Date of Approval: March 15, 2005

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-239

SPECTRAMAST DC Sterile Suspension

(ceftiofur hydrochloride)

"For the treatment of subclinical mastitis in dairy cattle at the time of dry off associated with *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*."

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

TABLE OF CONTENTS

1. GENERAL INFORMATION:

a. File Number:	NADA 141-239
b. Sponsor:	Pharmacia & Upjohn Co. A Division of Pfizer, Inc. 235 East 42d St. New York, NY 10017 Drug Labeler Code: 000009
c. Established Name:	Ceftiofur hydrochloride
d. Proprietary Name:	SPECTRAMAST DC Sterile Suspension
e. Dosage Form:	Sterile oil suspension
f. How Supplied:	10 mL plastic syringe (PLASTET) with cannula
g. How Dispensed:	Rx
h. Amount of Active Ingredients:	Each PLASTET contains ceftiofur hydrochloride equivalent to 500 mg ceftiofur (50 mg/mL).
i. Route of Administration:	Intramammary infusion
j. Species/Class:	Dairy cattle (dry)
k. Recommended Dosage:	One syringe (500 mg ceftiofur) per affected quarter at the time of dry off
l. Pharmacological Category:	Antimicrobial
m. Indications:	SPECTRAMAST DC Ceftiofur Hydrochloride Sterile Suspension is indicated for the treatment of subclinical mastitis in dairy cattle at the time of dry off associated with *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*.

2. EFFECTIVENESS:

a. Dosage Characterization:

A preliminary study (Report No. 705-9690-98-001) was conducted in dairy cows at dry off using ceftiofur hydrochloride sterile suspension (500 mg of ceftiofur per 10 mL), administered as an intramammary infusion in each quarter on the day of enrollment. A total of 583 cows with somatic cell counts (SCC) greater than or equal to 400,000 cells/mL or a linear score greater than or equal to 5 were enrolled. A single quarter milk sample was aseptically obtained from all four quarters for bacterial culture prior to treatment and on Days 3 and 5 post-calving. The results of this study also provide substantial evidence of effectiveness, and are summarized below (*2.b*.2). Based on the results, the 500 mg dosage was selected for further testing.

b. Substantial Evidence:

1. Field Dose Confirmation and Dose Determination Study for Ceftiofur Hydrochloride as a Dry Cow Treatment. Study No. 792-7922-O-JWH-98-001; Report No. a0093462. January 2000-February 2001.

a. Type of Study: A multi-location field dose confirmation study

b. Investigators:

Dr. Dale Ottosen, Cayuga Veterinary Services, Auburn, NY
Dr. Paul Busman, Ravenna, MI (two sites)
Dr. Ron Erskine, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI
Dr. Keith Salmon, South Kent Veterinary Hospital, Caledonia, MI
Dr. Meg Cattell, Windsor Dairy, Windsor, CO
Dr. Niles Jennett, Dairy Veterinary Services, Chandler, AZ
Dr. Bruce Vande Steeg, Oakdale, CA
Dr. Steve Carlson, Dairy Management Services, Tulare, CA
Dr. John Kirk, University of California - Davis, VMTRC, Tulare, CA
Dr. John Day, Dairy Health Services/MPS, Jerome, ID (two sites)
Dr. Andrew Keeter, Dairy Oz, Deerfield, KS
Dr. Tom Graham, Veterinary Consulting Services, Davis, CA
Dr. David Reid, Rocky Ridge Veterinary Service, Hazel Green, WI
Dr. Andrew Johnson, Total Herd Management Services, Inc., Seymour, WI
Dr. David Ohman, Dairy Production Services, Glenbeulah, WI
Dr. Jim Bennett, Northern Valley Animal Clinic, Plainview, MN
Drs. Ynte Schukken and Linda Garrison, Ithaca, NY
Dr. Ron Harrison, Professional Veterinary Research, Inc., Brownstown, IN
Dr. Darren Bryant, Professional Veterinary Research, Inc., Brownstown, IN

c. Study Design:

1) *Objective:* To confirm the effectiveness of a single intramammary infusion of ceftiofur hydrochloride sterile suspension (125 mg, 250 mg, or 500 mg

ceftiofur per quarter) for the treatment of intramammary infections present at the time of dry off.

2) *Animals:* A total of 533 cows in 21 herds were enrolled in the study. Cows with whole udder somatic cell counts greater than 400,000 cells/mL or a linear somatic cell count score greater than or equal to 5 were enrolled. Of the 533 cows enrolled, 431 cows were used in the investigation.

3) *Experimental Design:* Cows were blocked by lactation with first through third lactation cows blocked together as a group and fourth and greater lactation cows blocked together as another group. Within each block, all five treatments (125 mg, 250 mg, or 500 mg ceftiofur, negative control, and positive control) were included.

4) *Test Article Administration:* Ceftiofur hydrochloride sterile suspension (either 125 mg, 250 mg, or 500 mg of ceftiofur per 10 mL) was administered as an intramammary infusion on the day of enrollment. Cows in the positive control group were treated with an approved dry cow mastitis product. Cows in the negative control group were left untreated.

5) *Measurements and Observations:* A single quarter milk sample was aseptically obtained from all four quarters of each cow for bacterial culture prior to treatment and at Days 3 and 5 post-calving. The decision variable was the therapeutic (microbiologic) cure, defined as elimination of any pathogen found in the pre-treatment quarter milk sample in both post-calving quarter milk samples.

d. Results: The purpose of this study was to determine the effective dose(s) for the treatment of subclinical mastitis at the time of dry off, defined as the dose(s) significantly better than the negative control group at the one-sided 5% level. The proportion of quarters cured was analyzed using the GLIMMIX macro of SAS. Using the logit link with the binomial error, Treatment was a fixed effect while Site, Site x Treatment, and Cow-within-Site by Treatment were the random effects. The results are shown in Table 2.1.

Table 2.1. Overall therapeutic cure rates (all mastitis pathogens combined)

Treatment	Number of Animals	Therapeutic Cure Rate	
		% Cure rate*	P-value
0 mg	43	29/79 = 36.7%	-
125 mg	41	46/77 = 59.7%	0.017
250 mg	49	38/84 = 45.2%	0.137
500 mg	51	61/108 = 56.5%	0.012

*The number of quarters cured of mastitis pathogens isolated at dry off divided by the total number of quarters with at least one mastitis pathogen isolated at dry off.

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

f. Conclusion: Ceftiofur hydrochloride administered once by intramammary infusion at a 500 mg dose was effective for the treatment of subclinical mastitis in dairy cattle at the time of dry off associated with *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis.*

2. Preliminary Examination of Efficacy for Intramammary Formulation of Pirlimycin Hydrochloride, Ceftiofur Hydrochloride and Ceftiofur Crystalline Free Acid as Dry Cow Therapies. Report No. 705-9690-98-001. August 1996-June 1997.

a. Type of Study: A multi-location field effectiveness study conducted in herds and cows representative of the target population.

b. Investigators:

Dr. Walt Guterbock, Hanford, CA (two sites)
Dr. Keith Sterner, Ionia, MI
Dr. Ron Erskine, Dept. of Large Animal Clinical Science, Michigan State University, E. Lansing, MI
Dr. David Wolfgang, Dept. of Veterinary Science, Penn State University, University Park, PA (two sites)
Dr. Andrew Keeter, Lake Arthur, NM (two sites)
Dr. Mike Kerfoot, Bakersfield, CA (two sites)
Dr. Arthur Sherman, Keseca Veterinary Clinic, Geneva, NY
Dr. Meg Cattell, Loveland, CO

c. Study Design:

1) *Objective:* To evaluate the effectiveness of a single intramammary infusion of pirlimycin hydrochloride, ceftiofur hydrochloride, or ceftiofur crystalline free acid (CCFA) as a dry cow therapy compared to non-treated control cows.

2) *Animals:* In eleven study herds, 583 cows with somatic cell counts (SCC) greater than or equal to 400,000 cells/mL or a linear score greater than or equal to 5 were enrolled.

3) *Experimental Design:* Cows with a dry period of at least 45 days were blocked by lactation, with first and second lactation cows blocked together as a group and third and greater lactation cows blocked together as another group. Cows were assigned to one of four treatment groups – pirlimycin hydrochloride, ceftiofur hydrochloride, ceftiofur crystalline free acid, or negative control.

4) *Test Article Administration:* The test article was ceftiofur hydrochloride sterile suspension (500 mg of ceftiofur per 10 mL), administered as an intramammary infusion in each quarter on the day of enrollment. Cows in the negative control group were left untreated.

5) *Measurements and Observations:* A single quarter milk sample was aseptically obtained from all four quarters of each cow for bacterial culture prior to treatment and on Days 3 and 5 post-calving. The decision variable was the therapeutic (microbiologic) cure, defined as elimination of any pathogen found in the pre-treatment quarter milk sample in both post-calving quarter milk samples.

d. Results: The purpose of this study was to determine if the ceftiofur hydrochloride dry cow therapy cured the existing infections at a rate significantly greater than the non-treated control cows at the one-sided 5% level. The proportion of quarters cured was analyzed using the GLIMMIX macro of SAS. Using the logit link with the binomial error, Treatment was a fixed effect while Site, Site x Treatment, and Cow-within-Site by Treatment were the random effects. For therapeutic cure, 303 cows provided 666 quarters with at least one mastitis pathogen in the pre-treatment milk sample. The results for cows treated with ceftiofur hydrochloride and the non-treated control group are presented in Table 2.2.

Table 2.2. Quarter therapeutic cure rates

Treatment	Number of Animals	Therapeutic Cure Rate	
		% Cure rate*	P-value
Control	84	82/182 = 45.0%	-
Ceftiofur HCl	73	115/178 = 64.6%	0.004

*The number of cured quarters that had mastitis pathogens isolated at dry off divided by the total number of quarters that had mastitis pathogens isolated at dry off.

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

f. Conclusion: Ceftiofur hydrochloride administered once by intramammary infusion at a 500 mg dose was effective for the treatment of subclinical mastitis in dairy cattle at the time of dry off associated with *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis.*

3. Summary of Cure Rates by Pathogen:

The microbiologic data from the two effectiveness studies described above were combined for the purpose of evaluating ceftiofur cure rate for each major mastitis pathogen isolated. The results are presented in Table 2.3. Based on the data, ceftiofur hydrochloride is effective for the treatment of subclinical mastitis in dairy cattle at the time of dry off associated with *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*.

Table 2.3. Summary of combined cure rates by mastitis pathogen

Organism	Ceftiofur hydrochloride treated cows		Non-treated cows	
	No. cured	No. treated	No. cured	No. treated
Staphylococcus aureus	12 (44.4%)	27	6 (20.7%)	29
Streptococcus dysgalactiae	12 (80%)	15	2 (25%)	8
Streptococcus uberis	10 (83%)	12	5 (62.5%)	8

c. Microbiology:

The minimum inhibitory concentration (MIC) of ceftiofur was determined *in vitro* for isolates obtained from cows during a multi-site clinical field study in the U.S. and isolates from diagnostic laboratories in the U.S. and Canada. Susceptibility testing was conducted at the Pharmacia and Upjohn Company laboratory according to the methods described by the National Committee for Clinical Laboratory Standards (NCCLS). Table 2.4 contains MIC data for isolates from the clinical field study in dry cows. Table 2.5 contains MIC data for isolates from diagnostic laboratories. Appropriate reference strains were included in each test. MICs were determined by the standardized dilution technique with ceftiofur sodium standard reference powder; 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 2.6.

Table 2.4. Ceftiofur MIC values for isolates from clinical field study #792-7922-O-JWH-98-001 in the U.S. during 2000

Organism	No.	MIC_{90}* (µg/mL)	MIC range (µg/mL)
Staphylococcus aureus	300	1.0	≤ 0.06 to 2.0
Streptococcus dysgalactiae	55	≤ 0.06	≤ 0.06 to > 64.0
Streptococcus uberis	58	1.0	≤ 0.06 to 4.0

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

Table 2.5. Ceftiofur MIC values* for mastitis pathogens from diagnostic laboratories in the U.S. and Canada

Organism	Reference**	No.	Date Isolated	MIC_{90}*** (μg/mL)	MIC range (μg/mL)
Staphylococcus aureus	1	135	1991-92	1.0	0.13 to 2.0
	2	10	1993	1.0	0.25 to 1.0
	3	107	1995	1.0	0.25 to 2.0
	5	61	2000	1.0	≤ 0.06 to 2.0
Coagulase (-) staphylococci	6	139	2000-2001	1.0	≤ 0.06 to 2.0
Streptococcus dysgalactiae	1	15	1991-92	1.0	≤ 0.06 to 2.0
	2	15	1993	≤ 0.0039	No range†
	4	152	1997-1999	0.25	0.25 to 4.0
	5	64	2000	≤ 0.06	≤ 0.06 to 0.5
Streptococcus uberis	1	22	1991-92	0.5	≤ 0.06 to 4.0
	2	15	1993	0.03	≤ 0.0039 to 0.06
	4	133	1997-1999	0.5	0.5 to 8.0
	5	20	2000	1.0	< 0.06 to 2.0
Escherichia coli	1	39	1991-92	1.0	0.25 to 1.0
	2	40	1993	0.5	0.13 to 1.0
	5	52	2000	0.5	≤ 0.06 to 1.0

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

Table 2.6. 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)	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

*All testing performed using a 30 μg disk.

References:

1. Watts, J.L., S.A. Salmon, C.A. Case, and R.J. Yancey Jr. Antimicrobial susceptibility of microorganisms isolated from the mammary glands of dairy heifers. Upjohn Technical Report 705-7923-94-018, dated September 8, 1994.
2. Salmon, S.A., J.L. Watts, and R.J. Yancey Jr. *In vitro* activity of ceftiofur and its primary metabolite, desfuroylceftiofur, against organisms of veterinary importance. Upjohn Technical Report 705-7923-94-015, dated July 25, 1994.

3. Watts, J.L., C.A. Case, and S.A. Salmon. Activity of penicillin/novobiocin and pirlimycin against β-lactamase-negative and -positive strains of *Staphylococcus aureus* isolated from bovine intramammary infections. Pharmacia & Upjohn Technical Report 705-7923-95-017 dated December 15, 1995.
4. Rossitto, P.V., L. Ruiz, Y. Kikuchi, K. Glen, K. Luiz, J.L. Watts, and J.S. Cullor. Streptococcal antibiotic susceptibility: Antibiotic susceptibility patterns for environmental streptococci isolated from bovine mastitis in central California dairies. Pharmacia & Upjohn Study Report No. a0093503, dated March 2001.
5. Salmon, S.A., L. Ruiz, K.S. Glen, J.W. Hallberg, and J.L. Watts. *In vitro* activity of ceftiofur, pirlimycin, and comparators against bovine mastitis pathogens isolated from dairy cows in the United States and Canada. Pharmacia & Upjohn Study Report No. a0093317, dated March 2, 2001.
6. Salmon, S.A., E. Ruiz, K.S. Glen, and M.B. Wachowski. *In vitro* activity of ceftiofur, penicillin/novobiocin, pirlimycin, and comparators against bovine mastitis pathogens isolated at the time of dry cow therapy from dairy cows in the United States. Pharmacia & Upjohn Study Report No. a0096850, dated June 20, 2001.

3. TARGET ANIMAL SAFETY:

a. Udder Irritation in Lactating Dairy Cows Following Intramammary Infusion of a Sterile Formulation of Ceftiofur HCl (PNU-64279A) Containing 500 mg of Ceftiofur Free Acid Equivalents per 10 mL Plastet, the Maximum Effective Dose for Dry Cow Therapy. Pharmacia & Upjohn Study Report No. a0095242.

1. Type of Study: Target animal safety study to assess udder irritation in lactating dairy cows. The study was performed in accordance with Good Laboratory Practice (GLP) requirements.

2. Study Director: William M. Moseley, Ph.D., WW Animal Health Product Development, Pharmacia Animal Health, Kalamazoo, MI

3. Study Design:

    a. Objective: To assess udder irritation following intramammary infusion of 500 mg ceftiofur HCl once in all four quarters of lactating dairy cattle and milked out 12 hours after treatment.

    b. Animals: Twenty-seven healthy, lactating adult female Holstein dairy cows, approximately 2-5 years of age, in their first or greater lactation.

    c. Experimental Design: Lactating dairy cows were grouped by parity and production and assigned to receive 500 mg ceftiofur HCl by intramammary infusion once into each quarter. Two control cows were left untreated. Only cows with negative milk cultures, clinically normal udders (no abnormal milk and no evidence of inflammation), and low SCCs (less than 200,000 cells/mL) throughout the three day pre-treatment observation period were enrolled.

    d. Test Article Administration: The test article was ceftiofur hydrochloride sterile suspension (500 mg of ceftiofur per 10 mL), administered as an intramammary infusion once in each quarter.

    e. Measurements and Observations: The study duration was 14 days (27 milkings), including a 3-day pre-treatment period. Measurements included individual quarter somatic cell counts, milk weights, strip cup observation of the milk, udder palpation, and rectal temperatures.

4. Results: The cow was the experimental unit in this study. Post-treatment SCCs were compared to pre-treatment values. Cows, regardless of lactation or production level, exhibited a transient rise in SCC following treatment. SCCs remained elevated above pre-treatment levels throughout the 10 days following treatment but remained below the 200,000 cells/mL threshold the National Mastitis Council associates with inflammation. No clinical signs of mastitis (udder swelling or abnormal milk appearance) were observed. Rectal temperature (as a systemic measure of inflammation) and milk production were not affected by ceftiofur treatment.

5. Conclusions: These data support the conclusion that the sterile oil-based formulation containing 500 mg ceftiofur per 10 mL is safe and not irritating to mammary tissue in lactating dairy cattle when infused as a single intramammary dose in each quarter.

b. Determination of Residue Concentrations of Ceftiofur in the Milk of the Dairy Cow After Freshening and Post-Infusion Udder Irritation Following the Administration of Ceftiofur HCl (PNU-64279A) into the Udder at Dry Off and in the Kidney and Liver Tissues of the Neonatal Calf. Pharmacia & Upjohn Study Report No. a0086834.

1. Type of Study: Residue study. Animal safety observations conducted during the study to assess udder irritation in non-lactating (dry) dairy cows are summarized here. Results of residue analyses are summarized in Section 4 (see 4.b.1.b., page 14). The study was performed in accordance with Good Laboratory Practice (GLP) requirements.
2. Study Director: Rex E. Hornish, Ph.D., Animal Health Preclinical Development, Pharmacia Corp., Kalamazoo, MI
3. Study Design:
    a. Objective: To assess udder irritation following intramammary infusion of 250 mg or 500 mg of ceftiofur HCl into all four quarters of dairy cows at the end of lactation (dry off).
    b. Animals: Seventy-four healthy pregnant adult female Holstein dairy cattle, approximately 2 to 7 years of age, at the end of their first or greater lactation.
    c. Experimental Design: The cow was the experimental unit. The design of this study was a 2 x 2 factorial with lactation and length of dry period being the two factors with repeated measures within the cow. Thirty-six cows were administered 250 mg of ceftiofur into each of the four quarters of the udder at dry off. Thirty-eight cows were administered 500 mg of ceftiofur into each of the four quarters of the udder at dry off. A control group was not included.
    d. Test Article Administration: The test article was ceftiofur hydrochloride sterile suspension, (250 or 500 mg of ceftiofur per 10 mL), administered as an intramammary infusion once in each quarter.
    e. Measurements and Observations: Udders were evaluated for visible signs of irritation for 14 days following infusion.
4. Results: Two cows in the 250 mg ceftiofur/quarter group were disqualified from the post-freshening phase of the study because they developed either milk fever or mastitis. Three cows in the 500 mg ceftiofur/quarter group were disqualified from the post-freshening phase of the study because they freshened greater than 70 days post-treatment.

No clinical evidence of irritation was observed in the udders of any of the treated animals in either the 250 mg ceftiofur/quarter or 500 mg ceftiofur/quarter groups during the 14-day post-infusion observation period.

5. Conclusions: The study supports the conclusion that the sterile formulation containing up to 500 mg ceftiofur per 10 mL is safe and not irritating to the udder of non-lactating dairy cattle when infused as a single intramammary dose in each quarter.

c. Adverse Effects Observed in Multi-location Field Studies:

Field Dose Confirmation and Dose Determination Study for Ceftiofur Hydrochloride as a Dry Cow Treatment. Study No. 792-7922-O-JWH-98-001; Report No. a0093462. January 2000-February 2001.

In this study, described in the Effectiveness section (*2*.a.1), 51 cows were treated with 500 mg ceftiofur/quarter as an intramammary infusion at the time of dry off. No drug-related adverse reactions were reported.

Preliminary Examination of Efficacy for Intramammary Formulation of Pirlimycin Hydrochloride, Ceftiofur Hydrochloride and Ceftiofur Crystalline Free Acid as Dry Cow Therapies. Report No. 705-9690-98-001. August 1996-June 1997.

In this study, described in the Effectiveness section (*2*.a.2), 73 cows were treated with 500 mg ceftiofur/quarter as an intramammary infusion at the time of dry off. No drug-related adverse reactions were reported.

4. HUMAN FOOD SAFETY:

a. Toxicology:

1. General Toxicology

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

2. Microbial Safety

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

b. Residue Chemistry:

1. Residue Chemistry Studies

a. Total Residue Metabolism Study

1) Principal Investigators: R.E. Hornish and T.S. Arnold, Pharmacia Animal Health, Kalamazoo, MI

2)

Animal Species:	Bovine
Breed/Sex:	Holstein cows, lactating
Number of Animals:	14; five sacrificed at 0-day (12 hours), and three each at 2 days, 4 days, and 6 days post-last-treatment
Stage of Lactation:	Middle of 2^{nd} or 3^{rd} lactation
Health Status:	Healthy, mastitis free

3) Route of Administration: Intramammary (IMM)

4) Dose Rate: 125 mg ceftiofur HCl/quarter in all four quarters

5) Duration of Dosing: Two doses/quarter at a 24-hour interval

6) Radioisotope: ^{14}C located in the thiazole ring

7) Total Ceftiofur-Related Residue Disposition and Depletion Data: Samples of milk collected at 12-hour intervals were assayed for total ^{14}C residue by direct liquid scintillation counting (LSC) techniques. Samples of all edible tissues

(liver, kidney, muscle, and fat) and udder (injection site) were assayed for total ^{14}C residue by combustion analysis and LSC techniques. Samples of urine and feces collected at 12-hour intervals were also radioassayed for total ^{14}C residue by these techniques. The concentration of total ^{14}C residue in the tissues at the various time points and the recovery of the dose in the various fluids and solids are summarized in Table 4.1.

Table 4.1: Total ^{14}C-residue concentration (mean ±SD) by combustion analysis and liquid scintillation counting

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

Acceptable radiolabel accountability was demonstrated.

8) Milk Residue Depletion: Milk samples were assayed by a variety of assays; LSC for total ^{14}C-related residue, a cylinder-plate assay to measure the biological activity of the residue, HPLC methods to measure the desfuroylceftiofur-related residue (HPLC-DCA), and an HPLC-profiling procedure to assess the relative amounts of parent ceftiofur and of other metabolites in milk (HPLC-RAM). These results are summarized in Table 4.2.

Table 4.2. Concentration of ceftiofur residues in milk by several assays

Milking # and 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	2.79 ± 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	0.56 ± 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

LOQ = 0.015 µg/mL (HPLC-DCA); 0.02 µg/mL (cylinder plate); 0.01 µg/mL (HPLC-RAM)

9) Metabolic Profiles: 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 5th (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.

b. Study Establishing the Milk Discard Period in Freshened Dairy Cows

The following study addressed the milk discard time in terms of when the marker residue (desfuroylceftiofur) fell below the tolerance of 100 ppb in milk.

1) Principal Investigator: R.E. Hornish, Pharmacia Animal Health, Kalamazoo, MI

2)

Animal Species:	Bovine
Strain/Breed:	Holstein
Sex:	Female, lactating
No. of Animals:	74 treated, 70 healthy freshened
Stage of Lactation:	1st-4th lactation, mid lactation
Health Status:	Normal healthy cows

3) Route of Administration: Intramammary (IMM)

4) Dose Rate: 250 mg/quarter in all four quarters (36 cows)
   500 mg/quarter in all four quarters (38 cows)

5) Duration of Dosing: A single IMM infusion at the time of dry cow therapy approximately 30-60 days prepartum.

6) Ceftiofur Residues as Desfuroylceftiofur-Related Residue Depletion Data in Milk: Residues of ceftiofur were measured using the determinative HPLC-DCA method for desfuroylceftiofur-related residues. The results are summarized in Table 4.3.

Table 4.3. Analysis of milk for desfuroylceftiofur-related residues following a single IMM administration of ceftiofur HCl at 250 or 500 mg/quarter into all four quarters

Milking After Freshening for Cows With Freshening Interval of ≥ 30 Days	Mean Concentration Found, μg/mL*	
	250 mg/quarter	500 mg/quarter
Milking #1	< 0.015	< 0.015
Milking #2	< 0.015	< 0.015
Milking #3	< 0.015	< 0.015
Milking #4	< 0.015	< 0.015
Milking #5	< 0.015	< 0.015

* LOQ = 0.050 μg/mL, LOD = 0.015 μg/mL.

c. Study Establishing the Pre-Slaughter Withdrawal Period in the Non-Lactating Dairy Cow and in the Neonatal Calf

The following two studies addressed the pre-slaughter withholding time in terms of when the marker residue (DCA) decreased below the tolerance of 8.0 μg/g in kidney, below 2.0 μg/g in liver, and below 1.0 μg/g in muscle:

Residue Study 1 – Residues in the Treated Cow

1) Principal Investigator: R.E. Hornish, Pharmacia Animal Health, Kalamazoo, MI

2) Animal Species: Bovine
   Strain/Breed: Holstein
   Sex: Female, lactating
   No. of Animals: 15 treated
   Stage of Lactation: 1^{st}-2^{nd} lactation, at dry off
   Health Status: Normal healthy cows

3) Route of Administration: Intramammary (IMM)

4) Dose Rate: 500 mg/quarter into all four quarters

5) Duration of Dosing: One full four-quarter treatment once at dry off

6) Ceftiofur Residue as Desfuroylceftiofur-Related Residue Depletion Data in Tissues: Residues of ceftiofur as desfuroylceftiofur-related residue were measured using the determinative HPLC-DCA method. The results are summarized in Table 4.4.

Table 4.4. Mean concentration of desfuroylceftiofur-related residue (HPLC-DCA assay) in tissues following the single IMM administration of 500 mg ceftiofur HCl per quarter into all four quarters at dry off

Tissue	Tolerance (μg/g)	Mean Concentration, μg/g (LOQ = 0.10 μg/g)		
		1-Day (n=5)	3-Day (n=5)	7-Day (n=5)
Kidney	8.0	1.87 ± 1.10	0.594 ± 0.322	0.462 ± 0.232
Liver	2.0	0.710 ± 0.410	0.212 ± 0.110	0.160 ± 0.070
Muscle	1.0	0.101 ± 0.052	< 0.10*	< 0.10

* assay validated to 0.10 μg/g.

Residue Study 2 – Residues in the Neonatal Calf

1) Principal Investigator: R.E. Hornish, Pharmacia Animal Health, Kalamazoo, MI

2) Animal Species: Bovine
   Strain/Breed: Holstein
   Sex: Female, lactating
   No. of Animals: 74 cows treated - all male and twin-female calves were slaughtered (47 total)
   Stage of Lactation: 1^{st}-2^{nd} lactation, at dry off
   Health Status: Normal healthy cows

3) Route of Administration: Intramammary (IMM)

4) Dose Rate: 250 mg/quarter into all four quarters (36 cows),
   500 mg/quarter into all four quarters (38 cows)

5) Duration of Dosing: full four-quarter treatment approximately 30-60 days prepartum

6) Slaughter Groups: 1 day (27 male and twin-female calves) and 4 days (20 male calves) postpartum

7) Ceftiofur Residue as Desfuroylceftiofur-Related Residue Depletion Data in Tissues: Residues of ceftiofur were measured using the determinative HPLC-DCA method. The results are summarized in Table 4.5.

Table 4.5. Analysis of calf tissue for ceftiofur residue by HPLC-DCA assay

Dose Group	Freshening Interval	No. of Calves per Slaughter Time	Mean Concentration, μg/g (LOQ = 0.10 μg/g)			
			Day 1 Slaughter Group		Day 4 Slaughter Group	
			Liver	Kidney	Liver	Kidney
250 mg/quarter	26-46 days	10 and 6	< 0.10	< 0.10	< 0.10	< 0.10
250 mg/quarter	53-63 days	4 and 4	< 0.10	< 0.10	< 0.10	< 0.10
500 mg/quarter	32-45 days	10 and 7	< 0.10	< 0.10	< 0.10	< 0.10
500 mg/quarter	49-64 days	3 and 3	< 0.10	< 0.10	< 0.10	< 0.10

2. Target Tissue and Marker Residue Assignments

The target tissue and marker residue assignment are codified under 21 CFR 556.113.

3. Tolerance Assignments

Tissue	Concentration
Milk	100 ppb
Kidney	8 ppm
Liver	2 ppm
Muscle	1 ppm
Fat	Not Established

4. Withdrawal and Milk Discard Assignments

a. Calculation of a Milk Discard Period

The study described in 1.b. above provides the data needed to determine the milk discard period for treated cows. The mean concentration of desfuroylceftiofur-related residues in the milk from cows freshening with a minimum 30-day dry cow period is essentially non-detectable and well below the tolerance of 100 ng/mL. Therefore, these data support a zero milk discard if a cow has a minimum 30-day dry cow period following the IMM infusion of up to 500 mg ceftiofur HCl/quarter into all four quarters at dry off.

b. Calculation of the Pre-Slaughter Withdrawal Period for the Treated Cow

The first study described in 1.c. above, *Residue Study 1 – Residues in the Treated Cow*, provides the data needed to determine the pre-slaughter withdrawal period for treated cows. The tissue residue data were analyzed by a statistical method which determines the statistical tolerance limit for the 99th percentile of the population with a 95% confidence as outlined in the *Agency's Guideline for Establishing a Withdrawal Period.* These data support a 3-day pre-slaughter withdrawal period.

c. Calculation of the Pre-Slaughter Withdrawal Period for the Neonatal Calf

The second study described in 1.c. above, *Residue Study 2 – Residues in the Neonatal Calf*, above provides the data needed to determine the pre-slaughter withdrawal period for neonatal calves born to treated cows. The data demonstrated that there are no detectable residues in any tissues of the neonatal calf. Therefore, these data support a 0-day pre-slaughter withdrawal period for neonatal calves born to treated cows with a minimum dry period of 30 days.

c. Microbial Food Safety:

Microbial food safety information for ceftiofur hydrochloride was evaluated using a qualitative risk assessment procedure. The dosage regimen evaluated was 500 mg of ceftiofur as a single infusion per affected quarter. The indication associated with the dosage regimen is "for the treatment of subclinical mastitis in dairy cattle at the time of dry off associated with *Staphylococcus aureus*, *Streptococcus dysgalactiae,* and *Streptococcus uberis.*"

The qualitative risk assessment procedure involved conducting: 1) a release assessment to describe the probability that ceftiofur hydrochloride and its use in non-lactating dairy cattle will result in the emergence of resistant bacteria or resistance determinants in treated non-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. Analytical Method for Residues:

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:

Discard empty container: Do not reuse. 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 DC Sterile Suspension (ceftiofur hydrochloride), when administered as an intramammary infusion, is safe and effective for the treatment of subclinical mastitis in dairy cattle at the time of dry off associated with *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*.

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 subclinical 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 DC Sterile Suspension - PLASTET Label
B. SPECTRAMAST DC Sterile Suspension – Carton Label
C. SPECTRAMAST DC Sterile Suspension - Package Insert