Date of Approval: April 23, 2015

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-238

SPECTRAMAST LC Sterile Suspension

Ceftiofur Intramammary Suspension

Cattle (lactating dairy)

For the treatment of diagnosed subclinical mastitis associated with coagulase-negative staphylococci and *Streptococcus dysgalactiae* in lactating dairy cattle.

Sponsored by:

Zoetis Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-238

B. Sponsor

Zoetis Inc.
333 Portage St.
Kalamazoo, MI 49007

Drug Labeler Code: 054771

C. Proprietary Name

SPECTRAMAST LC Sterile Suspension

D. Established Name

Ceftiofur intramammary suspension

E. Pharmacological Category

Antimicrobial

F. Dosage Form

Sterile suspension

G. Amount of Active Ingredient

Each syringe contains 125 mg ceftiofur equivalents as ceftiofur hydrochloride

H. How Supplied

10 mL syringe with cannula

I. Dispensing Status

Rx

J. Dosage Regimen

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.

K. Route of Administration

Intramammary infusion

L. Species/Class

Lactating dairy cattle

M. Indications

SPECTRAMAST® LC (ceftiofur intramammary suspension) Sterile Suspension is indicated for use in lactating dairy cattle for (1) the treatment of clinical mastitis associated with coagulase-negative staphylococci, *Streptococcus dysgalactiae*, and *Escherichia coli* and (2) the treatment of diagnosed subclinical mastitis associated with coagulase-negative staphylococci and *Streptococcus dysgalactiae*.

N. Effect of Supplement

This supplement provides for a new indication, "for the treatment of diagnosed subclinical mastitis associated with coagulase-negative staphylococci and *Streptococcus dysgalactiae"* in lactating dairy cattle.

II. EFFECTIVENESS

A. Dosage Characterization

This supplemental approval does not change the previously approved dosage. The Freedom of Information (FOI) Summary for the original approval of NADA 141-238 dated February 9, 2005, contains dosage characterization information for cattle.

B. Substantial Evidence

1. Clinical Field Study

a. Title: "Efficacy and Safety of SPECTRAMAST LC for Intramammary Treatment of Subclinical Mastitis in Lactating Dairy Cattle." Study Number A131C-US-12-127. July 2013 to December 2013.

b. Investigators:

Table 1. Clinical Investigators.

Site	Clinical Investigators
A	Paul Busman, DVM Sparta Animal Clinic, Sparta, MI
B	Clint Walhof, DVM Valley Veterinarians Inc., Tulare, CA
C	Kenneth Mitchell, DVM Valley Veterinarians Inc., Tulare, CA
D	Gregory M. Goodell, DVM Advanced Dairy Analysis, LLC, Greeley, CO
E	Chad Wright, DVM Vet Outlet - Kern County, Bakersfield, CA
F	Kevin Crandall, DVM Rocky Mountain Veterinary Services, Shelley, ID
G	David Kolb, DVM LVH Research and Development, LLC, Lodi, WI
H	Michael Capel, DVM Perry Veterinary Clinic, Perry, NY

Site	Clinical Investigators
J	Keith Salmon, DVM Southkent Veterinary Hospital, Caledonia, MI
K	Chris Koeller, DVM Advanced Dairy Analysis, LLC, Greeley, CO

c. Study Design:

1) Objective: To evaluate the effectiveness of SPECTRAMAST LC Sterile Suspension (referred to as SPECTRAMAST LC) in comparison to a saline-treated control as an intramammary treatment for subclinical mastitis in lactating dairy cattle.

2) Study Animals: A total of 336 lactating cross- and pure-bred Holstein, Jersey, and Brown Swiss dairy cattle from commercial and university dairy herds, in the first to fifth parity and at least 3 days in milk, met the criteria for enrollment and were considered evaluable cases in the analysis.

3) Treatment Groups: Animals were assigned to one of two treatment groups as shown in Table 2.

Table 2. Treatment Groups.

Group	Dosage	Treatment Regimen	Number of Evaluable Animals
SPECTRAMAST LC	10 mL syringe containing 125 mg ceftiofur HCl	One syringe infused into the selected quarter once daily for 2 days.	164
Saline	Syringe containing 10 mL of sterile saline	One syringe infused into the selected quarter once daily for 2 days	172

4) Drug Administration: The test article was SPECTRAMAST LC (ceftiofur intramammary suspension) Sterile Suspension (NADA 141-238), commercially available in sterile, pre-filled 10 mL syringes. The control product was 10 mL saline, commercially available in sterile, pre-filled 10 mL syringes. The test article or saline was infused in the selected quarter with a short cannula attached to a syringe. The test article or saline was administered after milking, in two doses, 24 hours apart.

5) Experimental Design: The study was a randomized, masked, 10-location field study comparing a two-dose treatment of SPECTRAMAST LC with a two-dose treatment of saline. Animals were considered candidates for enrollment if they had a composite somatic cell count (SCC) >400,000 cells/mL and one or more quarters was positive for a Gram-positive mastitis-causing organism in the pre-treatment screening samples. Milk was collected and cultured once daily (Days -2, -1, and 0) to establish a pre-treatment bacterial profile. If a candidate animal exhibited no signs of clinical mastitis, illness, or other medical problem, the animal was randomly assigned to the SPECTRAMAST LC treatment group or the saline treatment group. One quarter was randomly selected for treatment if an animal had more than one quarter that met the pre-treatment bacterial culture criteria. The first treatment was infused after milking, following collection of the third pre-treatment milk sample for culture (Day 0). Subsequent dosing of SPECTRAMAST LC or saline was administered after milking approximately 24 hours after the first dose (Day 1). Two post-treatment milk samples were collected for bacterial culture 14 days and 20 days after the second dose (Days 15 and 21, respectively).

   Bacteria were initially identified using traditional laboratory methods. Isolates of *S. dysgalactiae* were identified to the species level using a Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry system.

   For an enrolled animal to be considered a qualified, evaluable case and included in the analysis, the following additional criteria were required:

   - one of three pre-treatment bacterial cultures was positive for a single contagious Gram-positive mastitis pathogen (*Staphylococcus aureus* or *Streptococcus agalactiae*) OR two of three pre-treatment bacterial cultures were positive for a single non-contagious (environmental or opportunistic) Gram-positive mastitis pathogen; and

   - both post-treatment milk samples were collected for bacterial culture; and

   - there were no signs of clinical mastitis or other health problems resulting in removal from the study.

   There were 336 qualified, evaluable cases at the end of the study across both groups. A treatment success (bacterial cure) was defined as an animal found to be culture-negative in both post-treatment milk cultures for the bacterial pathogen identified in the pre-treatment milk sample(s).

6) Measurements and Observations:

   a) Milk samples were collected for bacterial culture once daily during the pre-treatment period (Days -2, -1, 0) and at the time of the two post-treatment milk collections (Days 15 and 21).

b) Milk quality from all quarters was recorded (normal, clots, flakes, watery, stringy, bloody, etc.) once daily from Day -2 through Day 21. Quarter health from all quarters was recorded (normal, redness, firmness, swelling, pain, etc.) once daily from Day -2 through Day 21.

c) General health was recorded daily as normal or abnormal. If general health was recorded as abnormal, a veterinarian conducted a physical examination.

d. Statistical Analysis: The primary outcome of the presence or absence of an overall treatment success (bacterial cure rate) was analyzed using a generalized linear mixed model assuming a binomial distribution and a logit link function. The model included treatment as the fixed effect. Site and treatment x site interaction terms were included as the random effects. The treatment effect was tested at a 0.05 significance level. The Least Squares (LS) Means estimates of the cure rate for the two treatment groups were calculated by back-transformation from the logit scale.

e. Results:

1) Overall treatment success: The cure rate in the SPECTRAMAST LC-treated group was statistically significantly different ($P < 0.0001$) from the saline-treated group among all evaluable cases (without regard to the pathogen used to qualify the case). The SPECTRAMAST LC-treated group had a higher cure rate (LS Means estimate of 44%) compared to the saline-treated group (LS Means estimate of 4%). The results are shown in Table 3.

Table 3. Overall Bacterial Cure Rates.

Treatment Group	Total Cases	Cure Numbers	Failure Numbers	Cure Rates (LS Means)
SPECTRAMAST LC	164	75	89	44%
Saline	172	8	164	4%
Total	336	83	253	--
P-value	--	--	--	<0.0001

2) Microbiology: The most prevalent group of pathogens was the coagulase-negative staphylococci (CNS), present in 218 of 336 qualified cases. Eighteen qualified cases of *Streptococcus dysgalactiae* were observed. Additional cases defined by other bacteria were observed, but the frequency and relative cure rates between the treatment groups for these pathogens were not considered to be clinically relevant for microbiologic comparison. The response to treatment by pathogen is represented in Table 4.

Table 4. Summary of Bacterial Cure Rates by Mastitis Pathogen.

Indicated Pathogen	Treatment	Cure Numbers (% of Group)	Failure Numbers (% of Group)
CNS	SPECTRAMAST LC	59 (60.2)	39 (39.8)
CNS	Saline	5 (4.2)	115 (95.8)
S. dysgalactiae	SPECTRAMAST LC	6 (85.7)	1 (14.3)
S. dysgalactiae	Saline	1 (9.1)	10 (90.9)

A clinically-relevant ratio of clinical successes to failures was observed in the SPECTRAMAST LC-treated group compared to the saline-treated group for evaluable cases of subclinical mastitis associated with CNS and with *S. dysgalactiae.*

f. Adverse Reactions: No adverse reactions were reported in this study.

g. Conclusions: The results of this study demonstrate that SPECTRAMAST LC Sterile Suspension administered by intramammary infusion at 125 mg ceftiofur HCl/quarter for two treatments, 24 hours apart, is effective for the treatment of diagnosed subclinical mastitis associated with coagulase-negative staphylococci and *Streptococcus dysgalactiae* in lactating dairy cattle.

III. TARGET ANIMAL SAFETY

CVM did not require target animal safety studies for this supplemental approval. The FOI Summary for the original approval of NADA 141-238 dated February 9, 2005, contains a summary of a target animal safety study for lactating dairy cattle when 125 mg ceftiofur HCl is administered as an intramammary infusion every 24 hours for a minimum of 2 days and up to 8 consecutive days.

IV. HUMAN FOOD SAFETY

A. Antimicrobial Resistance

The impact of the addition of an indication for the treatment of diagnosed subclinical mastitis associated with coagulase-negative staphylococci and *Streptococcus dysgalactiae* in lactating dairy cattle at a dose of 125 mg ceftiofur, administered twice by intramammary infusion, 24 hours apart (for extended duration therapy, once daily treatment may be repeated for up to 8 consecutive days) was carefully considered by the Agency. For this supplemental approval, the firm provided information to CVM through a qualitative microbial food safety risk assessment.

The firm provided a qualitative microbial food safety risk assessment that included:

1. A *release assessment* to describe the probability that the use of ceftiofur in lactating dairy cattle will result in emergence or selection of ceftiofur (or other 3rd generation cephalosporin)-resistant *Escherichia coli* and *Salmonella* spp., a thorough assessment of the spectrum of antibacterial activity of ceftiofur, mechanisms of 3rd generation cephalosporin resistance in *E. coli* and

Salmonella, and the current prevalence of *E. coli* and *Salmonella* in dairy cattle and retail beef.

2. An *exposure assessment* to describe the likelihood of human foodborne exposure to *E. coli* and *Salmonella* following consumption of beef from treated dairy cattle.

3. A *consequence assessment* to describe potential human health consequences (attributable to exposure to the defined foodborne pathogens or resistance determinants) by considering the human medical importance of 3rd generation cephalosporins in the treatment of human gastrointestinal diseases.

The Agency evaluated the information submitted by the firm, considered the current cephalosporin susceptibility profiles of *Salmonella* spp. and *E.coli*, including their prevalence in the food commodity of concern (beef) and target animal (dairy cattle), and worked with the firm to develop the following language to be included in the PRECAUTION section of the drug labeling and on all marketing and promotional materials:

> *"SPECTRAMAST LC Sterile Suspension is intended for use in lactating dairy cattle with mastitis associated only with the specified labeled pathogens. To assure responsible antimicrobial drug use, it is expected that subclinical mastitis will be diagnosed using a positive culture or other pathogen-specific test in addition to any other appropriate veterinary medical evaluation prior to treatment."*

Based upon this evaluation and mitigating factors, the Agency concludes that the use of ceftiofur in dairy cattle for the treatment of diagnosed subclinical mastitis will not result in a significant risk with respect to the development of cephalosporin resistance in foodborne *E. coli* and *Salmonella* originating from treated lactating dairy cattle.

Decision Statement:

The overall risk estimation associated with the use of ceftiofur intramammary infusion in dairy cattle under the proposed conditions of use is high, based on individual rankings of medium for the *release assessment*, medium for the *exposure assessment*, and high for the *consequence assessment*. The latter ranking of high for the consequence assessment is based on 3rd generation cephalosporins being *critically important* in human medicine as it is an empiric drug class of choice to treat a majority of clinical gastrointestinal infections, particularly in children. However, the Agency thinks that there is a reasonable certainty of no harm from the use of ceftiofur in lactating dairy cattle for treatment of subclinical mastitis when risk management strategies such as prescription only (Rx) marketing status under the direction of a veterinarian, intramammary route of administration, individual animal use, the mitigating statement (described above), extra-label use prohibition for certain uses, and monitoring by the National Antimicrobial Resistance Monitoring System (NARMS) are put in place. The Agency, therefore, concludes that the proposed conditions of use, including appropriate use parameters to determine if lactating dairy cattle are eligible to receive ceftiofur for treatment of diagnosed subclinical mastitis, are adequate to support the use of ceftiofur in lactating dairy cattle, and will help to ensure that risks to public health from cephalosporin resistant *E. coli* and *Salmonella* originating from treated lactating dairy cattle are minimal.

B. Impact of Residues on Human Intestinal Flora

1. Determination of the need for establishing a microbiological acceptable daily intake (ADI)

A step-by-step approach was followed to determine whether there is a concern for effects of ceftiofur residues on human intestinal flora.

a. Step 1: Are residues of ceftiofur and/or its metabolites microbiologically active against representative human intestinal bacteria?

Yes, by default, ceftiofur has activity against representative human intestinal bacteria.

b. Step 2: Do residues enter the human colon?

Yes, ceftiofur residues enter the human colon.

c. Step 3: Do the residues entering the human colon remain microbiologically active?

Yes, ceftiofur residues remain microbiologically active in the colon, but quantities are very low, as concluded from a comprehensive, pivotal, multi-phased study summarized below.

Title of Study: Anaerobic Degradation of Ceftiofur by Human GI Tract Microflora in Human Fecal Slurries.

Study Number: 788-7926-I-REH-93-001

Report Date: January 26, 1994

Study Director: Susan Kotarski, PhD

Study Location: Veterinary Medicine Research & Development, Pharmacia & Upjohn Co., Kalamazoo, MI

Experiment Design: The objective of the study was to examine the degradation of ceftiofur in human fecal slurries, and to identify degradation products. Fecal samples from 11 donors were collected, diluted 1:1 in anaerobic buffer, and ceftiofur was added to the slurries at concentrations of 0, 10, 100, or 500 µg/mL. Untreated and autoclaved slurries were used in this experiment. Samples were incubated at 37 °C for up to 24 hours. Ceftiofur was used in the studies because its metabolites contain an intact β-lactam ring, and any degradation detected for ceftiofur is very likely to apply also to its metabolites.

The study was conducted in three phases:

1) Phase I determined whether rapid loss of microbiological activity occurs during anaerobic incubation of ceftiofur (500 µg/mL) with fecal slurries (up to 24 hours) from 8 human volunteers. Loss of microbiological activity was measured by a microbiological cylinder plate assay with a bacterial strain of *Micrococcus luteus*.

2) Phase II determined whether the microbiological activity of ceftiofur was stable in a fecal slurry. Fresh and autoclaved fecal samples were fortified with 0 and 833 µg/mL of ceftiofur, and incubated for 0 or 4 hours.

3) Phase III included a series of experiments designed to study the capacity of inactivation of ceftiofur in buffer-diluted fecal slurries. 500 µg/mL of ceftiofur was added to different dilutions of slurries and incubated for 0, 1, 2, and 4 hours.

Results and Conclusions: The three-phase study revealed the following:

1) In Phase I, 1) ceftiofur immediately lost its activity in fecal slurries when fortified with low doses (1 and 10 µg/mL); 2) slurries from 7 of 8 donors fortified with 500 µg/mL immediately showed >90% loss of microbiological activity; 3) sterilized fecal slurries lost only 0 to 36% of the microbiological activity after 4 hours of anaerobic incubation at 37 °C, suggesting that part of the activity is due to chemical instability/degradation, binding, or both; 4) fresh and sterilized samples fortified with 100 or 500 µg/mL of ceftiofur also showed immediate and complete loss of activity at 100 µg/mL (fresh samples), and almost total loss at 500 µg/mL after 4 hours of incubation. Autoclaved samples had a small loss of activity at both concentrations of the drug; 5) fecal slurries, diluted and maintained under anaerobic conditions, are capable of degrading ceftiofur to non-active products at concentrations as high as 500 µg/mL.

2) Phase II results showed that there might be some capacity in the methanol-diluted solutions for the degradation of ceftiofur activity, but its microbiological activity is largely retained, as confirmed by microbiological assay and high-performance liquid chromatography (HPLC) methods. Because deactivating enzymes or other degrading factors are not present to degrade ceftiofur in the cylinders in the microbiological assay *per se*, the ceftiofur degradation or inactivation found in Phase I was not due to the processing method of the samples, but rather due to incubation with fecal materials.

3) Phase III included three groups of experiments (*i.e.*, degradative activity by dilution, protein binding by ultrafiltration, and a degradation profile), and demonstrated that ceftiofur, in undiluted samples, had a very high loss of activity at 0 hours. At 2 hours of incubation, all activity had been lost. The saturation point is reached between 50- and 250-fold dilutions, dependent on individual feces used, which would indicate that 1 gram of undiluted fecal material would have the capacity to metabolize 22.5 to 112 mg ceftiofur within 5 minutes.

These results reveal the tremendous capacity of undiluted fecal samples to metabolize ceftiofur. Fecal microflora had the capacity to inactivate ceftiofur. The loss of ceftiofur activity was not due to protein binding but was more likely through inactivation by enzymatic action in the fecal environment.

d. Step 4: Determination if there is any scientific justification to eliminate testing for either one or both endpoints of concern:

Yes, based on results from the study described above, it was concluded that ceftiofur residues in feces are rapidly inactivated, and biologically active residues were very low. Ceftiofur residues should not produce changes in human intestinal flora; therefore, testing of either endpoint of concern - colonization barrier disruption or resistance development - is not needed.

2. Determination of the final Microbiological ADI

Based on findings from the inactivation of ceftiofur in the fecal slurries study (above), there is no need to determine a microbiological ADI.

Decision Statement:

Due to effective inactivation under the proposed conditions of use, the amount of microbiologically active residues of ceftiofur in the edible tissues of treated cattle that reach the human colon is negligible, and would most likely not adversely affect the intestinal flora of human consumers.

C. Toxicology

Reassessment of the toxicological Acceptable Daily Intake (ADI) or Acceptable Single-Dose Intake (ASDI) was not needed for this supplemental approval. The FOI Summaries for the original approval of NADA 140-338 dated January 25, 1988, NADA 140-890 dated April 26, 1996, NADA 141-209 dated September 5, 2003, and NADA 141-235 dated June 18, 2004, contain summaries of all toxicology studies and information.

D. Assignment of the Final ADI and the ASDI

The final ADI is the toxicological ADI of 30 µg/kg body weight (bw)/day derived from 90-day oral studies in dogs and rats. The ASDI is 0.83 mg/kg bw derived from studies conducted in the guinea pig model of ceftiofur hypersensitivity and cross hypersensitivity between penicillin G and ceftiofur. The codified ADI and ASDI are listed under 21 CFR 556.113.

E. Safe Concentrations for Total Residues

The safe concentrations of total residues of ceftiofur in each edible tissue of cattle are 4.40 ppm for muscle, 13.20 ppm for liver, 26.40 ppm for kidney, 26.40 ppm for fat, 0.320 ppm for milk, and 166 ppm for the injection sites.

F. Residue Chemistry

CVM did not require residue chemistry studies for this supplemental approval. The FOI Summaries for the original approval of NADA 141-238 dated February 9, 2005, and for a supplemental approval dated June 2, 2006, contain summaries of residue chemistry studies for cattle.

G. Analytical Method for Residues

The FOI Summaries for the original approval of NADA 141-238 dated February 9, 2005, and for a supplemental approval dated June 2, 2006, contain the analytical method summaries for ceftiofur in cattle.

V. USER SAFETY

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

FOR USE IN ANIMALS ONLY – NOT FOR HUMAN USE

KEEP OUT OF REACH OF CHILDREN.

WARNINGS: 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 protective 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 safety data sheet contains more detailed occupational safety information. To report suspected adverse events, for technical assistance or to obtain a copy of the safety data sheet (SDS), contact Zoetis Inc. at 1-888-963-8471. For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS or online at http://www.fda.gov/AnimalVeterinary/SafetyHealth.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that SPECTRAMAST LC Sterile Suspension, when used according to the label, is safe and effective for the treatment of diagnosed subclinical mastitis associated with coagulase-negative staphylococci and *Streptococcus dysgalactiae* in lactating dairy cattle. Additionally, data demonstrate that residues in food products derived from species treated with SPECTRAMAST LC Sterile Suspension will not represent a public health concern when the product is used according to the label.

A. Marketing Status

Labeling restricts this drug to use by or on the 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 subclinical mastitis; and (b) restricting this drug to use by or on the order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues.

B. Exclusivity

This supplemental approval for SPECTAMAST LC Sterile Suspension qualifies for THREE years of marketing exclusivity under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act because the supplemental approval included an effectiveness study. This exclusivity begins as of the date of our approval letter and only applies to the indication "for the treatment of diagnosed subclinical mastitis associated with coagulase-negative staphylococci and *Streptococcus dysgalactiae*" in lactating dairy cattle .

C. Supplemental Applications

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

D. Patent Information

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