FREEDOM OF INFORMATION SUMMARY

Naxcel® Sterile Powder
(ceftiofur sodium)

"…for the control of early mortality
associated with *E. coli* in day-old turkey poults"

supplemental new animal drug application

Sponsored by:
The Upjohn Company

April 1996

I. GENERAL INFORMATION

A. NADA Number:

140-338

B. Sponsor:

The Upjohn Company
7000 Portage Road
Kalamazoo, Michigan 49001

C. Accepted Name:

ceftiofur sodium

D. Trade Name:

Naxcel® Sterile Powder

E. Marketing Status:

A prescription (Rx) product which carries the following caution statement "Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian."

F. Effect of Supplement:

Provides for an additional species, day-old turkey poults, to be added to the previously approved product, Naxcel® Sterile Powder; also provides for revision of the chick indication.

II. INDICATIONS FOR USE

Naxcel® Sterile Powder is indicated for the control of early mortality associated with E. coli organisms susceptible to ceftiofur in day-old chicks and day-old turkey poults.

III. DOSAGE FORM, ROUTE OF ADMINISTRATION, AND DOSAGE

A. Dosage Form

Naxcel® Sterile Powder is available in two package sizes: 1-gram and 4-gram vials.

Reconstituted product should be used within 12 hours if stored at controlled room temperature 15 to 30 °C (59 to 86 °F) or within 7 days if stored in a refrigerator at 2 to 8 °C (36 to 46 °F).

1-gram vial: Reconstitute with 20 milliliters (mL) sterile water for injection. Each mL of the resulting solution contains ceftiofur sodium equivalent to 50 mg ceftiofur.

4-gram vial: Reconstitute with 80 mL sterile water for injection. Each mL of the resulting solution contains ceftiofur sodium equivalent to 50 mg ceftiofur.

B. Route of Administration

Naxcel® Sterile Powder is to be mixed with sterile water and should be administered as a single subcutaneous injection.

C. Approved Dose Range

Naxcel® Sterile Powder should be administered to day-old turkey poults at a dosage of 0.17 to 0.5 mg per poult. Treatment should be administered once.

IV. EFFECTIVENESS

A. Pivotal Studies - Dose Determination

1. Overview:

Naxcel® Sterile Powder (ceftiofur sodium) was approved in August 1992 (NADA 140-338) for use in day-old chicks for control of colibacillosis associated with E. coli susceptible to ceftiofur at a dosage range of 0.08 to 0.20 mg ceftiofur/chick. After the efficacy of Naxcel® Sterile Powder was demonstrated in chicks, a development program was initiated for the control of early mortality associated with E. coli organisms susceptible to ceftiofur in day-old turkey poults.

Since pathogenesis, lesions and bacterial strains associated with early mortality due to E. coli are similar in poults and chicks, an effective dose in day-old turkey poults was determined by comparing plasma pharmacokinetic data from chicks and turkey poults. The primary plasma pharmacokinetic parameters of interest were AUC (area under the curve estimated to the last quantifiable value), Cmax (maximum plasma concentration), and Tmic (time above minimum inhibitory concentration for E. coli). The approach was to determine the dose in turkey poults that produces ceftiofur and desfuroylceftiofur metabolite concentrations (measured as derivatized desfuroylceftiofur acetamide) over time which correspond to the plasma concentration time profile produced in the chick for a known efficacious dose in chicks.

Use of this method of dose extrapolation requires an assumption of similar interspecies protein binding characteristics of the active moieties (TR 812-7926-94-002), comparable partition characteristics of drug from blood to lung (TR 812-7926-92-001 and 812-7926-94-003), similar pathogen response to ceftiofur and its desfuroylceftiofur metabolites (TR 705-7922-90-005 and 705-7923-95-009) and similar host responses to the pathogens (literature survey). The validity of these assumptions has been supported by the results of both pivotal and non-pivotal studies.

The dose-response relationships of the plasma pharmacokinetic parameters for both the turkey poult and the chick results were modeled as simple linear regressions. Using the fitted regression equations, the upper 90% confidence limit corresponding with the pharmacokinetic parameter associated with a dose of 0.08 mg/bird in chicks was matched to the turkey dose associated with the lower 92.5% confidence limit. Since Cmax and Tmic resulted in almost identical dose estimates, the modeled Cmax values for chicks and turkey poults were used to predict a dose in the turkey poult corresponding to the low end of the approved chick label dose range (0.08 mg ceftiofur/chick). The

predicted poult dose corresponding to a chick dose of 0.08 mg ceftiofur/chick is 0.17 mg ceftiofur/poult. The upper dose of the dose range (0.5 mg/poult) for day-old turkey poults is easily justified based on drug residue and target animal safety data.

Minimum inhibitory concentration (MIC) determinations show that the in vitro susceptibility of E. coli isolated from chicks and turkey poults to ceftiofur is similar. In addition, ceftiofur and its desfuroylceftiofur metabolite, demonstrated equal in vitro potency against E. coli..

2. Pharmacokinetics: Chicks

   a. Type of Study: These reports (Upjohn Technical Report 812-7926-91-003, Upjohn Technical Report 812-7926-95-003, Upjohn Technical Report 812-7926-95-004) describe the pharmacokinetics of total ceftiofur active moiety in plasma, liver, lung and yolk sac of chicks following a single subcutaneous injection of 14C-ceftiofur sodium at 0.04, 0.08 or 0.16 mg ceftiofur free acid equivalents/chick.

   b. Investigators: J.L. Nappier, G.A. Hoffman, J.F. Caputo, S.T. Chester, D.R. Reeves, T.F. Flook, T.S. Arnold, and T.D. Cox. The Upjohn Company, Kalamazoo, MI 49001

   c. General Design:

      (1) Purpose of Study: To determine pharmacokinetics of total ceftiofur active moiety in plasma, liver, lung and yolk sac after administration of a single subcutaneous injection of ceftiofur sodium to chicks.

      (2) Animals: chicks; Vantress Cross

      - 80 males and 80 females per treatment group
      - 30 to 40 g each
      - 48 to 60 hours old

      (3) Experimental Design: Chicks (5 birds per cage; mixed sex) were randomly assigned to one of three treatment groups (0.04, 0.08 or 0.16 mg ceftiofur/chick) for each week over four weeks.

      (4) Sampling: Chicks were euthanatized at 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, or 8.0 hours post-treatment. The yolk sac, liver, and lungs were collected as composite tissues from each of the five replicate chicks per cage and stored at -20°C until processed. Blood was collected as a composite sample (5 chicks within a cage) during euthanasia and was processed into plasma immediately (within 2 hours) after collection.

      (5) Assay Method: The samples were assayed by HPLC equipped with an ultraviolet (UV) detector and a radioactive monitor. The limit of quantitation for the chick plasma assay method is 0.093 µg/mL (Upjohn Technical Report 812-7926-95-004).

      (6) Dosage Form: A stock solution was prepared by dissolving purified 14C ceftiofur sodium in water for injection. The dose solutions were prepared by diluting the stock solution with water for injection.

(7) Pharmacokinetic Analysis Model: The pharmacokinetic parameters of interest were Cmax , AUC, and Tmic. The Cmax was defined as the first observed maximum plasma concentration that occurred post 0.1 hour. The 0.1-hour time point was excluded because incomplete mixing of the blood pool at this time point might have produced an erroneous Cmax. The AUC was determined by a trapezoidal algorithm implemented in SAS®, and was determined only to the last time point. The Tmic values were calculated by fitting the raw data (from 2 to 8 hours) to a mono-exponential model using the SAS NLIN procedure. The Tmic was then calculated from the following fitted model:

$$\frac{[LN(MIC90) - LN(A)]}{-k}$$

Where A equals the Y intercept based upon the fitted data and k equals the elimination rate constant based on the concentrations from Hours 2 to 8 postdose. An acceptance criteria for r2 was not established due to the random fluctuations associated with the plasma concentration versus time profile.

d. Results: Chick plasma pharmacokinetic data for the three dose levels of ceftiofur are presented in Table 4.1. AUC, Cmax and Tmic estimates were confirmed to be dose proportional within the dosage range of 0.04 to 0.16 mg/bird.

Table 4.1. Major plasma pharmacokinetic endpoints (means ± standard deviation) in chicks following a single subcutaneous injection of ceftiofur sodium at three dose levels.

Dose (mg/chick)	Cmax (µg/mL)	AUC (µg·hr/mL)	Tmic (hr)
0.04	0.562 ± 0.014	2.78 ± 0.94	1.95 ± 1.54
0.08	1.665 ± 0.125	6.13 ± 1.91	7.59 ± 5.51
0.16	2.724 ± 0.678	9.82 ± 2.07	8.40 ± 1.30

e. Conclusions: The dose-proportionality of the three pharmacokinetic measures, AUC, Cmax and Tmic is consistent with the use of linear regression models as a method for interspecies dose extrapolation. Therefore, these three measures were used to predict an appropriate low end for the dose range for poults.

3. Pharmacokinetics: Turkey Poults

a. Type of Study: These reports (Upjohn Technical Report 812-7926-94-003, Upjohn Technical Report 812-7926-95-003, Upjohn Technical Report 812-7926-95-004) describe the plasma pharmacokinetics of ceftiofur and desfuroylceftiofur metabolites (measured as derivatized desfuroylceftiofur acetamide) in the plasma of turkey poults following a single subcutaneous injection of 14C-ceftiofur sodium at 0.12, 0.24 or 0.48 mg ceftiofur free acid equivalents/poult.

b. Investigators: J.L. Nappier, G.A. Hoffman, J.F. Caputo, S.T. Chester, T.S. Arnold, T.D. Cox, T.F. Flook, R.L. Janose, and V.R. Lewis. The Upjohn

Company, Kalamazoo, Michigan 49001

c. General Design:

(1) Purpose of Study: To determine ceftiofur and desfuroylceftiofur metabolite (measured as derivatized desfuroylceftiofur acetamide) plasma pharmacokinetics after administration of ceftiofur sodium to turkey poults. These results and the pharmacokinetic data from chicks were used to determine an efficacious dose in turkeys poults.

(2) Animals: turkey poults; Kent Broad-Breasted Whites

- 72 males and 72 females per treatment group
- 42 to 83 g each
- 48 to 60 hours old

(3) Experimental Design: Poults (3 birds per cage; mixed sex) were randomly assigned to one of three treatment groups (0.12, 0.24 or 0.48 mg ceftiofur per poult) for each of 6 replicates.

(4) Sampling: Poults were euthanatized at 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, or 12 hours post-treatment. Blood was collected as a composite sample (3 birds within a cage) during euthanasia and was processed into plasma immediately after collection. The plasma was stored frozen until assayed.

(5) Assay Method: The samples were assayed by HPLC equipped with an ultraviolet (UV) detector and a radioactive monitor. The limit of quantitation of the plasma assay method is 0.189 µg/mL (Upjohn Technical Report 812-7926-95-004).

(6) Dosage Form: A dose solution was prepared by combining 14C ceftiofur sodium with NAXCEL® Sterile Powder (ceftiofur sodium).

(7) Pharmacokinetic Analysis Model: The pharmacokinetic parameters of interest were Cmax , AUC, and Tmic. The Cmax value was defined as the first observed maximum plasma concentration that occurred post 0.1 hour. The AUC was determined by a trapezoidal algorithm implemented in SAS®, and was determined only to the last time point. The TMIC value was calculated by fitting the raw data (from 2 to 12 hours) to a mono-exponential model using the SAS® NLIN procedure. The TMIC was then calculated from the following fitted model:

$$\frac{[LN(MIC90) - LN(A)]}{-k}$$

where A = the Y intercept based upon the fitted data and k equals the elimination rate constant based on the concentrations from Hours 2 to 12 postdose. An acceptance criteria for r2 was not established due to the random fluctuations associated with the plasma concentration versus time profile.

d. Results:

A summary of the pharmacokinetic data for the three dose levels of ceftiofur in turkey poults is presented in Table 4.2. As seen with chicks, these measures suggest a linear correlation between dose and total blood ceftiofur concentrations, thereby justifying the use of linear regression models for the extrapolation of a minimum effective dose in poults. The target pharmacokinetic endpoints (means and upper 90% confidence limits) for the 0.08 (lowest approved dose in chicks), 0.12 and 0.20 mg/chick (maximum approved dose in chicks) are presented in Table 4.3. For a particular pharmacokinetic endpoint, the across-week-average intercept and slope, as well as the sample variance-covariance estimates, were computed. The dose at which the poult's lower 92.5% prediction interval equaled the target of the endpoint (corresponding to chick doses of 0.08, 0.012 and 0.20 mg/bird) is shown in Table 4.4.

The pharmacokinetic endpoints (Cmax, AUC and Tmic) did not estimate the exact same dose. These differences were in part attributable to error introduced by comparing AUC values estimated over different time point ranges (0 to 8 hr in chicks; 0 to 12 hr in poults). However, from dose estimates based on Cmax and Tmic values, it was concluded that this interspecies extrapolation would be most accurate if based upon observed Cmax values.

Table 4.2. Major plasma pharmacokinetic endpoints (means ± standard deviation) in turkey poults following a single subcutaneous injection of ceftiofur sodium at three dose levels.

Dose (mg/poult)	Cmax (µg/mL)	AUC (µg·hr/mL)	Tmic (hr)
0.12	1.674 ± 0.662	13.84 ± 4.49	8.50 ± 3.70
0.24	4.259 ± 1.276	22.77 ± 4.61	19.99 ± 11.45
0.48	9.071 ± 2.564	46.14 ± 18.86	15.97 ± 7.43

Table 4.3. Target pharmacokinetic values, represented as means and 90% levels (in parentheses), for selected chick doses of ceftiofur sodium

Variable	Dose of ceftiofur sodium (mg/chick)		
	0.08	0.12	0.20
Cmax (µg/mL)	1.60 (1.75)	2.43 (2.80)	4.08 (4.91)
AUC (µg·hr/mL)	6.44 (7.83)	9.45 (11.28)	15.46 (18.76)
Tmic (hr)	5.75 (7.82)	8.40 (10.15)	13.72 (15.31)

Table 4.4. Predicted poult doses of ceftiofur sodium (mg/poult) based on the mean and 90% upper confidence limit (in parentheses) for chick pharmacokinetic responses at various chick doses

Variable	Dose of ceftiofur sodium (mg/chick)		
	0.08	0.12	0.20
Cmax (µg/mL)	0.16 (0.17)	0.22 (0.24)	0.34 (0.42)
AUC (µg·hr/mL)	0.08 (0.10)	0.14 (0.21)	0.26 (0.36)
Tmic (hr)	0.15 (0.19)	0.21 (--2)	--2 (--2)

e. Conclusions: The poult doses corresponding to the chick doses of 0.08, 0.12, and 0.20 mg/chick as determined by comparison of the Cmax values are 0.17, 0.24 and 0.42 mg/poult, respectively.

4. Minimum Inhibitory Concentration (MIC) Study with E. coli isolated from chicks

a. Type of Study: In this study (Upjohn Technical Report 705-7922-90-005) the in vitro susceptibility of 223 E. coli strains to ceftiofur was determined. These strains of E. coli were isolated from broiler chicks collected in the conduct of a field efficacy study. The isolates were collected at eight locations in the US.

b. Investigators: L.K. Klein, R.J. Yancey, Jr., C.A. Case, T.R. Schriemer, and J.M. Brown. The Upjohn Company, Kalamazoo, MI 49001

c. General Design:

(1) Purpose of Study: To determine the in vitro susceptibility of microorganisms associated with early chick mortality to ceftiofur.

(2) Microorganisms: The microorganisms used in this study were obtained from cull chicks and chicks that became moribund during the course of a field study in which ceftiofur was evaluated for control of terminal bacterial infections in day-old broiler chickens.

(3) Test Method: The MIC determinations were conducted by the microdilution broth procedure using cation-supplemented Mueller-Hinton broth. The MIC50, MIC90 and MIC range for each species were determined. In addition to the clinical isolates, the following National Committee on Clinical Laboratory Standards (NCCLS) recommended American Type Culture Collection (ATCC) isolates were included as quality control strains: Staphylococcus aureus (ATCC 29213), Enterococcus faecalis (ATCC 29212), E. coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853).

d. Results: The MIC50 and MIC90 for ceftiofur against 223 E. coli isolates tested in this study are 0.25 and 0.5 µg/mL, respectively. The range of MICs for ceftiofur against E. coli field isolates and quality control organisms are shown in Table 4.5.

Table 4.5. Range of Minimum Inhibitory Concentration (MICs) for ceftiofur (µg/mL) for E. coli isolated from chicks and quality control strains

Organism	MIC Range
E. coli (Field isolates)	≤0.06 to 1.0
S. Aureus (ATCC 29213)	0.13 to 1.0
E. faecalis (ATCC 29212)	1 to 64
E. coli (ATCC 25922)	≤0.06 to 0.25
P. Aeruginosa (ATCC 27853)	8 to 32

e. Conclusions: The results of the in vitro susceptibility testing indicate that E. coli strains isolated from day-old chicks at different locations were

susceptible to ceftiofur.

5. Minimum Inhibitory Concentration (MIC) Study with E. coli isolated from poults

   a. Type of Study: In this study (Upjohn Technical Report 705-7923-95-009) the in vitro susceptibility of 802 E. coli isolates to ceftiofur was determined. These E. coli strains were isolated from moribund or dead poults collected in the conduct of an eight location field efficacy study.

   b. Investigators: J.L. Watts, S.A. Salmon, K.R. Goodenough, and B. L. Lee. The Upjohn Company, Kalamazoo, MI 49001

   c. General Design:

      (1) Purpose of Study: To determine the in vitro susceptibility of microorganisms associated with early poult mortality to ceftiofur.

      (2) Microorganisms: The microorganisms used in this study were obtained from poults that became moribund or died during the course of a field study in which ceftiofur was evaluated for control of terminal bacterial infections in day-old turkey poults.

      (3) Test Method: The MIC determinations were conducted by the microdilution broth procedure using cation-supplemented Mueller-Hinton broth. The MIC50, MIC90 and MIC range for each species were determined. In addition to the clinical isolates, the following NCCLS recommended ATCC isolates were included as quality control strains: S. aureus (ATCC 29213), E. faecalis (ATCC 29212), E. coli (ATCC 25922), P. aeruginosa (ATCC 27853).

   d. Results: The MIC50 and MIC90 for ceftiofur against 802 E. coli isolates tested in this study are 0.5 and 2.0 µg/mL, respectively. The MICs for 87.8% of these isolates range from 0.25 to 1.0 µg/mL. The range of MICs for ceftiofur against E. coli field isolates and quality control organisms are shown in Table 4.6.

      Table 4.6. Range of Minimum Inhibitory Concentration (MICs) for ceftiofur (µg/mL) for E. coli isolated from poults and quality control strains

Organism	MIC Range
E. coli (field isolates)	≤0.03 to >32
S. Aureus (ATCC 29213)	1 to 2
E. faecalis (ATCC 29212)	2 to 8
E. coli (ATCC 25922)	0.51
P. Aeruginosa (ATCC 27853)	16 to >32

      1No range, all MICs were the same

   e. Conclusions: The similar in vitro susceptibility of E. coli isolated from chicks and turkey poults to ceftiofur support the use of comparative pharmacokinetic data from the chick and poult to select a dose for poults.

6. Comparison of in vitro susceptibility of E. coli to ceftiofur and desfuroylceftiofur

   a. Type of Study: In this study (Upjohn Technical Report 705-7923-93-007)

the in vitro susceptibility of E. coli isolated from infected turkey poults to ceftiofur sodium and its active desfuroylceftiofur metabolite, was determined.

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

c. General Design:

(1) Purpose of Study: To determine the in vitro susceptibility of E. coli isolated from turkey poults to ceftiofur and desfuroylceftiofur.

(2) Microorganisms: The 40 E. coli isolates used in this study were originally isolated form infected turkey poults and are stored in the Upjohn Animal Health Discovery Research culture collection.

(3) Test Method: The MIC determinations were conducted using the NCCLS microbroth method. Mueller-Hinton broth (Sensititre®; Westlake, OH) was used as the growth medium. In addition to the clinical isolates, the following NCCLS recommended ATCC isolates were included as quality control strains: S. aureus (ATCC 29213), E. faecalis (ATCC 29212), E. coli (ATCC 25922), P. aeruginosa (ATCC 27853). The MIC50, MIC90 and MIC range were determined.

d. Results: As shown in Table 4.7, ceftiofur and desfuroylceftiofur have equal in vitro potency against E. coli.

Table 4.7. Summary of Minimum Inhibitory Concentrations (MIC) of ceftiofur and desfuroylceftiofur against 40 E. coli Isolates

	Ceftiofur (µg/mL)	**Desfuroylceftiofur (µg/mL)**
MIC50	0.25	0.25
MIC90	0.5	0.5
Range	0.125-1.0	0.06-1.0
Mode	0.25	0.25

e. Conclusions: The similar in vitro susceptibility of E. coli isolated from turkey poults to ceftiofur and desfuroylceftiofur support the use of comparative pharmacokinetic data from the chick and poult to select a dose for poults.

B. Pivotal Study - Dose Confirmation/Field Trial

1. Type of Study:A multi-location dose confirmation/clinical field study was conducted using Naxcel® Sterile Powder in one-day-old turkey poults. The study was conducted at eight sites; seven in the United States and one in Canada.

2. Investigators:

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3. General Design:

   a. Purpose: To evaluate the effectiveness of Naxcel® Sterile Powder administered to day-old turkey poults for control of early poult mortality associated with E. coli organisms susceptible to ceftiofur.

   b. Animals: Day-old BUTA and Nicholas large white turkeys from commercial hatcheries.

   c. Experimental Design:

      The study included eight trial sites. A randomized complete block design with 12 blocks in each of the trial sites was used. The experimental unit

was a pen containing 50 poults (same sex). A block consisted of four adjacent pens. Within each block, the treatment design was a 2 x 2 factorial. The factors of the factorial design were experimental group (control and ceftiofur) and sex (male and female poults). The design included a total of 19,200 poults (9,600 males and 9,600 females); the actual number of poults placed was 19,189 (9,589 males and 9,600 females).

d. Experimental Groups:

- Group 1 (control) Male poults injected with 0.2 mL Sterile Water for Injection
- Group 2 (control) Female poults injected with 0.2 mL Sterile Water for Injection
- Group 3 (ceftiofur) Male poults injected with a single dose of 0.17 mg of ceftiofur free acid equivalents in 0.2 mL Sterile Water for Injection.
- Group 4 (ceftiofur) Female poults injected with a single dose of 0.17 mg of ceftiofur free acid equivalents in 0.2 mL Sterile Water for Injection

e. Diagnosis: Morbidity/mortality was defined as having been associated with E. coli only if the final diagnosis by the poultry diagnostician indicated that E. coli was a causative microorganism, and all of the following conditions were met: 1) death occurred after treatment was initiated, 2) lesions indicative of E. coli were present, and 3) culture results were positive for E. coli..

f. Dosage Form of Test Articles: The dosage form was an aqueous solution containing 50 mg ceftiofur per mL (stock solution). The stock solution was prepared using Naxcel® Sterile Powder and Sterile Water for Injection. The stock solution was diluted with Sterile Water for Injection to prepare a dose solution of 0.17 mg/0.2 mL.

g. Route of Administration: Test articles were administered as a single subcutaneous injection in the back of the neck.

h. Dose: 0.17 mg/poult

i. Test Duration: 14 days at each site

j. Pertinent Variables Measured: The pivotal response variable was poult morbidity/mortality associated with E. coli, and was defined as the cumulative number of moribund plus dead poults in a pen over the 14 day trial period divided by the total number of poults in the pen at the beginning of the trial.

4. Results: The mean cumulative percent morbidity/mortality associated with E. coli for the control and ceftiofur group throughout the 14-day test period for all trials are shown in Table 4.8.

Table 4.8. Mean percent morbidity/mortality for all trials*

Dose ceftiofur (mg)	Morbidity / mortality	
	Total	E. coli-positive
0	6.7 (565/8387)	4.1 (341/8387)
0.17	4.0 (336/8352)	1.9 (158/8352)

*Data from one site not included due to technical problems.

5. Statistical Analysis

The pivotal response variable was analyzed by the method of weighted analysis of variance with trial location being considered random. The weight was the number of poults in a pen. There was no significant treatment group x sex interaction at $\alpha = 0.05$, so the effects of groups were tested by averaging across sex. To conclude that ceftiofur at 0.17 mg/poult was efficacious, the pivotal response variable for the ceftiofur group had to be significantly lower compared to the control group at $\alpha = 0.05$. The analysis of the pooled data (excluding the one site mentioned above) showed that ceftiofur at 0.17 mg/poult significantly ($p = 0.038$) reduced the morbidity/mortality associated with *E. coli* compared to the control group.

6. Conclusion:

It was concluded that Naxcel® Sterile Powder is effective at the 0.17 mg dose for control of early mortality associated with *Escherichia coli* (colibacillosis) susceptible to ceftiofur in one-day-old turkey poults.

7. Adverse Reactions: No adverse reactions were observed.

V. ANIMAL SAFETY

A. Pivotal Study

1. Target Animal Safety Study

a. Type of Study: This was a 7-day study in turkeys in which a single injection of Naxcel® Sterile Powder at a dose of 0, 100, 400, or 800 mg/kg of body weight was administered subcutaneously at 1 day of age followed by a 7-day observation period. The study was conducted and inspected according to Good Laboratory Practice (GLP) regulations.

b. Study Director:

Charles E. Hunt, DVM, Ph.D.
The Upjohn Company
301 Henrietta
Kalamazoo, Michigan 49001

c. General Design:

(1) Purpose: This study was designed to determine the toxicological effects of ceftiofur administered by subcutaneous injection to turkeys.

(2) Animals: A total of 258 one-day-old Nicholas broad-breasted white turkey poults were used for this study. Twenty-nine/sex (initial) and 10/sex (repeated) Nicholas broad-breasted white day-old turkey poults

were assigned to each of four treatment groups.

(3) Dosage Form of Test Articles: The dosage form was an aqueous solution containing 25, 100, or 200 mg ceftiofur/mL prepared using Naxcel® Sterile Powder and Sterile Water for Injection. The 0-mg dose group received Bacteriostatic Sodium Chloride for Injection.

(4) Dose: The doses were 0, 100, 400, or 800 mg ceftiofur/kg body weight.

(5) Route of Administration: Test articles were administered by subcutaneous injection.

(6) Study Duration: The study spanned 7 days beginning at 1 day of age.

(7) Pertinent measurements/observations:

Daily clinical observations, mortality, hematological measurements, (Day 4), body weight (control period and terminal), gross necropsy, and histopathology were evaluated to assess potential toxicity in turkeys for all birds in the initial portion of the study.
Due to problems encountered during the analysis of the blood samples collected on Day 4 from the birds in the initial portion of the study, additional poults were treated and blood samples collected four days later. Since birds used in the repeated portion of the study were intended to provide only the hematological measurements (Day 4) not obtained from the initial part of the study, birds from the repeated part of the study were not necropsied. Findings listed in the Results section below were derived from data collected from birds used in the initial portion of the study, with the exception of the hematological results which were obtained from birds in the repeated portion of the study.

d. Results:

(1) Clinical Observations

Of the three dosages tested, only the 800 mg/kg dose produced clinical signs of toxicity, including depression, ataxia and prostration. No clinical signs were noted in any of the mid dose poults (400 mg/kg). One female poult in the low dose group (100 mg/kg) showed mild signs of depression.

(2) Mortality

The only mortalities that appeared to be drug related occurred in the high dose group. Table 5.1 summarizes poult mortality for the initial portion of the study.

Table 5.1. Mortality by study day* and sex for poults administered an aqueous solution of Naxcel® Sterile Powder (ceftiofur sodium) subcutaneously

Day	Dose Group							
	0 mg/kg		100 mg/kg		400 mg/kg		800 mg/kg	
	M	F	M	F	M	F	M	F
1	0/29	0/29	0/20	0/20	0/20	0/20	0/20	0/20
2	0/29	0/29	0/20	0/20	0/20	0/20	8/20	3/20
3	0/29	0/29	0/20	0/20	0/20	0/20	6/12	3/17
4	0/29	0/29	0/20	1/19	0/20	0/20	0/6	0/14
5	0/29	0/29	0/20	0/19	0/20	0/20	0/6	0/14
6	0/29	0/29	0/20	0/19	0/20	0/20	0/6	0/14
7	1/29	0/29	0/20	0/19	0/20	0/20	0/6	0/14
TOTAL	1/29	0/29	0/20	1/20	0/20	0/20	14/29	6/29

*for initial portion of study

(3) Body Weight

Poults from the 800-mg/kg dose group showed reduced gains at Day 4, probably due to toxicity. There were no adverse effects on weight gain in the other treatment groups.

(4) Hematology

Blood samples were collected and analyzed on Day 4 from birds in the repeated portion of the study. There was no evidence of treatment effects on red blood cell count, hemoglobin or hematocrit.

(5) Gross Necropsy Observations

Only birds in the 800-mg/kg group showed gross changes in tissues that were thought to be drug related. Observations included: large blood clots surrounding the heart (5/20 males, 1/20 females); roughened, white heart surface (4/20 males, 1/20 females); white peritoneal surface (5/20 males, 2/10 females); liver and intestinal adhesions (3/20 males, 1/20 females). Additional observations (also from the high dose group) included mottled or pale liver with attached small pale or red nodules, and fluid in the thoracic or abdominal cavity.

(6) Histological Observations

There were no histological observations that were treatment related in the 100- or 400-mg/kg treatment groups. Microscopic lesions that were thought to be indications of drug related toxicity in the 800-mg/kg group included: mineralization of the heart muscle, necrosis of the heart muscle, and alterations of the lymphoid tissue in the bursa of Fabricius, spleen, and thymus.

e. Conclusion

The subcutaneous injection of ceftiofur sodium to day-old turkey poults at 100 and 400 mg/kg [twelve times (12X) and fifty times (50X) the

maximum label dose] caused no adverse effects that were believed to be drug related in this study. This, in conjunction with the lack of adverse effects in the clinical field trial indicates that Naxcel® Sterile Powder is safe when used in day-old turkeys in accordance with label directions.

B. Corroborative Study: Pivotal Dose Confirmation Field Trial (Section 4.B., page 11)

VI. HUMAN SAFETY

A. Toxicity Studies:

All issues concerning toxicity testing of ceftiofur are addressed in the previous Freedom of Information Summary for NADA 140-338, the approval notice for which appeared in the Federal Register on April 12, 1990 (55 FR 13768): Freedom of Information Summary for Naxcel® Sterile Powder (ceftiofur sodium) for Bovine Respiratory Disease. This summary was updated in June 1992.

B. Safe Concentration of Total Residues:

1. No Observed Effect Level (NOEL):

   Studies include mutagenicity, oral feeding, and hypersensitivity studies. The lowest no observed effect level (NOEL) from the 90-day oral feeding studies in both dogs and rats was 30 mg ceftiofur per kg body weight (bw). Since the drug is considered a Low Use Drug intended for therapeutic use in specific animals and because it has undergone extensive safety testing, chronic toxicity studies were not required. Thus, the ADI was based, without the 25 µg/kg bw/day limitation, on the 90-day studies; a safety factor of 1000 is used in the safe concentration calculations.

2. Calculation of Allowable Daily Intake (ADI)

$$Allowable\ Daily\ Intake\ (ADI) = \frac{Lowest\ NOEL}{Safety\ Factor}$$

   The lowest NOEL is 30 mg/kg, so:

$$ADI = \frac{30\ mg/kg}{1000}$$

$$ADI = 0.03 mg/kg\ bw/day$$

3. Allocation of ADI:

   The revised General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals (FDA-CVM July 1994) provides for the reservation of a portion of the ADI for milk. Since 1) ceftiofur sodium is approved for parenteral use in lactating dairy cattle, 2) both salts, hydrochloride and sodium, accede to the same primary metabolite, and 3) total residues are indistinguishable quantitatively or qualitatively, the ADI for both salts is the same.

The portion of the ceftiofur ADI reserved for milk is 27%.
ADI for milk = 27% x total ADI
= 0.27 x 0.03 mg/kg body weight (bw)/day
= 0.008 mg/kg body weight/day

ADI for edible tissues = total ADI - ADI for milk
= 0.030 mg/kg bw/day - 0.008 mg/kg body weight//day
= 0.022 mg/kg bw/day

4. Safe Concentration (SC) Calculations: The following are the new SC for edible tissues of turkey:

$$Safe\ Concentration(SC) = \frac{Acceptable\ Daily\ Intake\ (ADI) \times Human\ Weight}{Consumption\ Factor}$$

The average human weight is approximated as 60 kg. The daily consumption values of tissues are approximated as 300 g muscle, 100 g liver, 50 g kidney, and 50 g skin/fat.

$$SC\ (muscle) = \frac{0.022mg/kg/bw/day \times 60kg}{300\ g/day} = 4.40mg/kg = 4.40\ ppm$$

$$SC\ (liver) = \frac{0.022mg/kg/bw/day \times 60kg}{100\ g/day} = 13.20\ mg/kg = 13.20\ ppm$$

$$SC\ (skin/fat) = \frac{0.022mg/kg/bw/day \times 60kg}{50\ g/day} = 26.40\ mg/kg = 26.40\ ppm$$

Using the revised food consumption factors, the permitted Safe Concentrations for total residues in edible tissues from turkeys are as summarized in Table 6.1.

Table 6.1. Safe Concentrations for total residues in edible tissues from turkeys

Tissue	Daily Consumption (grams)	Calculated Safe Concentration (ppm)
Muscle	300	4.40
Liver	100	13.20
Skin/Fat	50	26.40

C. Total Residue Depletion and Metabolism Studies

1. Investigators: G.A. Hoffman, J.L. Nappier, C. Ho, T.J. Gilbertson, M.A. Travis, T.S. Arnold, R.L. Janose, T.D. Cox, T.F. Flook, and V.R. Lewis. The Upjohn Company, Kalamazoo, MI 49001

2. Animals: Twenty-four Kent broad-breasted white turkeys (12 males and 12 females) were used in the study. Poults were 48 to 60 hours old at time of treatment and weighed 52 to 76 grams.

3. Dose: Each poult received a single subcutaneous dose containing 0.091 mg 14C ceftiofur sodium in 0.2 mL in the dorsal side of the neck.

4. Results: The total residue concentrations of 14C-ceftiofur free acid equivalents in edible tissues of turkey poults at 21 days post injection are shown in Table 6.2. Analysis of the tissues at 21 days post treatment revealed that all residues were significantly below the safe concentration for ceftiofur (4.4, 13.2, 26.4, ppm for muscle, liver, and skin/fat, respectively).

Table 6.2. Mean concentrations of 14C-ceftiofur sodium in pooled edible tissues (n = 23) at 21 days post injection of a single dose of 0.091 mg/poult compared to Safe Concentrations for edible tissues of turkeys.

Tissue	Safe Concentration (ppm)	Mean Concentration (ppm)
Muscle	4.40	0.03553 ± 0.013
Liver	13.20	0.00300 ± 0.001
Skin/Fat	26.40	0.00977 ± 0.015

5. Conclusion: This residue study was conducted at approximately two times the maximum dose level. Since total ceftiofur residue levels for edible tissues at 21 days after treatment are markedly below (over 1000 times) the safe concentration, this study supports assignment of a zero-day withdrawal period.

D. Metabolism and Comparative Metabolism

The metabolism and comparative metabolism requirements in turkey poults are minimal and are waived for the following reasons:

1. Under current commercial practices, tom turkeys are marketed at approximately 122 days, and hen turkeys are marketed at approximately 98 days.

2. Ceftiofur sodium will only be used in day-old turkey poults which provides an inherent withdrawal time of at least 21 days.

3. The total residues at 21 days post treatment were well below the safe concentration.

E. Tolerance for the Marker Residue

Total residues at 21 days following the injection of ceftiofur sodium in turkey poults are markedly lower than the calculated safe concentrations. Therefore, a tolerance is not required.

F. Withdrawal Time

The residue data showed that the mean concentrations of total ceftiofur residues at 21 days post treatment were significantly below the safe concentration (over 1000 times lower) for each of the edible tissues of turkey poults treated with approximately two times the maximum dose. Poultry husbandry practices assure that no turkey poult will be handled more than once and therefore would not receive more than one injection, nor will the poults be brought to market prior to

21 days post treatment. Therefore, a withdrawal period will not be required for the use of ceftiofur sodium in day-old turkey poults. Since a withdrawal period will not be required, a target tissue, marker residue, and tolerance have not been assigned.

G. Regulatory Method

An official regulatory method is not required because the toxicology and residue data support a zero-day withdrawal for the use of Naxcel® Sterile Powder (ceftiofur sodium) in day-old turkey poults.

VII. Agency Conclusions

The data submitted in support of this NADA supplement satisfy the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that Naxcel® Sterile Powder (ceftiofur sodium), when administered as a single subcutaneous injection to one-day-old turkeys, is safe and effective for the control of early mortality associated with *E. coli* organisms.

This supplement also provides for a change in the wording for the chick indication. Wording for the chick indication has been modified to more closely resemble that for the poult. Accordingly, the indications for both the chick and the turkey poult are: "...for the control of early mortality associated with E. coli organisms susceptible to ceftiofur".

The Allowable Daily Intake (ADI) for this product was revised reserving 27 percent of the ADI for milk. The safe concentrations were recalculated using the new consumption values. The safe concentrations for total residues of ceftiofur are 4.40 ppm in muscle, 13.20 ppm in liver, and 26.40 ppm in skin/fat. The total residue data showed that the mean concentrations of total ceftiofur residues at 21 days after injection were well below the permitted safe concentration in the edible tissues of growing turkey poults. Husbandry practices for turkeys are such that they will only be handled at one day of age for injection with ceftiofur and not enter the human food chain until 98 or more days of age. Therefore, a withdrawal period will not be required for this use of Naxcel® Sterile Powder in day-old turkey poults. An official regulatory method is not required because the residue and toxicology data support a zero-day withdrawal.

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 control early mortality associated with E. coli organisms, and (b) the rate of emergence of ceftiofur-resistant organisms may be reduced by the involvement of veterinarians in product use.
Public health concerns associated with potential increases in antimicrobial resistance have been satisfactorily addressed by establishing conditions of use intended to minimize inappropriate use of this product, and excretion of ceftiofur and ceftiofur-resistant zoonotic pathogens into the environment.

The agency has carefully considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human

environment and that an environmental impact statement is not required. The agency's finding of no significant impact (FONSI) and the evidence supporting that finding are contained in an environmental assessment, which may be seen in the Docket Management Branch (HFA 305), Parklawn Building (Room 1-23), 12420 Parklawn Dr., Rockville, Maryland 20857.

Under the Center's supplemental approval policy (21 CFR 514.106(b)(2)(v) and (vii)), these are Category II changes. The approval of these changes is not expected to have any adverse effect on the safety or effectiveness of this new animal drug and, therefore, did not require a reevaluation of the human food or target animal safety data in the parent application.

Under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act, this approval for turkey poults qualifies for three years of marketing exclusivity beginning on the date of approval because the application contains reports of new clinical or field investigations and new human food safety studies essential to the approval of the application and conducted or sponsored by the applicant.

Naxcel® Sterile Powder is under patent number U.S. 4464367, expiring August 7, 2001.

VIII. Approved Labeling

A copy of the draft facsimile labeling is attached to this document.

1. naxcel® Sterile Powder 1-Gram Vial Label
2. naxcel® Sterile Powder 1-Gram Packer Front Label
3. naxcel® Sterile Powder 1-Gram Packer Back Label
4. naxcel® Sterile Powder 1-Gram Packer Top Label
5. naxcel® Sterile Powder 1-Gram Packer End Label
6. naxcel® Sterile Powder 4-Gram Vial Label
7. naxcel® Sterile Powder 4-Gram Packer Front Label
8. naxcel® Sterile Powder 4-Gram Packer Back Label
9. naxcel® Sterile Powder 4-Gram Packer Top Label
10. naxcel® Sterile Powder 4-Gram Packer End Label