Date of Approval: February 9, 2005

# FREEDOM OF INFORMATION SUMMARY

## ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-238

SPECTRAMAST LC Sterile Suspension
(ceftiofur hydrochloride)

"For the treatment of clinical mastitis in lactating dairy cattle associated with coagulase-negative staphylococci, *Streptococcus dysgalactiae*, and *Escherichia coli*."

Sponsored by:
Pharmacia & Upjohn Co.,
A Division of Pfizer, Inc.

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## *1. GENERAL INFORMATION:*

| | |
|---|---|
| a. File Number: | NADA 141-238 |
| b. Sponsor: | Pharmacia & Upjohn Co.<br>A Division of Pfizer, Inc.<br>235 East 42d St.<br>New York, NY 10017<br><br>Drug Labeler Code: 000009 |
| c. Established Name: | Ceftiofur hydrochloride |
| d. Proprietary Name: | SPECTRAMAST LC Sterile Suspension |
| e. Dosage Form: | Sterile oil suspension |
| f. How Supplied: | 10 mL plastic syringes (PLASTETS) with cannula |
| g. How Dispensed: | Rx |
| h. Amount of Active Ingredient: | Each PLASTET contains ceftiofur hydrochloride equivalent to 125.0 mg ceftiofur (12.5 mg/mL). |
| i. Route of Administration: | Intramammary infusion |
| j. Species/Class: | Bovine/lactating dairy cattle |
| k. Recommended Dosage: | Infuse one (1) syringe into each affected quarter. Repeat this treatment in 24 hours. For extended duration therapy, once daily treatment may be repeated for up to 8 consecutive days. |
| l. Pharmacological Category: | Antimicrobial |
| m. Indications: | SPECTRAMAST LC Sterile Suspension (ceftiofur hydrochloride) is indicated for the treatment of clinical mastitis in lactating dairy cattle associated with coagulase-negative staphylococci, *Streptococcus dysgalactiae*, and *Escherichia coli*. Cows with systemic clinical signs caused by mastitis should receive other appropriate therapy under the direction of a licensed veterinarian. |

## *2. EFFECTIVENESS:*

### *a. Dosage Characterization:*

The following dose characterization study was conducted using a non-final (peanut oil based) formulation of ceftiofur hydrochloride. The study enabled the selection of a dose to be used in the dose confirmation (field) study, which was conducted using the final cottonseed oil formulation of ceftiofur hydrochloride.

**"Evaluation of Ceftiofur Free Acid, Provided as Ceftiofur Hydrochloride, During an Intramammary Dose-Response Efficacy Study in Lactating Dairy Cows with Clinical Mastitis." Report No. 794-9690-89-001.**

The objective of this study was to evaluate the effectiveness of intramammary administered ceftiofur, as a peanut oil based formulation, for the treatment of clinical mastitis in lactating dairy cows. The investigators were T. Fuhrmann (Tempe, AZ), N. Jennett (Higley, AZ), S. Smalley (Chandler, AZ), W. Guterbock (Ontario, CA), Drs. Harmon & Doherty (Chino, CA), D. Braay (Gainesville, FL), D. Hardin (Mississippi State, MS), V. Anspaugh (Clovis, NM), and J. Reed (Dublin, TX). A total of 619 cows from 38 herds were enrolled. Cows in their second or greater lactation, with an elevated somatic cell count (SCC) and either a positive milk culture for a mastitis pathogen or signs of clinical mastitis were assigned to one of four treatment groups – 0 mg, 50 mg, 100 mg, and 200 mg of ceftiofur per quarter. Cows were infused twice at a 24-hour interval. A quarter was considered cured if there were no clinical signs of mastitis, visibly normal milk, and a negative milk culture 11 days post-treatment. Using these criteria, cure rates were 25.7% (0 mg), 43.5% (50 mg), 41.4% (100 mg), and 41.1% (200 mg).

The results generated using alternate definitions of enrollment (clinical infection) and cure (return to normal milk and no clinical signs 11 days post-treatment) were analyzed using the Walker-Carmer procedure to compute the optimal dosage. The optimal dosage was determined to be 119 mg of ceftiofur. A dosage of 125 mg of ceftiofur (provided as ceftiofur HCl in an oil vehicle) per 10 mL syringe administered twice by intramammary infusion at a 24-hour interval was selected as the dosage regimen for further testing.

### *b. Substantial Evidence:*

1. **"Field Dose Confirmation for Ceftiofur for the Treatment of Clinical Mastitis". Trial No. 794-9690-O-JWH-98-001.**

   a. Type of Study: A thirteen-location field study using lactating dairy cows with clinical mastitis.

b. Investigators:

| Investigator | Address |
|---|---|
| Dr. David Tomsche | Melrose-Albany-Upsala Vet Associates, Melrose, MN |
| Dr. Jim Bennett | Northern Valley Animal Clinic, Plainview, MN |
| Dr. Walt Guterbock | Sandy Ridge Dairy, LLC, Scotts, MI |
| Dr. Andy Johnson | Total Herd Management Services, Inc., Seymore, WI |
| Dr. Pam Ruegg | Dept. of Dairy Science, Madison, WI |
| Dr. Niles Jennett | Dairy Veterinary Services, Chandler, AZ |
| Dr. John Kirk | UC Davis, VMTRC, Tulare, CA |
| Dr. Jerry Roberson | VMRCVM-LACS, Blacksburg, VA |
| Dr. Leslie De Groff | Perry Veterinary Clinic, Perry, NY |
| Dr. Ron Harrison | Professional Veterinary Research, Brownstown, IN |
| Dr. Jim Cullor | Milk Quality Laboratory, UC Davis, VMTRC, Tulare, CA |
| Dr. Ken Leslie (2 sites) | University of Guelph, Guelph, Ontario, Canada |

c. Study Design:

1) *Objective:* To evaluate the effectiveness of intramammary infusion of ceftiofur hydrochloride sterile suspension twice at a 24-hour interval at 62.5 mg or 125.0 mg dosage in the affected quarter in comparison to a negative control for the treatment of clinical mastitis in lactating dairy cattle.

2) *Animals:* A total of 13 trial sites enrolled 352 cows in the study. Of these, 207 (58.8%) were first or second lactation animals with the remaining 145 (41.2%) cows being in their third or higher lactation. Cows were enrolled in the study when they had visually abnormal milk (clots, flakes, or watery secretion) or if udder swelling, heat, pain or redness were present and milk was not yet visually abnormal, but the California Mastitis Test (CMT) gave results of 2 or greater. A pre-treatment milk sample was obtained from each affected quarter(s) and cultured for the presence of organisms associated with mastitis; the culture data were not available prior to treatment assignments.

3) *Experimental Design:* Cows were grouped by parity, with first and second parity animals forming one group, and third or higher forming a second group. Within parity group, the cows were assigned in blocks of three to receive either 62.5 mg ceftiofur HCl, 125 mg ceftiofur HCl, or no treatment. Enrollment was restricted to cows with one quarter affected with clinical mastitis.

4) *Test Article Administration:* SPECTRAMAST LC Sterile Suspension (either 62.5 mg or 125 mg ceftiofur) was administered as an intramammary infusion twice at a 24-hour interval beginning on the day of enrollment (Day 0). Cows assigned to the control group were left untreated.

5) *Measurements and Observations:* Visual evaluations of milk and udders were conducted daily through 24 days after enrollment. Milk was cultured on 14 and 21 days post-treatment. Quantitative somatic cell counts (SCC) were determined on Day 0 and 14 and 21 days post-treatment.

   Three different definitions of cure were used for analysis purposes: (a) a clinical cure was defined as the milk and udder returning to normal 14 days after the last treatment and remaining normal at 21 days post-treatment; (b) a bacterial cure was defined as the absence of the pre-treatment pathogen at 14 and 21 days post-treatment; and (c) a protocol cure was defined as the absence of the pre-treatment pathogen at 14 and 21 days post-treatment and normal udder and milk evaluations at 14 and 21 days post-treatment.

d. Results: Analysis was conducted using 337 cows for clinical, bacterial, and protocol cure rates. Fifteen cows were not included in the analyses due to incorrect treatment administration. The purpose of the study was to determine the effective dose that is significantly better than control at the one-sided 5% level. The primary endpoint was the clinical cure, bacterial cure, and protocol cure rates. The proportion of cows cured within each herd was transformed using the Freeman-Tukey transformation, and a weighted ANOVA was performed with these values (with weights n+½, where n=number of cows within each herd x treatment group). The results are shown in Table 2.1.

**Table 2.1. Results of analysis of cure rates[1]**

| Type of cure | Treatment | Cure rate | P-value[2] |
|---|---|---|---|
| Clinical cure[3] | 0 mg | 64/117 = 54.7% | -- |
| | 62.5 mg | 75/108 = 69.4% | 0.041 |
| | 125 mg | 88/112 = 78.6% | 0.002 |
| Bacterial cure[4] | 0 mg | 19/46 = 41.3% | -- |
| | 62.5 mg | 21/46 = 45.6% | 0.356 |
| | 125 mg | 38/54 = 70.4% | 0.006 |
| Protocol cure[5] | 0 mg | 11/46 = 23.9% | -- |
| | 62.5 mg | 19/46 = 41.3% | 0.068 |
| | 125 mg | 34/54 = 63.0% | 0.001 |

[1]The quarter cure results were analyzed using PROC MIXED from SAS Version 6.12 following an angular (Freeman-Tukey) transformation of the proportion of quarters cured within each herd x treatment group. Treatment was a fixed effect, while herd was a random effect. A weighted analysis was conducted with weights n+½, n is the number of quarters within the herd x treatment group.
[2]One-sided pairwise comparison of the 62.5 mg and 125 mg doses with the negative control (0 mg). The test was associated with 23 degrees of freedom for the revised protocol and bacterial cure analyses, and with 24 degrees of freedom for the other cure three analyses.

[3]A quarter was cured if it was a clinical cure. The variance component estimates for the analysis were 0.0 and 1.598 for herd and residual, respectively.
[4]A quarter was cured if the pathogen isolated at treatment was not present in either post-treatment sample. The variance component estimates for the analysis were 0.027 and 1.333 for herd and residual, respectively.
[5]A quarter was cured if it was both a clinical and microbiologic cure. The variance component estimates for the analysis were 0.0 and 1.429 for herd and residual, respectively.

The results show that there were statistically significant improvements in clinical, bacterial, and protocol cure rates in the 125 mg dose treatment group compared with the non-treated control group. A clinically relevant number of coagulase-negative staphylococci (33), *Streptococcus dysgalactiae* (32), and *Escherichia coli* (35) isolates were obtained from cows in the study.

e. Adverse Reactions: There were no drug related adverse reactions reported in this study.

f. Conclusions: Based on clinical, bacterial, and protocol cure rates of the study results, the final cottonseed oil sterile suspension of ceftiofur hydrochloride administered twice at a 24-hour interval by intramammary infusion at the 125 mg dose was effective in the treatment of clinical mastitis in lactating dairy cows associated with coagulase-negative staphylococci, *Streptococcus dysgalactiae*, and *Escherichia coli*.

***c. Microbiology:***

The minimum inhibitory concentration (MIC) of ceftiofur was determined *in vitro* for isolates obtained from cows with intramammary infection in the United States during clinical trials and from diagnostic laboratories in the U.S. and Canada. Susceptibility testing was conducted at the Pharmacia & Upjohn Company laboratory according to the methods described by the National Committee for Clinical Laboratory Standards (NCCLS). The MIC data for clinical isolates and isolates from diagnostic laboratories are presented in Tables 2.2 and 2.3, respectively. Appropriate reference strains were included in each testing; MICs were determined by the standardized dilution technique with ceftiofur sodium standard reference powder and zone diameters were determined by the disk diffusion technique with a 30 µg disk. The MIC values and zone diameters of the reference strains are presented in Table 2.4.

**Table 2.2. Ceftiofur MIC values for isolates from field studies evaluating clinical mastitis in dairy cows in the U.S. during 2000**

| Organism | No. | $MIC_{90}$* (µg/mL) | MIC range (µg/mL) |
|---|---|---|---|
| Coagulase-negative staphylococci | 33 | 1.0 | ≤0.06 to 2.0 |
| *Streptococcus dysgalactiae* | 32 | ≤0.06 | ≤0.06 to 0.5 |
| *Escherichia coli* | 35 | 0.5 | ≤0.06 to 1.0 |

*The minimum inhibitory concentration for 90% of the isolates.

**Table 2.3. Ceftiofur MIC values* for mastitis pathogens from diagnostic laboratories in the U.S. and Canada**

| Organism | No. | Date isolated | $MIC_{90}$** (µg/mL) | MIC range (µg/mL) |
|---|---|---|---|---|
| *Staphylococcus aureus* | 135 | 1991-1992 | 1.0 | 0.13 to 2.0 |
| | 10 | 1993 | 1.0 | 0.25 to 1.0 |
| | 107 | 1995 | 1.0 | 0.25 to 2.0 |
| | 61 | 2000 | 1.0 | ≤ 0.06 to 2.0 |
| Coagulase (-) staphylococci | 139 | 2000-2001 | 1.0 | ≤ 0.06 to 2.0 |
| *Streptococcus dysgalactiae* | 15 | 1991-1992 | 1.0 | ≤ 0.06 to 2.0 |
| | 15 | 1993 | ≤ 0.0039 | No range† |
| | 152 | 1997-1999 | 0.25 | 0.25 to 4.0 |
| | 64 | 2000 | ≤ 0.06 | ≤ 0.06 to 0.5 |
| *Streptococcus uberis* | 22 | 1991-1992 | 0.5 | ≤ 0.06 to 4.0 |
| | 15 | 1993 | 0.03 | ≤ 0.0039 to 0.06 |
| | 133 | 1997-1999 | 0.5 | 0.5-8.0 |
| | 20 | 2000 | 1.0 | < 0.06 to 2.0 |
| *Escherichia coli* | 39 | 1991-1992 | 1.0 | 0.25 to 1.0 |
| | 40 | 1993 | 0.5 | 0.13 to 1.0 |
| | 52 | 2000 | 0.5 | ≤ 0.06 to 1.0 |

* The above *in vitro* data are available, but their clinical significance is unknown.
** The minimum inhibitory concentration for 90% of the isolates.
† No range, all isolates yielded the same value.

**Table 2.4. Acceptable quality control ranges for ceftiofur against National Committee for Clinical Laboratory Standards recommended American Type Culture Collection (ATCC) reference strains**

| Organism (ATCC No.) | Zone diameter (mm) (disk content 30 µg/mL) | MIC range (µg/mL) |
|---|---|---|
| *Escherichia coli* (25922) | 26 to 31 | 0.25 to 1.0 |
| *Staphylococcus aureus* (29213) | -- | 0.25 to 1.0 |
| *Staphylococcus aureus* (25923) | 27 to 31 | -- |
| *Pseudomonas aeruginosa* (27853) | 14 to 18 | 16.0 to 64.0 |

## *3. TARGET ANIMAL SAFETY:*

**Milk Residue Decline and Udder Irritation in Lactating Dairy Cows Following Intramammary Infusion of a Sterile Formulation of Ceftiofur HCl (PNU-64279A) Containing 125 mg of Ceftiofur Free Acid Equivalents per 10 mL Plastet Into All Four Quarters. Part 1-Udder Irritation. Pharmacia & Upjohn Study Report No. a0098213.**

a. Type of Study: combined pivotal milk residue decline and target animal safety study

b. Study Director: Jeffrey L. Watts, Ph.D., R & D Discovery Biology, Pharmacia & Upjohn Company, a Division of Pfizer Inc, Kalamazoo, MI.

c. Study Design:

1) *Objective:* To evaluate the safety of intramammary infusion of 125 mg ceftiofur hydrochloride sterile cottonseed oil suspension in each of four quarters daily for either two days or eight consecutive days with an approximate 24-hour interval between infusions.

2) *Animals:* Forty lactating female Holstein dairy cattle ranging from 2 to 5 years of age and representing low milk producers (13.2 kg to 24.0 kg/day) and high milk producers (25.0 kg to 31.9 kg/day) were used in the study. Cows were enrolled based on the absence of mastitis pathogens, absence of clinical mastitis (normal strip cup and udder palpation), a somatic cell count (SCC) less than or equal to 200,000 per mL, and no edema or teat lesions.

3) *Experimental Design:* Within each of the lactation/production subgroups ($1^{st}$ lactation high producers, $1^{st}$ lactation low producers, $\geq$ $2^{nd}$ lactation high producers, or $\geq$ $2^{nd}$ lactation low producers), cows were assigned to receive either two doses or eight doses of ceftiofur by intramammary infusion. There were no non-treated controls.

4) *Study Schedule:* Following a 3-day pre-treatment clinical observation and milk sample collection period, animals received their assigned treatment. Measurements and observations continued during the treatment period and through Day 12 (two dose group) or Day 21 (eight dose group).

5) *Test Article Administration:* Ceftiofur hydrochloride sterile cottonseed oil suspension (125 mg) was administered as an intramammary infusion into all four quarters twice at a 24-hour interval (two dose group) or once daily for eight consecutive days (eight dose group).

6) *Measurements and Observations:* The following clinical and production measures were evaluated for a total of either 24 milkings (two dose group) or

42 milkings (eight dose group): appearance of foremilk (strip cup evaluation), physical condition of udder, somatic cell count (SCC), milk production, rectal temperature, and general health.

d. Results: Individual udder palpation and strip cup scores were normal during the 3-day pre-treatment period. The mean SCC ranged from 1,000/mL to 69,000/mL in the two dose group, and 2,000/mL to 20,000/mL in the eight dose group during the pre-treatment period. None of the animals in either the two dose or the eight dose group showed any abnormality in their general health or body temperature during the pre-treatment period.

The SCC of the two dose group showed a transient increase following intramammary infusion, with a post-treatment least square mean SCC of 34,300, which was well below the level of concern (200,000 SCC/mL). The SCC levels of one animal rose over 200,000 and remained elevated during the 14-day study period. Culture results from this animal showed several contaminated milk samples and one sample positive for *Pseudomonas fluorescens*.

The SCC values for the eight dose group during treatment also showed a transient increase following intramammary infusion. The post-treatment least square mean SCC value was 59,800, which was well below the level of concern (200,000 SCC/mL). Five animals had elevated mean SCC values (> 100,000) relative to the rest of the animals in the eight dose group. Examination of the culture data for all five of these animals showed the presence of *Mycoplasma* organisms. In addition, the milk cultures of three of these animals were positive for a fungus and one was positive for a *Corynebacterium* spp.

There were no overall differences in udder physical condition scores, strip cup analyses, body temperature, or milk production (milk weight and percent milk fat) in response to treatment with two doses or eight doses of ceftiofur.

d. Conclusions: These data support the conclusion that the sterile cottonseed oil formulation containing 125 mg ceftiofur per 10 mL dose is clinically safe and not irritating to the mammary tissue of lactating dairy cattle when infused once daily for up to eight consecutive days.

## *4. HUMAN FOOD SAFETY:*

### a. Toxicology:

Summaries of all pivotal toxicology studies supporting ceftiofur, as either the sodium, hydrochloride, or crystalline free acid, are incorporated by reference to the approved NADAs 140-338, 140-890, and 141-209.

### b. Residue Chemistry:

1) Summary of Residue Chemistry Studies

a) Total Residue and Metabolism Study:

A GLP-compliant total residue and metabolism study was conducted in 14 healthy, lactating Holstein cows. Cows were in the middle of their 2nd or 3rd lactation. The principal investigators were RE Hornish and TS Arnold, Pharmacia Animal Health, Kalamazoo, MI.

Ceftiofur HCl labeled with $^{14}C$ in the thiazole ring was administered as an intramammary infusion at a dose of 125 mg ceftiofur HCl/quarter into all four quarters. Cows received two infusions in 24 hours.

Samples of all edible tissues (liver, kidney, muscle, and fat) were assayed for total $^{14}C$ residue by combustion analysis and LSC techniques. The concentration of total $^{14}C$ residue in the tissues at various time points are summarized in Table 4.1.

**Table 4.1. Mean total $^{14}C$ residue by combustion analysis and liquid scintillation counting (LSC) techniques**

| Withdrawal (days) | Concentration of total residues (µg/g) by combustion analysis | | | |
|---|---|---|---|---|
| | Kidney | Liver | Muscle | Fat |
| 0 | 1.25±0.26 | 0.255±0.064 | 0.051±0.008 | 0.052±0.051 |
| 2 | 0.329±0.048 | 0.051±0.010 | 0.013±0.004 | 0.013±0.004 |
| 4 | 0.140±0.016 | 0.058±0.025 | 0.011±0.001 | 0.010±0.003 |
| 6 | 0.125±0.045 | 0.094±0.102 | 0.010±0.000 | 0.020±0.017 |

Acceptable radiolabel accountability was demonstrated.

Samples of milk collected at 12-hour intervals were assayed for total $^{14}C$ residue by direct liquid scintillation counting (LSC) techniques. Residues were also determined using the regulatory HPLC-DCA assay (desfuroylceftiofur-related residues), the cylinder plate assay (microbiologically active residues), and an HPLC-RAM UV assay (parent ceftiofur). The results of the various assays are summarized in Table 4.2.

**Table 4.2. Concentration of ceftiofur residue in milk by several assays**

| Milking #, Sampling Time after Dose 2 | Mean Concentration, µg/mL | | | |
|---|---|---|---|---|
| | Total $^{14}C$ Ceftiofur | HPLC-DCA | Cylinder Plate | HPLC-RAM UV |
| #1, 12 hr | 49.66±17.61 | 45.05±16.02 | 29.66±12.45 | 21.56±8.78 |
| #2, 24 hr | 13.67±6.37 | 12.44±5.81 | 2.76±2.18 | 2.19±1.65 |
| #3, 36 hr | 4.74±2.27 | 4.00±1.94 | 0.31±0.24 | 0.29±0.19 |
| #4, 48 hr | 2.20±1.17 | 1.88±1.01 | 0.09±0.07 | 0.08±0.03 |
| #5, 60 hr | 0.79±0.44 | 0.55±0.34 | 0.04±0.04 | 0.04 |
| #6, 72 hr | 0.57±0.40 | 0.19±0.07 | 0.04±0.02 | 0.02 |
| #7, 84 hr | 0.32±0.16 | 0.14±0.07 | 0.04±0.02 | < LOQ |
| #8, 96 hr | 0.21±0.15 | 0.10±0.08 | 0.11 | < LOQ |
| #9, 108 hr | 0.15±0.10 | 0.06±0.03 | 0.11 | < LOQ |
| #10, 120 hr | 0.11±0.08 | 0.06±0.01 | < LOQ | < LOQ |
| #11, 132 hr | 0.09±0.06 | 0.04 | 0.02 | < LOQ |
| #12, 144 hr | 0.07±0.06 | < LOQ | < LOQ | < LOQ |

Limit of Quantitation (LOQ)=0.015 mg/mL (HPLC-DCA); 0.02 µg/mL (cylinder plate) assay; 0.01 µg/mL HPLC-RAM)

Milk was analyzed for the various metabolites of ceftiofur by HPLC-RAM analysis. The principle metabolites found in milk were the desfuroylceftiofur cysteine disulfide (DCD, or DFC-cysteine) and desfuroylceftiofur dimer (DFC-Dimer). Parent ceftiofur was the predominant milk residue in the first and second milkings post-treatment, but was not detected in the 5$^{th}$ (Dose 2 + 60 hour) and subsequent milkings post-last-treatment.

DCD was the only residue metabolite detected in kidney. There were no identifiable metabolites found in liver, nor was there a single component in the HPLC-RAM chromatogram that was ≥10% of the total residue. The concentrations of residue in muscle and fat were low (<0.20 µg/g), precluding metabolite profiling in these tissues.

Total residue data were not collected for the 8-dose treatment regimen. To address concerns for potential residue accumulation associated with this longer duration of dosing, reference is made to total residue accumulation studies conducted with ceftiofur sodium. In these studies, total residue concentrations resulting from the intramuscular administration of ceftiofur sodium at a dose of 2.2 mg/kg body weight for either three or five consecutive days were comparable (see FOI Summary NADA 140-338), indicating a lack of residue accumulation with extended treatment.

A similar lack of residue accumulation is expected for the intramammary use of ceftiofur at the extended 8-dose treatment. However, as data were not provided

for this treatment regimen, a worst-case extrapolation was made using the data from the 2-dose study. In this scenario, total residue concentrations from the 2-dose study were multiplied by four to approximate residues that might result from an 8-dose treatment regimen. Projected tissue residues for all tissues were significantly less than the respective tissue safe concentrations. Results are summarized in Table 4.3.

**Table 4.3. Comparison of mean total $^{14}$C-residue concentration (0-withdrawal), projected 4X residues and safe concentrations (all µg/mL)**

| Tissue | Mean total residues | Projected 4X total residues | Safe concentrations |
|---|---|---|---|
| Kidney | 1.125 | 4.5 | 26.4 |
| Liver | 0.255 | 1.02 | 13.2 |
| Muscle | 0.051 | 0.204 | 4.4 |
| Fat | 0.052 | 0.208 | 26.4 |

b) Milk Discard Study - 2 doses:

A GLP-compliant total residue and metabolism study was conducted in 24 healthy, lactating Holstein cows. Cows were in the middle of their 1st to 4th lactation. The principal investigator was RE Hornish, Pharmacia Animal Health, Kalamazoo, MI.

Ceftiofur HCl was administered as an intramammary infusion at a dose of 125 mg ceftiofur HCl into all four quarters. Cows received two infusions/quarter in 24 hours.

Residues of ceftiofur were measured using the determinative HPLC-DCA method for desfuroylceftiofur-related residues. The results are summarized in Table 4.4.

**Table 4.4. Mean residues of desfuroylceftiofur-related residues in milk following two daily intramammary doses of ceftiofur HCl at 125 mg/quarter into all four quarters**

| Sampling time (hours after second dose) | Mean concentration (µg/mL)* |
|---|---|
| D2+8 | 29.73±14.25 |
| D2+24 | 5.43±2.33 |
| D2+32 | 1.79±1.41 |
| D2+48 | 0.61±0.55 |
| D2+56 | 0.30±0.38 |
| D2+72 | 0.11±0.08 |
| D2+80 | 0.17±0.15 |
| D2+96 | 0.09±0.04 |
| D2+104 | 0.1±0.03 |
| D2+120 | 0.07 |
| D2+128 | < LOQ |
| D2+144 | < LOQ |

*LOQ = 0.05 µg/mL; Limit of Detection (LOD) = 0.015 µg/mL

c) Milk Discard Study - 8 doses:

A GLP-compliant total residue and metabolism study was conducted in 24 healthy, lactating Holstein cows. Cows were in the middle of their 1st to 4th lactation. The principal investigator was RE Hornish, Pharmacia Animal Health, Kalamazoo, MI.

Ceftiofur HCl was administered as an intramammary infusion at a dose of 125 mg ceftiofur HCl/quarter into all four quarters. Cows received eight infusions/quarter at a 24-hour interval.

Residues of ceftiofur were measured using the determinative HPLC-DCA method for desfuroylceftiofur-related residues. The results are summarized in Table 4.5.

**Table 4.5. Mean residues of desfuroylceftiofur-related residues in milk following intramammary doses of ceftiofur HCl at 125 mg/quarter into all four quarters once daily for eight consecutive days**

| Sampling time (hours after eighth dose) | Mean concentration (µg/mL)* |
|---|---|
| D8+12 | 22.83±7.15 |
| D8+24 | 6.66±2.77 |
| D8+36 | 1.62±0.76 |
| D8+48 | 0.51±0.38 |
| D8+60 | 0.19±0.10 |
| D8+72 | 0.09±0.03 |
| D8+84 | 0.07±0.02 |
| D8+96 | 0.09 |
| D8+108 | 0.05 |
| D8+120 | <LOQ |
| D8+132 | <LOQ |
| D8+144 | <LOQ |

*LOQ = 0.05 µg/mL; LOD = 0.015 µg/mL

2) Target Tissue and Marker Residue Assignment:

The target tissue for ceftiofur is kidney (21 CFR 556.113).

The results of the total residue and metabolism study indicate that a procedure that converts all of the desfuroylceftiofur-related residues to desfuroylceftiofur, which is then derivatized to the acetamide derivative, or DCA, is appropriate for the analysis of ceftiofur residue in milk and tissues. Thus, DCA is the appropriate marker substance for residue analysis of ceftiofur residue in milk and tissues.

3) Tolerance Assignments:

Tolerances for desfuroylceftiofur-related residues determined using the HPLC-DCA assay were established under NADA 140-890 (63 FR 53579, October 6, 1998) and are codified under 21 CFR 556.113 as follows:

**Table 4.6. Tolerances for ceftiofur (as desfuroylceftiofur)**

| Tissue | Concentration |
|---|---|
| Milk | 0.100 µg/mL |
| Kidney | 8.0 µg/g |
| Liver | 2.0 µg/g |
| Muscle | 1.0 µg/g |
| Fat | Not established |

4) Withdrawal and Milk Discard Times:

a) Pre-slaughter Withdrawal Period Assignment:

The total residue study described in 4.b.1) a) was used to determine the pre-slaughter withdrawal period for the intramammary use of ceftiofur HCl in lactating dairy cows. Total residue values in liver, kidney, and muscle for the zero withdrawal sampling time were compared against their respective tissue safe concentrations: 4.4 ppm in muscle, 13.2 ppm in liver, and 26.4 ppm in kidney and fat. All of the total residues were less than the tissue safe concentrations. Therefore, a zero pre-slaughter withdrawal period is assigned for the intramammary use of ceftiofur HCl in lactating dairy cattle. The results are summarized in Table 4.7.

**Table 4.7. Mean total $^{14}C$ residue by combustion analysis and liquid scintillation counting (LSC) techniques**

| Withdrawal (days) | Concentration of total residues (µg/mL) by combustion analysis | | | |
|---|---|---|---|---|
| | Kidney | Liver | Muscle | Fat |
| 0 | 1.125±0.26 | 0.255±0.064 | 0.051±0.008 | 0.052±0.051 |
| 2 | 0.329±0.048 | 0.051±0.010 | 0.013±0.004 | 0.013±0.004 |
| 4 | 0.140±0.016 | 0.058±0.025 | 0.011±0.001 | 0.010±0.003 |
| 6 | 0.125±0.045 | 0.094±0.102 | 0.010±0.000 | 0.020±0.017 |

Tissue residues also were determined using the validated HPLC-DCA assay. The results are summarized in Table 4.8.

**Table 4.8. Mean residues of desfuroylceftiofur-related residues in tissues following administration of two intramammary doses of ceftiofur HCl at 125 mg/quarter into all four quarters at a 24-hour interval**

| Withdrawal (days) | Concentration of total residues (µg/mL) by HPLC-DCA analysis | | |
|---|---|---|---|
| | Kidney | Liver | Muscle |
| 0 | 0.589±0.144 | 0.144±0.032 | 0.033±0.007 |
| 2 | 0.090±0.026 | <LOQ | <LOQ |
| 4 | <LOQ | <LOQ | <LOQ |

The data were analyzed using a statistical tolerance limit algorithm for the 99$^{th}$ percentile with 95% confidence and the tolerances described in Table 4.3. Again, the data support a zero pre-slaughter withdrawal period.

b) Milk Discard Period Assignment:

The milk residue depletion studies described in 4.b.1) b) and 4.b.1) c) were used to determine the milk discard period for the 2-dose and 8-dose intramammary use of ceftiofur HCl in lactating dairy cows. Based on the data from these studies, a milk discard period of 72 hours is assigned for both the 2-dose and the 8-dose treatment regimens.

## c. Microbial Food Safety:

Microbial food safety information for ceftiofur hydrochloride was evaluated using a qualitative risk assessment procedure. The dosage regimen evaluated was 125 mg of ceftiofur infused per affected quarter, and repeated at least once in a 24 hour interval, up to a maximum dose and duration of treatment in all four quarters daily up to eight consecutive days. The indication associated with the dosage regimen is for the treatment of mastitis associated with coagulase negative staphylococci, *Streptococcus dysgalactiae, and Escherichia coli.*

The qualitative risk assessment procedure involved conducting: 1) a release assessment to describe the probability that ceftiofur hydrochloride and its use in lactating dairy cattle will result in the emergence of resistant bacteria or resistance determinants in treated lactating dairy cattle under proposed conditions of use; 2) an exposure assessment to describe the likelihood of human exposure to resistant bacteria or resistance determinants through consumption of edible products from treated animals (in this case, beef); and 3) a consequence assessment to describe potential human health consequences arising from exposure to the defined resistant bacteria or resistance determinants by considering the human medical importance of cephalosporins used in the treatment of human infectious disease.

It was determined that the risk of development of transferable resistance elements from this use of ceftiofur hydrochloride in dairy cattle is HIGH, leading to an overall risk estimation of HIGH. The proposed conditions of use are compatible with the Agency's

risk management strategies associated with a product having an overall risk estimation of HIGH.

**d. Regulatory Methods:**

The regulatory method for determination of DCA in bovine kidney, muscle, and milk is the HPLC-DCA assay that 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.

***5. USER SAFETY:***

Studies to evaluate the safety of ceftiofur to users are discussed in detail in the original FOI Summary for NADA 140-338 (NAXCEL Sterile Powder, ceftiofur sodium).

Human Warnings are provided on the product labeling as follows:

Keep out of reach of children.

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

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

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

The material safety data sheet contains more detailed occupational safety information. To report adverse effects in users, to obtain more information or to obtain a material safety data sheet, call 1-800-366-5288.

***6. 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 SPECTRAMAST LC Sterile Suspension (ceftiofur hydrochloride), when administered as an intramammary infusion, is safe and effective for the treatment of clinical mastitis in lactating dairy cattle associated with coagulase-negative staphylococci, *Streptococcus dysgalactiae*, and *Escherichia coli*.

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 clinical mastitis, (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, and (c) the rate of emergence of ceftiofur-resistant organisms may be reduced by the involvement of veterinarians in product use.

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.

No patents were submitted with this application.

## *7. ATTACHMENTS:*

Facsimile labeling is attached as indicated below.

A. SPECTRAMAST LC Sterile Suspension - PLASTET Label

B. SPECTRAMAST LC Sterile Suspension – Carton Label

C. SPECTRAMAST LC Sterile Suspension - Package Insert