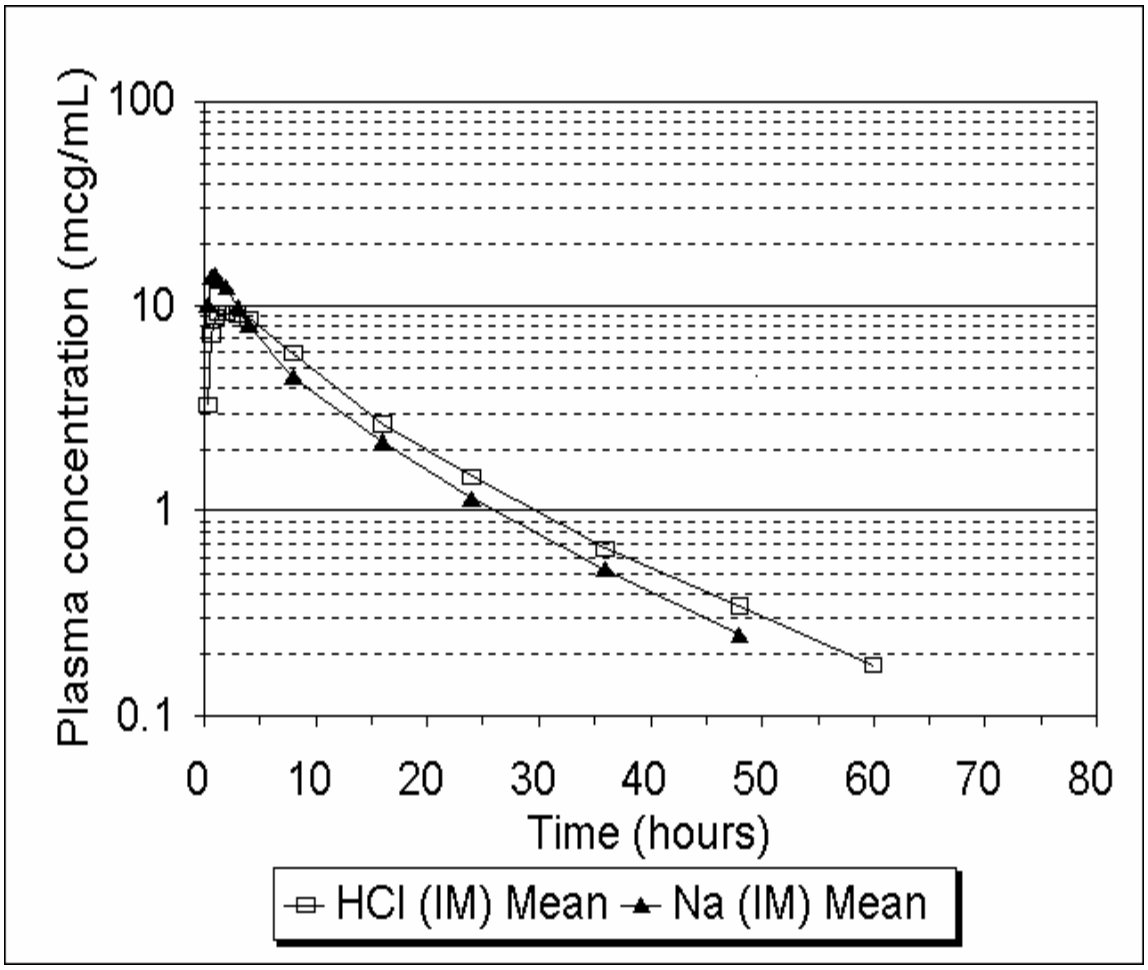

Figure 4.1. Mean plasma concentrations (mcg/mL) of ceftiofur and desfuroylceftiofur metabolites (measured as desfuroylceftiofur acetamide by the HPLC-DCA assay) after IM administration of ceftiofur hydrochloride or ceftiofur sodium to cattle at 1.0 mg CE/lb
(2.2 mg CE/kg) BW (data from Period 1 only)

e. Conclusions: Ceftiofur hydrochloride sterile suspension administered IM provides comparable bioavailability to ceftiofur sodium administered IM. This conclusion is based on the comparison of the $AUC_{0\text{-}LOQ}$, C_{max}, and $t_{>0.2}$. Ceftiofur hydrochloride provides similar plasma concentrations of ceftiofur and desfuroylceftiofur metabolites when administered at the approved dose range of ceftiofur sodium (0.5 to 1.0 mg CE/lb; 1.1 to 2.2 mg CE/kg BW).

2. Plasma Disposition and Pharmacokinetics Study #2: TR 788-7926-94-010.

   Pharmacokinetic comparison of ceftiofur and metabolites after ceftiofur hydrochloride (EXCENEL Ô Sterile Suspension) administered subcutaneously and ceftiofur sodium (NAXCEL® /EXCENEL Ô Sterile Powder) administered intramuscularly to cattle as a single dose of 2.2 mg ceftiofur free acid equivalents/kg body weight. S.A. Brown, P.J. Hamlow, A.K. Speedy, V.L. Hubbard, J.K. Callahan, T.S. Arnold, S.T. Chester, T.D. Cox, T.F. Flook, R.L. Janose, V.R. Lewis, D.L. Kiefer, B. Hibbard, and R.C. Faulstich.

   a. Purpose: The purpose of this study was to compare the pharmacokinetics of ceftiofur and desfuroylceftiofur metabolites following a single SC administration of ceftiofur hydrochloride and a single IM administration of ceftiofur sodium at 1.0 mg CE/lb (2.2 mg CE/kg) BW.

   b. Investigators: S.A. Brown, P.J. Hamlow, A.K. Speedy, V.L. Hubbard, J.K. Callahan, T.S. Arnold, S.T. Chester, T.D. Cox, T.F. Flook, R.L. Janose, V.R. Lewis, D.L. Kiefer, B. Hibbard, and R.C. Faulstich. Pharmacia & Upjohn Company, Kalamazoo, MI 49001.

   c. General Design:

      1) Experimental Animals: Sixteen Hereford steers, weighing 274 to 321 kg BW at initial dose.

      2) Dosage Form: Ceftiofur hydrochloride sterile suspension (50 mg CE/mL), ready to use formulation, clinical supplies lot #40583. Ceftiofur sodium sterile powder reconstituted with sterile water. This formulation (50 mg CE/mL) of ceftiofur sodium is the approved NAXCEL® Sterile Powder for Injection (Lot #438HT).

      3) Experimental Design: Cattle were randomly assigned to two groups of eight animals (Sequence 1 and Sequence 2).

         - Cattle in Sequence 1 were administered a single injection of ceftiofur sodium in the first period and a single injection of ceftiofur hydrochloride in the second period.

         - Cattle in Sequence 2 were administered a single injection of ceftiofur hydrochloride in the first period and a single injection of ceftiofur sodium in the second period.

         - There was a 14-day washout period between periods.

4) Sampling: Blood samples were collected before dosing and at 20 and 40 minutes and 1, 1.5, 2, 3, 5, 8, 12, 16, 24, 36, 48, 60, 72, and 96 hours after drug administration.

5) Assay Method: HPLC assay of ceftiofur and desfuroylceftiofur-related metabolites. LOQ is 0.15 µg CE/mL plasma. Each sample was analyzed as a single determination.

6) Pharmacokinetic Analysis Method: The main pharmacokinetic parameters were estimated using noncompartmental analysis. AUC values were calculated using the trapezoidal rule.

d. Results:

The upper end of the one-sided 90% confidence interval of the difference in C_{MAX} for ceftiofur hydrochloride and ceftiofur sodium was not greater than 10% of the mean C_{MAX} for ceftiofur sodium (upper end of the 90% confidence interval about difference in treatment means expressed relative to the ceftiofur sodium treatment group = 71%).

The lower limit of the one-sided 90% confidence interval of the difference in $t_{>0.2}$ for ceftiofur hydrochloride and ceftiofur sodium was not less than 10% of the mean $t_{>0.2}$ for ceftiofur sodium (lower end of the 90% confidence interval about the difference in treatment means expressed relative to the ceftiofur sodium treatment group = 92%).

The pharmacokinetic parameters (Mean ± standard deviation) after SC administration of ceftiofur hydrochloride or IM administration of ceftiofur sodium at 1.0 mg CE/lb (2.2 mg CE/kg) BW are presented in Table 4.2 and Figure 4.2.

Table 4.2. Pharmacokinetic parameters following SC administration of ceftiofur hydrochloride or IM administration of ceftiofur sodium to cattle at 1.0 mg CE/lb (2.2 mg CE/kg) BW

Parameter	Ceftiofur HCl	Ceftiofur Na
C_{max} (µg/mL)	8.56 ± 1.89	14.4 ± 3.11
t_{max} (h)	1 to 5 (range)	0.67 to 3 (range)
$t_{>0.2}$ (h)	51.0 ± 6.53	50.7 ± 9.27
$AUC_{0\text{-}LOQ}$ (µg·h/mL)	95.4 ± 17.8	115 ± 25.3
$t_{1/2}$ (h)	11.5 ± 2.57	11.1 ± 2.37
$C_{24\ h}$ (µg/mL)	0.926 ± 0.257	0.860 ± 0.329
$C_{48\ h}$ (µg/mL)	0.271 ± 0.086	0.268 ± 0.091

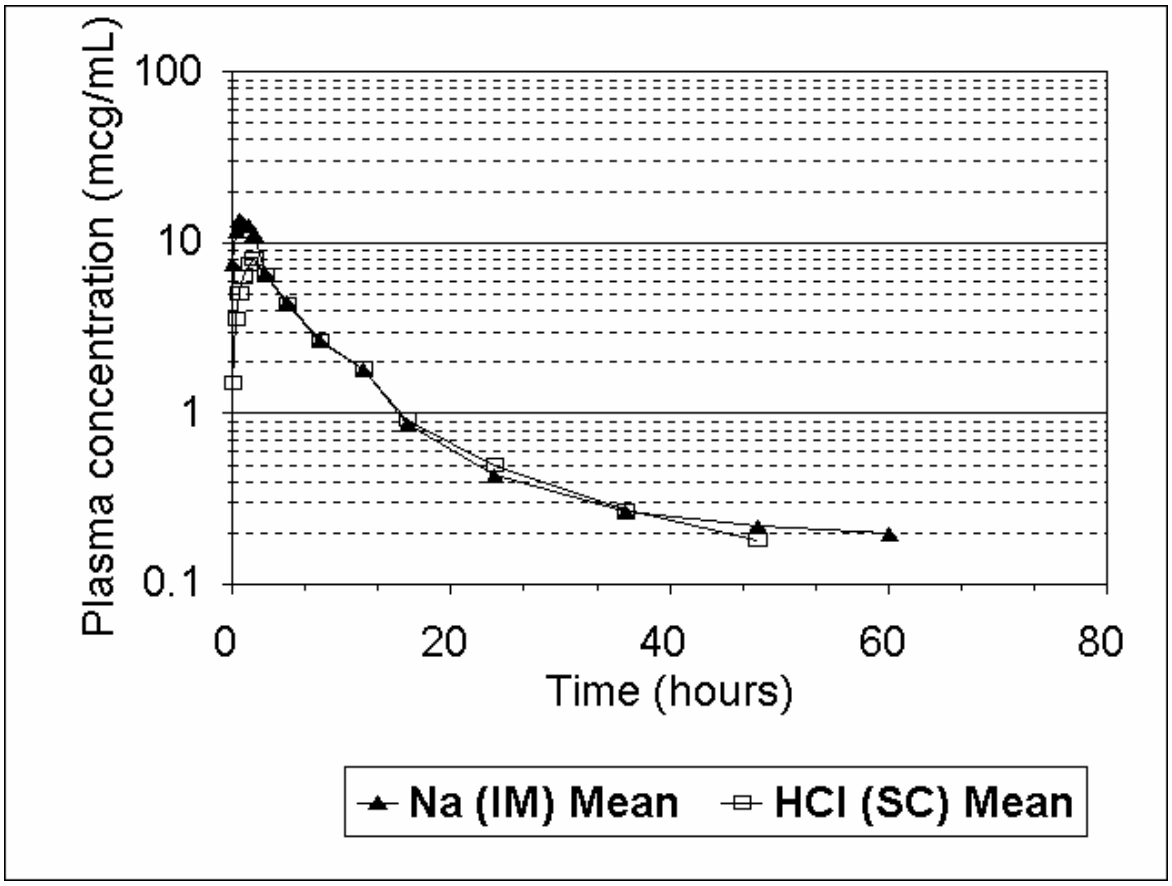

Figure 4.2. Mean plasma concentrations (mcg/mL) of ceftiofur and desfuroylceftiofur metabolites (measured as desfuroylceftiofur acetamide by the HPLC-DCA assay) after SC administration of ceftiofur hydrochloride or IM administration of ceftiofur sodium to cattle at 1.0 mg CE/lb (2.2 mg CE/kg) BW

e. Conclusions: In cattle, ceftiofur hydrochloride sterile suspension administered SC provides comparable plasma concentrations to ceftiofur sodium administered IM. This conclusion is based on the decision criteria defined in the protocol. Using these criteria, the C_{MAX} and $t_{>0.2}$ were determined to be therapeutically equivalent for the two salts. Therefore, equivalent doses of ceftiofur hydrochloride administered SC and ceftiofur sodium administered IM at the approved dose range for ceftiofur sodium (0.5 to 1.0 mg CE/lb; 1.1 to 2.2 mg CE/kg BW) provide similar plasma concentrations of ceftiofur and desfuroylceftiofur metabolites.

B. Dose Confirmation

Data from the following clinical field study (TR 788-9690-94-001) confirmed the efficacy of ceftiofur hydrochloride administered subcutaneously at 0.5 or 1.0 mg CE/lb (1.1 or 2.2 mg CE/kg) BW for for three consecutive days for the treatment of BRD.

Pivotal dose confirmation of U-64279A ceftiofur hydrochloride sterile suspension in bovine respiratory disease. B. Hibbard, E.J. Robb, S.T. Chester, K.J. Dame, L.L. Dinvald, and T.A. Jackson.

1. Purpose: The purpose of this study was to evaluate the clinical efficacy of ceftiofur hydrochloride administered daily at 0.5 or 1.0 mg CE/lb (1.1 or 2.2 mg CE/kg) BW for three consecutive days for the treatment of the bacterial component of naturally-occurring BRD.

2. Investigators: B. Hibbard, E.J. Robb, S.T. Chester, K.J. Dame, L.L. Dinvald and T.A. Jackson, Pharmacia & Upjohn Company, Kalamazoo, MI 49001, and T. TerHune and J. Davidson, Health Management Services, Tulare, CA 93274.

3. General Design

   a. Experimental Animals: Sixty male Holstein and Holstein-beef crossbred cattle (111 to 207 kg BW at assignment) were assigned to the study when uniform clinical signs of BRD were present. Twenty animals were assigned to each of the three treatment groups.

   b. Dosage Form: Ceftiofur hydrochloride sterile suspension (50 mg/mL), ready to use formulation, clinical supplies lot #40583.

   c. Negative Control: A placebo (vehicle, research supplies lot #V1478) was administered to all negative controls.

   d. Route of Administration: All treatments were administered by SC injection.

   e. Dosage: Placebo-treated negative controls were administered vehicle at 0.22 mL/kg BW. Calves in the ceftiofur hydrochloride treatment groups were administered ceftiofur hydrochloride at 0.5 or 1.0 mg CE/lb (1.1 or 2.2 mg CE/kg) BW. Treatments were administered daily for three consecutive days.

   f. Experimental Design: Randomized complete block design. When animals met the pre-defined inclusion criteria, they were ranked by descending rectal temperature (and body weight if necessary) and randomly assigned to one of the three treatment groups.

g. Microbiology: Prior to the administration of the first dose, a nasal swab was obtained from each animal assigned to the study, and tracheal aspirates were obtained from seven pre-selected blocks. These samples were cultured for the three primary BRD pathogens (*P. haemolytica, P. multocida* and, *H. somnus*).

h. Clinical Evaluations: Following assignment to the study (Day 1), the study veterinarian clinically evaluated all study animals daily through Day 15. The study veterinarian remained blinded to the assigned treatment groups throughout the study.

i. Necropsies: Animals that died during the study were necropsied by the study veterinarian. On Day 15, all surviving animals were euthanatized and necropsied, and the lung lesions were scored by the study pathologist.

j. Primary Decision Variables: The primary decision variables in this study were cumulative mortality through Day 15, rectal temperature 24 hours after the third treatment, and Day 15 calculated lung lesion score.

4. Results

The cultures from the nasal swabs and tracheal aspirates obtained at study entrance demonstrate that the study animals were exposed to the three primary BRD pathogens. The MIC for all *P. haemolytica* and *P. multocida* isolates was 0.12 µg/mL (the lowest dilution tested). The MIC for all *H. somnus* isolates was 0.3 µg/mL.

The cumulative mortality through Day 15, rectal temperature 24 hours after the third treatment, and Day 15 calculated lung lesion score are represented in Table 4.3.

Table 4.3. Mortality, rectal temperature 24-hr after third treatment, and Day 15 calculated lung lesion scores for negative control and ceftiofur hydrochloride administered SC to cattle at 0.5 or 1.0 mg CE/lb (1.1 or 2.2 mg CE/kg) BW for three consecutive days

Dose (mg/lb)	Mortality (%)	Rectal temp. 24 hours after third treatment (°F)	Day 15 calculated lung lesion scores (%)[1]
0	65	104.0	19.8
0.5	10[2]	103.1[3]	15.2
1.0	5[2]	102.8[3]	14.5

[1] There were no differences in Day 15 calculated lung lesion scores ($P > 0.05$).
[2] Different from negative control ($P < 0.0001$)
[3] Different from negative control ($P < 0.05$)

5. Conclusions: Ceftiofur hydrochloride administered SC at 0.5 or 1.0 mg CE/lb (1.1 or 2.2 mg CE/kg) BW daily for three consecutive days is an effective treatment for the bacterial component of naturally occurring BRD.

C. Every-Other-Day Administration for BRD

The following studies support the efficacy of ceftiofur hydrochloride when administered at a dose of 1.0 mg CE/lb (2.2 mg CE/kg) BW daily for three days, or every other day for two treatments, for the treatment of BRD.

A clinical field trial was conducted that evaluated the efficacy of ceftiofur hydrochloride when administered intramuscularly to cattle, daily for 3 consecutive days or every other day for two treatments, for the treatment of BRD. Since this clincal study was conducted with a different formulation of ceftiofur hydrochloride
(100 mg CE/mL) than that of the current product (50 mg CE/mL), data were provided to demonstrate that ceftiofur hydrochloride (the previous 100 mg CE/mL formulation) and ceftiofur sodium are bioequivalent (based on plasma concentrations of ceftiofur residues) and equally bioavailable (based on lung concentrations of ceftiofur residues).

As summarized previously, studies have demonstrated that the IM or SC administration of ceftiofur hydrochloride (50 mg CE/mL) to cattle provides similar plasma absorption and distribution profiles as the administration of an equivalent dose of ceftiofur sodium administered IM.

1. *A bioavailability and bioequivalence comparison of ceftiofur hydrochloride and sodium salts in the bovine.* (TR 788-9760-87-015) D.B. Johnson and D.D. Kratzer.

    a. Purpose: The purpose of this investigation was to determine if the Na and HCl salts are comparable with respect to lung and plasma total ceftiofur concentrations following five consecutive doses. By comparing the data generated in two independent residue studies, this technical report provides a critical pharmacokinetic bridge back to the original formulation of the HCl salt that was used to demonstrate the clinical effectiveness of a q48 hr dosage regimen.

    b. Investigators: D.B. Johnson and D.D. Kratzer, Pharmacia & Upjohn Company, Kalamazoo, MI 49001.

    c. General Design:

        1) Two independent total residue studies using ^{14}C-ceftiofur hydrochloride or ^{14}C-ceftiofur sodium were conducted to evaluate whole blood concentrations and lung tissue concentrations of ceftiofur in cattle.

2) Experimental Animals: Six calves (approximately 275 to 425 lbs) were used in each study.

3) Dosage Form: In the ^{14}C-ceftiofur hydrochloride study, the original ceftiofur hydrochloride formulation (100 mg CE/mL) was used. The ^{14}C-ceftiofur sodium study used a 50 mg CE/mL formulation.

4) Dosages: In each study, calves were administered ceftiofur at 1.0 mg CE/lb (2.2 mg CE/kg) BW daily for five consecutive days. All injections were administered IM.

5) Blood Samples: Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 16, and 24 hr after dosing.

6) Lung Samples: Calves were euthanatized 12 hours after the fifth dose in the ceftiofur hydrochloride study and 8 hours after the fifth dose in the ceftiofur sodium study.

7) Measurements: Comparable drug availability was confirmed on the basis of the area under the whole blood concentration vs. time profile and by the comparability of total ceftiofur concentrations in the lung.

d. Results: The lung concentrations and plasma AUC are presented in Table 4.4.

Table 4.4. ^{14}C-ceftiofur concentrations in lung and whole blood of cattle following intramuscular administration of ^{14}C-ceftiofur hydrochloride or ^{14}C-ceftiofur sodium at 1.0 mg CE/lb (2.2 mg CE/kg) BW daily for five consecutive days.

AUC	Ceftiofur		Ratio	Confidence Interval	
(µg·h/mL)	HCL	Na	(HCl/Na)	Lower	Upper
0-24 hours	42.9	51	0.84	69	99
24-48 hours	52.1	53.9	0.97	84	110
48-72 hours	58.6	58.7	1.00	83	116
72-96 hours	60	57.9	1.04	89	118
Lung Conc. (ppm)	1.15	1.18	1.02	86	118

The Day 5 AUC following administration of ceftiofur hydrochloride was demonstrated to be bioequivalent to the Day 5 AUC values following administration of ceftiofur sodium.

Based upon the 90% confidence interval about the difference in treatment means, the total exposure of lung tissue to ceftiofur and its desfuroylceftiofur metabolites is determined to be equivalent following repeated IM administrations of identical doses of ceftiofur hydrochloride and ceftiofur sodium.

e. Conclusions: Data from the two multiple-day studies demonstrate that the extent of ceftiofur absorption resulting from repeated IM injections of ceftiofur hydrochloride and ceftiofur sodium are comparable and will result in equivalent total systemic exposure to ceftiofur and its desfuroylceftiofur metabolites.

2. *Ceftiofur hydrochloride vs. ceftiofur sodium for the treatment of bovine respiratory disease.* (TR 788-9690-86-002) G.G. Stocking, D.D. Kratzer, F.B. Tesar and J.B. Paulissen.

a. Purpose: This study was designed to evaluate the efficacy of the original formulation of ceftiofur hydrochloride (100 mg CE/mL) for the treatment of the bacterial component of naturally occurring BRD.

b. Investigators: G.G. Stocking, D.D. Kratzer, F.B. Tesar, and J.B. Paulissen, Pharmacia & Upjohn Company, Kalamazoo, MI 49001; D.T. Bechtol, Canyon, TX; A.J. Edwards, Manhattan, KS; and D. Horton, Wellington, CO.

c. General Design

1) Experimental Animals: A total of 360 crossbred feeder cattle (350 to 450 lbs) were assigned to the study when uniform clinical signs of BRD were present.

2) Dosage Form: The original 100 mg CE/mL formulation of ceftiofur hydrochloride was used in this study (lot #40,418). The ceftiofur sodium formulation contained 50 mg CE/mL (lot #40,395).

3) Dosages: Calves were randomly assigned to one of the following treatment groups:

- Negative control
- Ceftiofur sodium at 0.5 mg CE/lb (1.1 mg CE/kg) BW daily for three days
- Ceftiofur hydrochloride at 1.0 mg CE/lb (2.2 mg CE/kg) BW daily for three days
- Ceftiofur hydrochloride at 1.0 mg CE/lb (2.2 mg CE/kg) BW on Days 1 and 3 only (every other day)
- Two additional ceftiofur hydrochloride treatment groups (2.0 mg CE/lb (4.4 mg/kg dose)) were also included in this study.

All treatments were administered by IM injection.

4) Clinical Evaluations: Cattle were evaluated on Days 1 to 4, 10, and 28. Temperature and body weight were also measured on the same days.

5) The primary decision variable was rectal temperature reduction from Day 1 to Day 4.

6) Ancillary variables included Day 4 rectal temperature and Day 28 clinical success.

d. Results

Table 4.5. Clinical parameters in cattle following intramuscular administration of ceftiofur hydrochloride (100 mg/mL formulation) or ceftiofur sodium for the treatment of BRD

Treatment group	Rectal Temp. Reduction, Day 1 to Day 4 (°F)	Day 4 Rectal Temp. (°F)	Day 28 Clinical Success (%)
Negative control	1.12	104.3	63
Ceftiofur Na (0.5 mg/lb)	2.65	102.9	67
Ceftiofur HCL (1.0 mg/lb; 3 days)	2.63	102.8	77
Ceftiofur HCL (1.0 mg/lb; Days 1 and 3)	2.54	102.9	80

e. Conclusions: This study confirmed the clinical effectiveness of IM administration of ceftiofur hydrochloride for the treatment of the bacterial component of BRD. Since the original formulation of ceftiofur hydrochloride is bioequivalent to ceftiofur sodium and the revised formulation has equal relative plasma bioavailability to ceftiofur sodium, the clinical conclusions obtained in this clinical study are relevant to the revised formulation of ceftiofur hydrochloride.

D. *In vitro* Activity Studies

The following studies were considered as supportive information regarding the *in vitro* susceptibility of bovine bacterial pathogens to ceftiofur.

1. *A 4-year survey of antimicrobial susceptibility trends for isolates from cattle with bovine respiratory disease in North America.* J.L. Watts, R.J. Yancey, Jr., S.A. Salmon, and C.A. Case. *J. Clin. Microbiol.* 32(3): 725-731, 1994.

a. Purpose: This study reports the susceptibility of clinical isolates to ceftiofur.

b. Investigators: J.L. Watts, R.J. Yancey, Jr., S.A. Salmon, and C.A. Case, Pharmacia & Upjohn Company, Kalamazoo, MI 49001.

c. General Design

1) Bacteria: A four-year study (1988-1992) was conducted to examine the MIC for ceftiofur against the three primary BRD pathogens, *P. haemolytica* (n=461), *P. multocida* (n=318), and *H. somnus* (n=109). The isolates were obtained from US and Canadian veterinary diagnostic laboratories, with the majority of isolates obtained from the lungs of animals that died from BRD. In addition to the clinical isolates, the following American Type Culture Collection (ATCC) isolates were included as quality control strains, as recommended by the National Committee on Clinical Laboratory Standards (NCCLS): *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853. In addition, *P. haemolytica* ATCC 43137, *P. multocida* ATCC 33396, and *H. somnus* ATCC 4326 were included in most assays.

2) Antibiotics: Ceftiofur sodium and other commercially available antibiotics

3) Minimum Inhibitory Concentrations: All *Pasteurella* spp. isolates were tested using NCCLS microdilution broth method. *H. somnus* isolates were tested using the agar dilution method.

d. Results: The results are presented in Table 4.6.

Table 4.6. Summary of ceftiofur MIC data from a 4-year (1988 to 1992) survey of BRD pathogens isolated from cattle in the US and Canada.

Organism	Number of isolates	MIC (µg /mL) 90%	MIC (µg /mL) Range	Percent Susceptible[a]
P. haemolytica	461	≤ 0.06	≤ 0.03-0.13	100
P. multocida	318	≤ 0.06	≤ 0.03-0.25	100
H. somnus	109	≤ 0.06	≤ 0.03-0.13	100

[a]Based on the NCCLS guideline which categorizes isolates with MIC of ≤ 2 µg/mL as susceptible.

e. Conclusions: The data demonstrate that the three primary BRD pathogens are susceptible to ceftiofur.

2. *Minimum inhibitory concentrations for ceftiofur and desfuroylceftiofur with isolates of veterinary importance.* S.A. Salmon, J.L. Watts, R.J. Yancey, Jr., and C.A. Case. (TR 705-7923-93-007)

   a. Purpose: This study reports the susceptibility of *H. somnus* clinical isolates to ceftiofur and desfuroylceftiofur.

   b. Investigators: S.A. Salmon, J.L. Watts, R.J. Yancey, Jr., and C.A. Case, Pharmacia & Upjohn Company, Kalamazoo, MI 49001.

   c. General Design:

      1) Bacteria: *H. somnus* isolates (n=59) from the four-year survey were tested. In addition, the following ATTC isolates were included as quality control organisms: *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853.

      2) Antibiotics: Ceftiofur and desfuroylceftiofur were tested.

      3) Minimum Inhibitory Concentrations: All isolates were tested using the NCCLS microdilution broth method. Mueller-Hinton broth (Sensititre, Westlake, OH) was used as the growth medium. Due to the instability of desfuroylceftiofur, all microdilution broth panels containing this compound were prepared and inoculated on the same day.

   d. Results: Results are presented in Table 4.7.

      Table 4.7. MIC data for ceftiofur and desfuroylceftiofur for *Haemophilus somnus* isolates (n=59)

	Ceftiofur	Desfuroylceftiofur
MIC_{90} (µg/mL)	≤ 0.0019	≤ 0.0019
MIC Range	No Range[1]	No Range[1]

[1]All isolates yielded the same MIC value

   e. Conclusions: The *H. somnus* isolates tested in this study were equally sensitive to ceftiofur and desfuroylceftiofur, its primary metabolite.

3. *Minimum inhibitory concentrations for ceftiofur and desfuroylceftiofur with bacterial isolates from porcine and bovine sources.* S.A. Salmon, J.L. Watts, R.J. Yancey, Jr., C.A. Case, A.R. Cazers, and C.L. Gatchell. (TR 705-7923-93-010)

a. Purpose: This study reports the susceptibility of *P. haemolytica* and *P. multocida* clinical isolates to ceftiofur and desfuroylceftiofur.

b. Investigators: S.A. Salmon, J.L. Watts, R.J. Yancey, Jr., C.A. Case, A.R. Cazers, and C.L. Gatchell, Pharmacia & Upjohn Company, Kalamazoo, MI 49001.

c. General Design

1) Bacteria: *P. haemolytica* (n=42) and *P. multocida* (n=48) isolates from the four-year survey were tested. In addition, the following ATTC isolates were included as quality control organisms: *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853.

2) Antibiotics: Ceftiofur and desfuroylceftiofur were tested.

3) Minimum Inhibitory Concentrations: All isolates were tested using the NCCLS microdilution broth method. Mueller-Hinton broth (Sensititre, Westlake, OH) was used as the growth medium. Due to the instability of desfuroylceftiofur, all microdilution broth panels containing this compound were prepared and inoculated on the same day.

d. Results: The results are presented in Table 4.8.

Table 4.8. MIC data for ceftiofur and desfuroylceftiofur for BRD pathogens

Organism	No. of isolates	Drug	MIC (µg /mL)	
			90%	Range
P. haemolytica	42	Ceftiofur	0.015	≤ 0.003-0.03
P. haemolytica	42	Desfuroylceftiofur	0.015	≤ 0.003-0.03
P. multocida	48	Ceftiofur	≤ 0.003	≤ 0.003-0.015
P. multocida	48	Desfuroylceftiofur	0.0078	≤ 0.003-0.0078

e. Conclusions: The *P. haemolytica* and *P. multocida* isolates tested were sensitive to both ceftiofur and its primary metabolite, desfuroylceftiofur.

V. ANIMAL SAFETY

A. Systemic Safety of Ceftiofur Hydrochloride

As previously discussed, ceftiofur hydrochloride administered either SC or IM to cattle at doses of 0.5 to 1.0 mg CE/lb (1.1 to 2.2 mg CE/kg) BW demonstrated equal relative plasma bioavailability when compared to an equivalent dose of ceftiofur sodium administered IM. The safety of ceftiofur sodium has been demonstrated in studies discussed in the FOI Summary for NAXCEL® Sterile Powder (ceftiofur sodium), NADA 140-338.

In addition to the previously submitted studies with the sodium salt solution, two injection site irritation studies were conducted using the ceftiofur hydrochloride salt suspension in oil.

B. Injection Site Tolerance

1. Injection Site Tissue Tolerance to Intramuscular Administration of Ceftiofur Hydrochloride. TR 7219-96-017.

U-64279A: Single-dose Injection Site Irritation Study of Ceftiofur Hydrochloride in Cattle When Administered by Intramuscular Injection Four Times Varying From 12 Hr to 60 Days Before Necropsy. W.J. Seaman and J.M. Marcek

a. Purpose: To define tissue tolerance and resolution following IM administration of EXCENEL® Sterile Suspension.

b. Investigators: W.J. Seaman and J.M. Marcek, Pharmacia & Upjohn Company, Kalamazoo, MI 49001

c. General Design:

1) Animals: Three groups of four castrated male Holstein cattle were used in this study. The animals had a body weight range of 380 to 592 pounds at initial dosing.

2) Dosage Form and Route of Administration: Each animal received an IM injection of ceftiofur hydrochloride (EXCENEL® Sterile Suspension, 50 mg CE/mL; Lot 40,597) in the neck and another IM injection in the rear leg on each of four treatment administration days. A sterile, 16-gauge, 1 inch needle was used to administer each injection. Different neck and rear leg injection sites were used for each injection day.

3) Dosage: 1.0 mg CE/lb (2.2 mg CE/kg) BW/injection was administered for each injection. The total dose received by each animal each dosing day was 2.0 mg CE/lb (4.4 mg/kg)/day.

4) Pertinent Parameters Measured: Daily clinical observations included injection site evaluations. Body weights were obtained before the start of the study and before necropsy. Injection sites were excised and examined grossly at necropsy on the following days post-injection:

Group 1: 60, 28, 24, 19

Group 2: 15, 13, 11, 9

Group 3: 7, 5, 3, 1

d. Results:

1) Clinical Observations: Visual and/or palpable tissue changes in response to injections in neck sites were observed in 4.1% of the animals (2/48 sites), whereas tissue changes in reponse to injections in rear leg sites were observed in 43.7% of the animals (21/48 sites).

2) Gross Injection Site Observations: Following intramuscular administration, areas of discoloration associated with the neck injection sites persisted through 11 days post-injection; whereas, areas of discoloration associated with the rear leg injection sites persisted through 28 days post-injection.

e. Conclusion: Evaluations made in this study suggest that intramuscular injection in the neck results in less tissue reaction than intramuscular injection in the rear leg. Intramuscular administration of ceftiofur hydrochloride produces grossly apparent blemishes at the injection site that may result in trim loss of edible tissue at slaughter.

2. Injection Site Tissue Tolerance to Subcutaneous Administration of Ceftiofur Hydrochloride. TR 7227-95-026.

U-64279A: Injection Site Tolerance Study of Ceftiofur Hydrochloride Sterile Suspension in Cattle When Administered Subcutaneously for 5 Days. T.A. Jackson, D.M. Brussee, and J.J. Cypher.

a. Purpose: To define tissue tolerance and resolution following SC administration of EXCENEL® Sterile Suspension.

b. Investigators: T.A. Jackson, D.M. Brussee, and J.J. Cypher, Pharmacia & Upjohn Company, Kalamazoo, MI 49001.

c. General Design:

1) Animals: Four groups of four mixed breed beef steers were used in this study. The animals had a body weight range of 450 to 550 pounds at initial dosing.

2) Dosage Form and Route of Administration: Ceftiofur hydrochloride (EXCENEL® Sterile Suspension, 50 mg/mL; Lot 40,584) was administered SC using a 16-gauge needle once daily for five consecutive days. The injections were administered in the right loin, right posterior neck, right anterior neck, left anterior neck, or left posterior neck.

3) Dosage: Animals received a single dose of 0.5 or 1.0 mg CE/lb (1.1 or 2.2 mg CE/kg) BW on each dosing day. Groups were dosed at the following times before necropsy:

Groups 1 and 2: 0.5, 1.5, 2.5, 3.5, and 4.5 days

Groups 3 and 4: 5.5, 6.5, 7.5, 8.5, and 9.5 days

4) Pertinent Parameters Measured: Daily clinical observations included injection site evaluations. Injection sites were excised and examined grossly at necropsy.

d. Results:

1) Clinical Observations: Both dose levels resulted in no indications of pain and only mild, and usually transient, visible tissue changes at the injection site. Palpable thickening was limited, but lasted somewhat longer than the visible reactions.

2) Gross Injection Site Observations: Dissection of injection sites indicated limited increase in thickness and color changes of the subcutaneous tissue and/or fascial surface of adjacent muscle, but the underlying muscle mass was not affected. The fascial surface of the muscle was visibly affected at time points out to and including 3.5 days after injection and, in most cases, through 9.5 days after injection.

e. Conclusion: Subcutaneous administration of ceftiofur hydrochloride produces grossly apparent blemishes at the injection that may result in trim loss of edible tissue at slaughter. There were no apparent differences in tissue response to administration of either dose.

3. Summary of Injection Site Irritation Data

Following administration in the neck (IM or SC), areas of discoloration associated with the injection site may persist beyond 11 days, resulting in trim loss of edible tissues at slaughter. Following IM administration in the rear leg, areas of discoloration associated

with the injection site may persist beyond 28 days resulting in trim loss of edible tissues at slaughter.

VI. HUMAN SAFETY

A. Toxicity and Comparative Metabolism (Salts)

The toxicity testing of ceftiofur has been summarized in previous FOI Summaries for NAXCEL® (ceftiofur sodium) Sterile Powder (NADA 140-338). Additional information concerning the toxicity testing of ceftiofur, as ceftiofur hydrochloride, is provided in the FOI Summary for the original approval of EXCENEL® (ceftiofur hydrochloride) Sterile Suspension (NADA 140-890) for use in swine. This FOI summarized two additional studies, one assessed the acute toxicity of ceftiofur hydrochloride, and the other demonstrated equal oral bioavailability of both salts in the rat.

B. Calculation of Safe Concentrations

Derivation of the following is in the above-referenced FOI Summaries for NADAs 140-338 and 140-890.

- The lowest No Observed Effect Level (NOEL) from the 90-day oral feeding studies in both dogs and rats is 30 mg/kg body weight (BW).
- The Allowable Daily Intake (ADI) is 0.03 mg/kg BW/day or 1.8 mg/day.
- The ADI for milk is 0.008 mg/kg BW/day and the ADI for edible tissues is 0.022 mg/kg BW/day.
- Ceftiofur is a Category A compound, which will be used in a manner consistent with a low-use drug. Accordingly, a Safety Factor of 1000 has been used in the Safe Concentration calculations.

Using the revised food consumption factors, the permitted Safe Concentrations for total residues of ceftiofur in edible tissues determined are presented in Table 6.1.

Table 6.1. Consumption factors and ceftiofur Safe Concentrations for edible tissues

Tissue	Daily Consumption (g)	Safe Concentration (mg/kg)
Muscle (non-injection)	300	4.40
Liver	100	13.2
Kidney	50	26.4
Fat	50	26.4
Milk	1500 (1.5 L)	0.320

C. Total Residue Depletion and Metabolism Study:

1. Purpose: This pivotal residue study was conducted to determine total residues in edible tissues, plasma, urine and feces following the IM administration of ceftiofur hydrochloride to cattle. Metabolites were characterized when sufficient radioactivity was present in the edible tissue samples, and in plasma and urine.

2. Study Director: Maria G. Beconi-Barker, Ph.D.
Animal Health Drug Metabolism, Unit 7926
Pharmacia & Upjohn, Kalamazoo, MI 49001

3. General Design:

   a. Test animals: Six mixed-breed cattle (3 steers/3 heifers) weighing approximately 236 to 287 kg were used.

   b. Dosage form, dosage, and route of administration: 14C-labeled ceftiofur hydrochloride suspension was administered at 1.11 mg CE/lb BW (2.45 mg/kg). Five IM injections were administered at 24-hr intervals.

   c. Assay: HPLC-RAM.

4. Results - Total Residues (based on total radioactivity):

Table 6.2. Total tissue residues (ppm) 12 hours after the last of 5 daily IM injections of ^{14}C-ceftiofur hydrochloride administered at 1.11 mg CE/lb BW (adjusted to 2.45 mg/kg). Data are reported as mean (sd).

Tissue	Safe Concentration (ppm)	Concentration (ppm)
Muscle	4.4	0.24 (0.06)
Liver	13.2	1.52 (0.23)
Kidney	26.4	7.37 (1.04)
Fat	26.4	0.41 (0.05)
Injection Site 5 (12 hr)	—	29.93 (12.93)
Injection Site 4 (36 hr)	—	9.50 (2.27)
Injection Site 3 (60 hr)	—	5.49 (2.94)
Injection Site 2 (84 hr)	—	6.16 (4.42)
Injection Site 1 (108 hr)	—	1.41 (0.84)

5. Conclusions for Total Residues in Edible Tissues**:** In the animals administered the 1.11X the maximum approved dose, total ceftiofur residues in all edible tissues other

than injection sites were less than half of the Safe Concentrations 12 hours after the last treatment.

6. Results - Metabolism: The metabolite profiles in cattle parenterally administered ceftiofur sodium or ceftiofur hydrochloride were compared.

   a. Plasma: For cattle administered either ceftiofur hydrochloride or ceftiofur sodium, desfuroylceftiofur (DFC)-cysteine was the only free plasma ^{14}C-metabolite. The remaining ^{14}C-residues were associated with macromolecules. Following incubation of the macromolecule-associated fraction with dithioerythritol (DTE), DFC was the only ^{14}C-residue released.

   b. Urine: DFC-dimer was the most abundant initial metabolite in the urine of cattle administered ceftiofur hydrochloride representing 38.0, 35.4, 31.3, 29.5, and 38.3 % of the total radioactivity in urine collected from the initial dose (+24 h) to 12 h after the fifth dose (+108 h) respectively (values represent 24-h collection periods). DFC-cysteine was the second most abundant metabolite initially in the urine of cattle administered ceftiofur hydrochloride, representing 31.1, 35.4, 35.9, 39.9, and 31.4 % of the total radioactivity in urine collected from the initial dose (+24 h) to 12 h after the fifth dose (+108 h) respectively. Parent ceftiofur represented 4.2 to 6.4 %. of the total radioactivity in urine collected. Minor components included parent ceftiofur and polar metabolites. DFC-cysteine, DFC-dimer, parent ceftiofur and polar metabolites. The type and proportion of ceftiofur-related residues are summarized in Table 6.3.

   Table 6.3. ^{14}C-residues found in the urine of cattle following intramuscular injection of ceftiofur HCl at 2.45 mg ceftiofur equivalents/kg BW daily for 5 days. Values correspond to the percentage of ^{14}C-components that represented, on the average, more than 5% of the radioactivity eluting from the HPLC.

	mean percent (s.d) of total eluting from the HPLC			
sample time[a]	polar and unknowns	DFC-cysteine	DFC-dimer	ceftiofur
24	21.2	31.1 (9.4)	38.0 (15.2)	4.6 (2.1)
48	20.7	35.4 (9.5)	35.4 (14.3)	4.2 (2.3)
72	22.9	35.9 (10.7)	31.3 (13.2)	5.4 (2.1)
96	14.5	39.9 (10.4)	29.5 (12.3)	4.4 (1.8)
108[b]	13.2	31.4 (8.9)	38.3 (12.3)	6.4 (2.5)

[a]Hours after first treatment; doses occurred at 0, 24, 48, 72 and 96 h;
[b]Collections 10 to 12 h after last treatment.

c. Tissues: In cattle administered ceftiofur hydrochloride, an average of 3.17, 0.56, 0.16, and 0.09 mg ceftiofur equivalents/g tissue were extracted from kidney, liver, muscle, and fat, respectively, using the extracting conditions previously used for rat tissues. These values represent 43.1, 37.2, 38.3, and 39.9% of the concentration of ceftiofur-related residues found by combustion, respectively.

1) Kidney: In cattle administered ceftiofur hydrochloride, an average of 88.9% of the total residue was macromolecule associated and 11.1% free. DFC-cysteine was the only free metabolite observed. In cattle administered ceftiofur sodium, the majority of the metabolites were determined to be bound to macromolecules and treatment of these samples with DTE yielded entirely DFC indicating that it was the primary metabolite.

2) Liver, Fat and Muscle: DFC-cysteine was the only radiolabeled metabolite found in liver and fat in the macromolecule-free fraction. Treatment with DTE of the macromolecule-associated fraction generated DFC. The concentration of free radiolabeled ceftiofur and related residues was insufficient to conduct metabolic profiles in muscle.

3) Overall: When cattle were administered either ceftiofur sodium or ceftiofur hydrochloride intramuscularly, the majority of the dose was excreted via urine (>55%), followed by feces (approximately 30%). Dose recoveries within urine and within feces were similar among doses and between salts. The distribution of the drug in tissues followed the same pattern regardless of the ceftiofur salt or of the dose level administered. ^{14}C-residue concentrations in these tissues were similar for both salts. The type of metabolites present in the urine of cattle was similar for both ceftiofur salts.

7. Conclusions**:** Quantitative and qualitative assessments of residue levels and profiles in bovine tissues, plasma, and urine demonstrate the similarity in metabolism of ceftiofur administered as either ceftiofur Na or ceftiofur HCl.

D. Comparative Metabolism of Ceftiofur in Cattle and Rats

Information concerning the toxicity testing of ceftiofur as ceftiofur hydrochloride is addressed in the FOI Summary for EXCENEL© (ceftiofur hydrochloride) Sterile Suspension (NADA 140-890) for use in swine. In that summary, the metabolic profiles of ceftiofur in the urine, kidney extracts, and plasma of rats treated orally with ^{14}C-labeled ceftiofur sodium at 700 mg ceftiofur equivalents(CE)/kg BW were compared to the urine, kidney extracts, and plasma of swine. This supplement provides a similar comparison to cattle dosed with ceftiofur hydrochloride intramuscularly for 5 days with 2.45 mg CE/kg BW and the metabolic profiles in urine of rats treated orally with ^{14}C-labeled ceftiofur sodium and ceftiofur hydrochloride at 200 mg CE/kg BW.

The toxicological bridge established between cattle and the rat for NAXCEL® Sterile Powder (NADA 140-338) was based on the contention that rats treated orally with ceftiofur sodium are autoexposed to the same metabolites found in the ceftiofur sodium-treated cattle. By extension, rats treated with ceftiofur sodium are autoexposed to the metabolites found in ceftiofur hydrochloride-treated cattle.

1. Urine: The most abundant metabolite in the 8- and 24-h urine sample from rats after oral dosing with ceftiofur sodium at a dose of 700 mg CE/kg BW was ceftiofur sulfoxide cysteine ester (31 and 37%, respectively). Other significant metabolites were polar devoid of a ß-lactam ring (27 and 24% in the 8- and 24-h samples, respectively) and DFC-dimer (11 and 10% in the 8- and 24-h samples, respectively). Parent ceftiofur and DFC-cysteine were found as minor components (<5%). Twelve hours after oral dosing of rats with either ceftiofur sodium or ceftiofur hydrochloride at 200 mg CE/kg BW, the most abundant metabolite was also ceftiofur sulfoxide cysteine ester (39 and 36%, respectively). Other significant metabolites were polar devoid of a ß-lactam ring (26 and 11%, respectively), desfuroylceftiofur (15 and 35%, respectively), and DFC-cysteine (13 and 9%, respectively). Parent ceftiofur and DFC-dimer were found as minor components (<5%). The most abundant metabolite in cattle urine collected from the time the last dose was administered until the animals were euthanatized 12 h later was DFC-dimer (38%). DFC-cysteine (31%), parent ceftiofur (6%) and polar metabolites (2%) were components of cattle urine collected during this period. The ceftiofur sulfoxide cysteine ester metabolite found in rat urine was not observed in cattle urine.

   All of the metabolites found in ceftiofur hydrochloride-treated cattle urine were found in ceftiofur sodium and ceftiofur hydrochloride-treated rat urine for a complete qualitative match. Quantitatively, cattle tended to produce DFC-dimer and DFC-cysteine in larger proportions than did rats.

2. Kidney: In cattle, approximately 75% of the kidney metabolites were conjugated to macromolecules, with the remainder free. DFC-cysteine was the predominant component of the fraction not conjugated to macromolecules. In rats, 63.9 to 70.2% of the kidney metabolites were found bound to macromolecules, with the remainder free.

   In both species, ceftiofur-related residues were predominantly found as macromolecule conjugates. The rat kidney extracts contained all the metabolites found in bovine kidney extracts plus additional metabolites not found in the bovine kidney extracts.

3. Plasma: On average, 90.0 to 94.2% of the ceftiofur-related residues found were conjugated in cattle plasma. DFC-cysteine was the only metabolite detected not conjugated to macromolecules. In rat plasma, approximately 72.3% of the ceftiofur-related residues found were conjugated with macromolecules, with the remainder free.

In both species, ceftiofur-related residues were predominantly found as macromolecule conjugates. Rat plasma contained all the metabolites found in bovine plasma plus additional metabolites not found in bovine plasma.

4. Conclusions: These data demonstrate that the rat was auto-exposed to metabolites to which humans would be exposed as a result of eating tissues from ceftiofur-treated cattle. Therefore, the toxicology studies in the rat accurately reflect the toxicity of the metabolites to which humans would be exposed.

E. Tissue Residues Depletion Study

1. Purpose: This study was designed to measure concentrations of ceftiofur and related metabolites using a non-radiolabeled HPLC-DCA assay in tissues of cattle 12-hr after the last of five daily subcutaneous injections of ceftiofur hydrochloride administered at the highest approved dosage.

2. Study Director: Scott A. Brown D.V.M., Ph.D.
Animal Health Drug Metabolism, Unit 7926
Pharmacia & Upjohn, Kalamazoo, Michigan 49001

The animal phase of this study took place at: Southwest Bio-Labs, Rancho Brazito, Mesilla Park, New Mexico.

3. General Design:

a. Test animals: Fourteen Hereford-cross cattle (7 steers, 7 heifers; 305 to 455 kg body weight) were used in this study. Two cattle served as untreated controls that were euthanatized along with the other cattle to obtain drug-naive tissues for assay purposes. Twelve cattle were in a single treatment group and randomly allotted by sex and weight into 2 separate blocks such that each block had an equal number of males and females. All cattle were humanely slaughtered approximately 12 hours after the last injection.

b. Dosage form and route of administration: Ceftiofur hydrochloride sterile suspension (50 mg/mL) was administered as a subcutaneous injection in the neck region. Injections 1, 3, and 4 were administered on the left side of the neck, and Injections 2 and 5 were administered on the right side of the neck.

c. Dosage: Each animal received 5 daily injections of 1.0 mg CE/lb (2.2 mg/kg) BW.

d. Pertinent parameters measured: Concentrations of ceftiofur and related metabolites were determined by HPLC-DCA assay in liver, kidney, and skeletal muscle (non-injection site) collected 12 h after the last dose and in the subcutaneous injection site

at 12 and 36 h after last injection. The total weight of injection site and underlying muscle removed was approximately 500 grams.

f. Results: No clinical signs of irritation (except for local transient swelling at injection sites) were observed during the course of the study. Tissue residue concentrations and concentrations of ceftiofur and desfuroylceftiofur metabolites for the subcutaneous injections site are provided in Table 6.4.

Table 6.4. Mean concentrations of ceftiofur and desfuroylceftiofur metabolites 12 and 36 h (inj. site only) after the last of 5 daily subcutaneous injections of ceftiofur hydrochloride administered at 1.0 mg CE/lb BW (2.2 mg/kg)

	Mean (s.d.) Concentration (ppm)			
Sample Time (hr)	Liver	Kidney	Muscle (non-injection)	Muscle Injection Site
12	0.990 (0.254)	4.06 (0.605)	0.204 (0.044)	96.3 (70.9)
36	-	-	-	7.86 (10.4)

g. Conclusions:

1) Kidney: Of the edible tissues, residues are highest in kidney, the target tissue. Mean kidney residues are approximately half the tolerance (see VI. G.) at 12 hours withdrawal.

2) Subcutaneous Injection Site: Residues at the subcutaneous injection site are high and extremely variable. At 12 hours withdrawal, drug levels at the injection site are unsafe and do not support a zero withdrawal (see VI. H.).

F. Milk Residue Decline Studies

1. Background: Metabolism studies conducted in the target species and rat demonstrated that ceftiofur, regardless of whether it is administered as the hydrochloride or the sodium salt, is rapidly metabolized to desfuroylceftiofur (DFC), the microbiologically active metabolite. Identical types of metabolites are found in the animal after administration of either salt of ceftiofur. Milk is an ultrafiltrate of plasma and all ceftiofur metabolites in milk resulting from the pareteral administration of ceftiofur are derived from plasma. The metabolites in blood provided to the udder are identical for either ceftiofur salt. Similarly assay methodology and relationships between marker and total residues are valid for both ceftiofur salts.

a. Residue foundation: The approval of ceftiofur sodium for use in lactating dairy cattle with no milk discard was based on total (radiolabeled) studies conducted at 2.2 mg CE/kg BW. This study demonstrated that residue concentration in milk following

administration of ceftiofur sodium during 5 days of treatment and for 120 h after the fifth treatment were significantly below the established Safe Concentration (lactating cattle supplement to NADA 140-338).

b. Analytical methodology: Since the metabolites detected after administration of either ceftiofur salt are identical, the same assay methodology used (for ceftiofur sodium) for determination and quantitation of "marker residues", is appropriate for determination of residue concentrations in milk after the administration of ceftiofur hydrochloride. Based on the indistinguishable metabolism between both salts, the conversion factor from "marker residue" to total residues derived for ceftiofur sodium is valid for ceftiofur hydrochloride.

c. Assays: Milk residue concentrations were re-calculated from the pivotal ceftiofur sodium ^{14}C study using the HPLC-DCA assay. Values initially determined using HPLC-DCA-IS assay, an assay employing an internal standard, were reevaluated using an HPLC-DCA-RE assay, which does not utilize an internal standard.

A conversion factor of 1.34 is accepted for estimating total residues [^{14}C-ceftiofur equivalents mg/kg (mg/L)] from concentrations obtained with the HPLC-DCA-RE assay for milk in cattle, sheep, and goats.

2. Pivotal residue study in lactating dairy cattle after ceftiofur hydrochloride administrations.

a. Purpose: This study was conducted to determine residues in milk of lactating cows after intramuscular and subcutaneous administration of ceftiofur hydrochloride. The residue concentrations were determined using the HPLC-DCA assay. Milk samples were also tested with common screening assays.

b. Objective: This pivotal study was designed to determine the concentrations of ceftiofur-related residues containing an intact ß-lactam ring, and the level of microbiologically-active and receptor-recognized residues in milk from individual cows collected at approximately 0, 12, and 24 h after the first and approximately 12, 24, 36, and 48 h after the last of 5 daily injections of ceftiofur hydrochloride sterile suspension administered intramuscularly at 1.0 mg CE/lb (2.2 mg/kg) BW. Additional treatment groups include ceftiofur hydrochloride sterile suspension administered subcutaneously at 0.45 mg CE/lb (1.0 mg/kg) BW or ceftiofur sodium administered intramuscularly at 0.45 mg CE/lb (1.0 mg/kg) BW.

c. Study Director: Edward Robb, D.V.M. M.S.
Worldwide Animal Health
Clinical Research and Product Development Unit 9690
Pharmacia and Upjohn., Kalamazoo, MI 49001

The animal phase of this study took place at Lockshore Farms, Richland, Michigan.

The analytical phase was conducted at Pharmacia and Upjohn, Kalamazoo, Michigan.

d. Test animals: Forty-eight (48) lactating Holstein cows with a weight range of 533 to 718 kg (1175 to 1584 lbs), not exceeding 270 days in lactation and a somatic cell count of < 600,000 cells/mL were used. Within blocks, animals were randomly assigned to one of the three treatments.

e. Dosage form and route of administration: Ceftiofur hydrochloride sterile suspension (50 mg/mL) was administered subcutaneously and intramuscularly, respectively. Ceftiofur sodium sterile powder (50 mg/mL) was administered intramuscularly. Intramuscular injections were administered in the semitendonosus muscle, and subcutaneous injections were administered in the cervical neck (pre-scapular). Injections were administered on alternating sides of the animal (right or left) from day to day.

f. Dosage: Each cow received five daily injections, at approximately 24-h intervals, of ceftiofur hydrochloride at 1.0 mg CE/lb (2.2 mg/ kg) BW administered intramuscularly or 0.45 mg CE/lb (1.0 mg/kg) BW administered subcutaneously or ceftiofur sodium at 0.45 mg CE/lb (1.0 mg/kg) BW administered intramuscularly.

g. Test duration: The cows were treated for a total of 5 consecutive days at 24-h intervals. Milk samples were collected at 12-h intervals for up to 36 h following the last treatment.

h. Pertinent parameters measured: The HPLC-DCA-IS assay was used for the determination and quantification of ϑ-lactam containing ceftiofur-residues (LOQ = 50 µg/L). Data obtained from the HPLC-DCA-IS assay was re-calculated using the HPLC-DCA-RE criteria (LOQ = 56 µg/L). The following milk screening tests were used: Bacillus stearothermophilus Disc Assay (BSDA-PMO), Idexx SNAP™ ϑ-lactam test, Gist brocades Delvotest® P MINI (Delvotest P), Charm-II® Tablet Beta-lactam Test (Competitive Assay) (Charm-II), Charm Bacillus stearothermophilus Disc Assay (Charm-BSDA), Idetek LacTek™ B-L (beta-lactam), Idetek LacTek™ CEF (ceftiofur) and the BR Blue Star Test (Brilliant Black Reduction Test.

i. Results:

1) HPLC - Only results from the ceftiofur hydrochloride 1.0 mg CE/lb (2.2 mg/kg) BW intramuscular treatment group are reported in this summary. Results are summarized in Table 6.5.

Table 6.5. Summary of Milk Assay/Screen Results: Ceftiofur hydrochloride 1.0 mg CE/lb (2.2 mg/ kg) BW intramuscular, for 5 days

	Assay Results HPLC-DCA-RE		Screening Results No. of PositivesTest/Total Tests		
Sample Time Pre- and Post-Treatment (days + hours)	No. >LOQ Total No.	Mean±SD (µg/L)	Idexx SNAP™ ß-Lactam Test	BSDA-Charm II Charm BSDA Idetek B-L Idetek CEF	BR Blue Star Delvotest-P
Pretreatment	0/16	NA	0/16	0/8	0/16
Day 1 + 12	1/16	58	4/16	0/8	0/16
Day 1 + 24	0/16	NA	0/16	0/8	0/16
Day 5 + 12	7/16	63.4± 9.0	12/16	0/8	0/16
Day 5 + 24	0/16	NA	1/16	0/8	0/16
Day 5 + 36	0/16	NA	0/16	0/8	0/16

2) Screening tests: The results for the BSDA-PMO, Delvotest-P, Charm Test II, Charm BSDA, LacTek ß-lactam and LacTek CEF Assays, Idexx SNAP Test and the BR Blue Star Tests are all negative except for the Idexx SNAP Test. The Idexx SNAP Beta-Lactam Test was positive for individual composite milk samples during treatment and for up to 24 hours post-treatment.

j. Conclusion: After intramuscular administration of ceftiofur hydrochloride to lactating dairy cattle at 1.0 mg CE/lb (2.2 mg/kg) BW, residue concentrations of ceftiofur-related metabolites containing an intact ß-lactam ring were below 56 µg/L (LOQ of the RE assay) in most instances. Exceptions were one sample at 12 h after the first dose (58 µg/L) and approximately 50% of the samples at 12 h after the last dose (concentrations ranging between 58 and 82 µg/L). The highest daily mean concentration was 63.4 µg/L on Day 5 +12 h; the highest observed individual sample concentration was 82.0 µg/L using the HPLC-DCA-RE recalculation procedures. When these values are multiplied by the milk total adjustment factor of 1.34, total residue concentrations are 85.0 (mean) and 109.8 µg/mL (individual), respectively. Therefore, all values observed are less than one half of the revised ceftiofur milk Safe Concentration of 320 µg/L.

G. Tolerances

At the present time, ceftiofur Na is approved for use in cattle, swine, day-old chickens, day-old turkey poults and sheep with a zero withdrawal. Ceftiofur HCl is approved for use in swine via the intramuscular route of administration with a zero withdrawal. Tolerances were not required for these approvals and there has been no official analytical method for the determination of residues of ceftiofur either in edible tissues or in milk.

Due to the unsafe injection site residues following subcutaneous administration of ceftiofur in cattle, ceftiofur HCl cannot be approved for use in cattle with a zero-day withdrawal. Therefore, to protect the public health, tolerances will be established for cattle residues in kidney, liver, and muscle.

The tolerance for the marker residue, desfuroylceftiofur, in the target tissue, kidney, is 8 ppm. Thus, when residues of the marker residue in kidney tissue have depleted to less than 8 ppm, residues in the four principal edible tissues will have depleted to below their safe concentration.

A tolerance of 2 ppm is established for residues of desfuroylceftiofur in liver, and a tolerance of 1 ppm is established for residues of desfuroylceftiofur in muscle. Since neither liver nor muscle is the target tissue, tolerances for desfuroylceftiofur residues in these tissues will serve to monitor residues in liver and muscle tissue, respectively, and cannot be applied to other tissues.

A tolerance of 100 ppb is established for residues of desfuroylceftiofur, the marker residue, in milk.

H. Withdrawal Time

Due to unsafe levels of drug at the injection site, a two (2) day withdrawal period is established for the use of ceftiofur HCl in cattle by BOTH the subcutaneous and intramuscular routes of administration.

I. Regulatory Method

The official regulatory analytical method for residues of desfuroylceftiofur in tissues is the HPLC-DCA-BF method. The official analytical method for residues of desfuroylceftiofur in milk is the HPLC-DCA-RE assay.

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 Sterile Powder).

VII. AGENCY CONCLUSIONS

The data submitted in support of this supplemental NADA comply with the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR 514 of the implementing regulations. The data demonstrate that EXCENEL® (ceftiofur hydrochloride) Sterile Suspension when administered as intramuscular or subcutaneous injection to cattle, is safe and effective for the treatment of bovine respiratory disease (BRD) associated with *Pasteurella multocida*, *P. haemolytica*, and *Haemophilus somnus*, and for the treatment of acute bovine interdigital necrobacillosis (foot rot) associated with *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*.

Tolerances are established for ceftiofur residues in kidney, liver, and muscle. The tolerance for the marker residue, desfuroylceftiofur, in the target tissue, kidney, is 8 ppm. The tolerances for residues of desfuroylceftiofur in liver and muscle are established at 2 ppm and 1 ppm, respectively. The official regulatory analytical method for residues of desfuroylceftiofur in tissues is the HPLC-DCA-BF method. A tolerance of 100 ppb is established for residues of desfuroylceftiofur, the marker residue, in milk. The official analytical method for residues of desfuroylceftiofur in milk is the HPLC-DCA-RE assay.

Due to the unsafe levels of drug at the injection site, a two (2) day withdrawal period is established for the use of ceftiofur HCl in cattle by both the subcutaneous and intramuscular routes of administration.

The product remains a prescription drug for safe and effective use by or on the order of a licensed veterinarian.

In accordance with 21 CFR 514.106(b)(2)(vii), this is a Category II change which did not require a reevaluation of the human food and target animal safety data in the parent application.

The agency has carefully considered the potential environmental effects of this action and has determined under 21 CFR 25.33(d)(5) that this action is of a type that does not individually or cumulatively have a significant impact on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

Under Section 512 (c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act, this approval for food producing animals qualifies for THREE years of marketing exclusivity beginning on the date of approval because the supplemental application contains substantial evidence of the effectiveness of the drug involved, any studies of animal safety, or, in the case of food producing animals, human food safety studies (other than bioequivalence or residue studies) required for the approval of the supplement and conducted or sponsored by the applicant. The THREE years of marketing exclusivity applies only to the indications in the new species (cattle) for which the supplemental application is approved.

EXCENEL® Sterile Suspension is under patent number U.S. 4,902,683 expiring February 20, 2007.

VIII. APPROVED PRODUCT LABELING

A copy of the facsimile labeling, including the package insert, is attached to this document.