I. GENERAL INFORMATION

A. File Number

NADA 140-338

B. Sponsor

The Upjohn Company
7000 Portage Road
Kalamazoo, Michigan 49001

C. Proprietary Name

Naxcel Sterile Powder

D. Established Name

ceftiofur sodium sterile powder

E. Form, Route of Administration, and Recommended Dosage

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°-30°C (59°-86°F) or within 5 days if stored in a refrigerator.

1 gram vial
Reconstitute with 20 ml Sterile Water for Injection or with Bacteriostatic 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 or with Bacteriostatic Water for Injection. Each ml of the resulting solution contains ceftiofur sodium equivalent to 50 mg ceftiofur. Naxcel Sterile Solution should be administered by intramuscular injection to cattle at the dosage of 0.5 mg ceftiofur per pound of body weight (1 ml reconstituted sterile solution per 100 lb body weight). Treatment should be repeated every 24 hours for a total of three treatments. Additional treatments may be given on days four and five for animals which do not show a satisfactory response (not recovered) after the first three treatments.

F. Indication

Naxcel Sterile Powder is indicated for treatment of bovine respiratory disease (shipping fever, pneumonia).

G. Effect of Supplement

This supplemental application provides for a change in the approved dosage of 0.5 mg/lb body weight to a range of 0.5 mg to 1.0 mg ceftiofur sodium/lb of body weight.

II. EFFECTIVENESS

A. Pivotal Study

A seven location clinical trial was conducted. This included 629 cattle with spontaneously occurring disease and three treatment groups. These were 1) non-medicated control, 2) ceftiofur at 0.5 mg/lb and 3) ceftiofur at 1.0 mg/lb body weight. Placebo and ceftiofur treatments were administered by intramuscular injection once per day for three or five days. Results indicated that each of the two ceftiofur doses (0.5 and 1.0 mg/lb) was significantly better than placebo. The two ceftiofur dosed groups provided similar responses.

1. Multi-location Clinical Trial

   a. Type of Study

      This study was a multi-location clinical trial in cattle with spontaneously occurring disease under typical feedlot conditions.

   b. Investigators

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   c. General Design

      (i) Purpose of this multi-location study was to determine the efficacy of ceftiofur sodium at 0.5 mg and 1.0 mg/lb body weight given intramuscularly for three to five days for the treatment of BRD under typical feedlot conditions.

      (ii) Animals: -629 head of feedlot cattle ranging in weight from 281 to 562 lbs.

      - calves ranged from six to 10 mos. in age
      - typical feedlot animals, mixed steers and bulls
      - 209 head per treatment group

(iii) Control was a non-drug sterile diluent administered intramuscularly at 8 ml/animal for three consecutive days.

(iv) Diagnosis: BRD was diagnosed by an elevated body temperature (104.5°F or greater) and the presence of at least two additional signs of the disease such as depressed physical appearance, increased or labored respiration, cough and loss of appetite. Nasal swabs were taken from each animal just prior to treatment to determine presence of BRD pathogens.

(v) Dosage Form: Dosage form was an aqueous solution containing 50 mg ceftiofur equivalents per ml.

(vi) Route of Administration: Intramuscular.

(vii) Dose: Control was the sterile vehicle (Bacteriostatic Water for Injection) given intramuscularly at 8 ml per animal. Ceftiofur was administered intramuscularly either at 0.5 or 1.0 mg/lb body weight three or five consecutive days. On day four of the trials, the investigators evaluated the condition of all surviving animals and determined whether or not they were recovered. If they were deemed "recovered", no further medication was given. If they were judged "not recovered" two more daily treatments were administered on days four and five. This procedure also included placebos.

(viii) Test Duration: This was a 28 day study.

(ix) Pertinent Parameters Measured: -body temperature drop at day 4

- clinical success at day 10
- clinical success at day 28
- mortality by day 28

d. Results

Nasal swabs were taken for pre-treatment culture and organism identification from 618 cattle used in this study.

The most commonly isolated pathogen was Pasteurella hemolytica which was present in 385 (47%) of the specimens. Pasteurella multocida was found in 163 (20%) of the swabs. Haemophilus somnus was present in 41 (5%) and Mycoplasma spp. in 309 (37%). There were 210 (25%) cattle from whose nasal swabs no organisms could be detected.

Treatment Means

Treatment	no.	Temperature Drop Day 4 (Degrees F)	Temperature Day 4 (Degrees F)	Percent Clinical Success Day 10	Percent Clinical Success Day 28	Percent Mortality Day 28
0.00	204	1.2	104.0	29	57	25
0.5 mg/lb ceftiofur	201	2.1	103.1	53	69	7
1.0 mg/lb ceftiofur	201	2.2	103.0	61	69	3
LSD-1 sided	-	.227	.226	7.4	7.4	5.1
LSD-2 sided	-	.270	.270	8.8	8.8	6.1

Based on necropsy results, all but two of the 79 calves which died during the 28 day study were caused by typical BRD syndrome. Of the two, one was diagnosed as septicemia and the other anemia, dehydration and nutritional deficiency complicated by the stress of handling and shipping.

e. Statistical Analysis

A randomized complete block design was used with five animals of similar body temperatures and similar body weights being assigned per block. Treatments (3) were randomized within each block. All variables were analyzed according to the following Analysis of Variance:

Source	DF	Test Term	Decision
Location	6	--	--
Block	180		
Doses	2	--	--
0 vs 0.5 mg/lb	(1)	error +	significance at one-sided .05 infers 0.5 dose is efficacious
0 vs 1.0 mg/lb	(1)	error +	significance at one-sided .05 infers 1.0 dose is efficacious
0.5 vs 1.0 mg/lb	(1)	error +	significance at one-sided .05 infers 1.0 dose is more effective than 0.5 dose
Pooled Error	417*		
dose* location	(12)	pure error	significance at .25 warrants use of dose*location as test term for contrasts
Pure error	405**		

+ error = dose*location if P < .25 for test of dose* location
* df = 400 for variables temperature reduction, and temperature on day four
** df = 388 for variables temperature reduction, and temperature on day four

Interpretations: Body temperature drop - Both ceftiofur treatment groups were significantly improved relative to non-medicated control, not different from each other.

Success rate at day 10 - Ceftiofur treatment groups were each significantly better than non-medicated control, and not significantly different from each other.

Success rate at day 28 - Ceftiofur treatment groups were each significantly better than non-medicated controls and not different from each other.

Mortality at day 28 - Ceftiofur treatment groups were each control and not significantly better than non-medicated different from each other.

Significance level used P ¾ 0.05.

f. Conclusions Results from this study demonstrated that ceftiofur at doses of either 0.5 or 1.0 mg/lb was effective for treatment of BRD under typical feedlot conditions.

g. Adverse Reactions

Except for an immediate and transient local pain reaction to the intramuscular injection of ceftiofur, there was no evidence of any adverse effect.

h. Special Issues: None

B. Corroborative Studies: (None)

III. TARGET ANIMAL SAFETY

A. Summary

Three specifically designed Target Animal (cattle) Safety Studies were conducted to address the tolerance to and safety of ceftiofur. Additionally, a clinical efficacy trial was conducted wherein target animal safety was also determined. Based on results of these seven trials, ceftiofur is safe when administered intramuscularly at doses up to 1.0 mg ceftiofur per lb body weight daily for five days. Local pain reaction to the injection is deemed transient and not detrimental to safety of an already sick animal.

B. Pivotal Studies

1. Safety/Toxicity Study

a. Type of Study

This was a five day study in cattle where drug was administered at daily doses of 0, 1 mg lb, 3 mg/lb, or 5 mg/lb body weight to determine the pharmacologic and toxicologic effects of ceftiofur following the intramuscular injection.

b. Investigator

Study Director:

A. D. Hall, D.V.M.
The Upjohn Company

Kalamazoo, Ml 49001

c. General Design

Purpose of this study was to determine the pharmacologic and toxicologic effects of ceftiofur following the intramuscular injection in the bovine. Potential target organs and tissues were to be identified through clinical observations, histologic examination, hematology and serum chemistries. Red blood cell counts, hemoglobin, packed cell volume, serum creatinine and blood urea nitrogen values were also parameters of interest. Finally, injection site irritation was evaluated by use of histopathology and serum creatinine phosphokinase (CPK).

(i) Animals

crossbred beef type steers
body weight range 370-450 lb
20 animals for four treatment groups

(ii) Control was a non drug containing sterile diluent administered intramuscularly daily at 5 ml/100 lb body weight.

(iii) Dosage Form - sterile aqueous solution containing 100 mg drug/ml.

(iv) Dosage Used - 0, 1, 3 or 5 mg/lb body (e.g., 1, 3, or 5 ml sterile solution containing 100 mg/ml per 100 lbs. Controls received 5 ml per 100 lb body weight.

(v) Route of Administration - intramuscular

(vi) Test Duration - 7 days

(vii) Pertinent Parameters Measured

Clinical observations, hematologic and serum chemistry measurements, body weight changes, organ weights and gross and microscopic evaluations were used to assess the potential toxicity in the steers.

d. Results

(i) Clinical Observations

No clinical signs of toxicity or illness were observed during this study. Occasionally a steer would react to the injection of the compound by vocalizing and/or exhibiting a limp on the injected rear leg for the first few strides out of the squeeze chute. The limp was always transient and was no longer noticeable by the time the steer returned to its pen.

(ii) Hematology

Statistical analysis of the data identified a slight but significant decrease in hematocrit (HCT) and hemoglobin (HGT) values when the high dose animals were compared to controls. However, the pretest hematology data contained a comparable statistically significant difference In hematocrit values between the control and high dose

group. It should also be noted that the hematocrit and hemoglobin values decreased similarly in all groups, including the untreated controls, over the duration of the study. All mean hematologic values were within the normal biologic range for the bovine, and since there was no significant decrease in red blood cell numbers, the hematologic differences noted were not attributed to treatment.

(iii) Serum Chemistries

Significantly elevated CPK values (P ¾ .05) were observed in the high dose animals when compared to the controls. The CPK values also increased in the 3 mg/lb group but were not statistically different from the control value. In contrast, the untreated control and 1 mg/lb group CPK values were similar. The elevated CPK values correlated with the muscle damage at the injection site which was observed histopathologically. There were no other treatment related differences between treated and controls regarding clinical chemistry measurements.

(iv) Body Weights

The five day treatment regimen with U 64,279 had no significant effect on body weight in any of the groups.

(v) Organ Weights

There were no significant differences between control and any of the dose groups for any of the five organs weighed.

(vi) Gross and Histologic Observations

The only gross change associated with the use of this drug was the focal areas of hemorrhage and/or muscle discoloration at the injection sites. Occasional, focal lung lesions were observed, but the incidence was uniformly distributed among the treatment groups. Drug-related histopathologic changes were confined to the skeletal muscle at the injection sites. There appeared to be greater muscle fiber degeneration and localized inflammation at the highest dose. Localized edema, hemorrhage, mononuclear inflammatory cell infiltration and myofibril degeneration and mineralization were observed at the injection sites, leading to the morphologic diagnosis of mild to severe subacute necrotizing myositis. However, this study was not designed to correlate sequential histopathologic changes in the intramuscular injection sites with time.

Two steers in the 5 mg/lb group had evidence of a mild abomasitis. One lesion was composed of a mild infiltrate of eosinophils, plasma cells and lymphocytes and may have been a response to a parasite or a piece of plant material that might have become lodged in that area of the abomasum. The other lesion was a focal lymphoid reaction that was probably triggered by a local irritation. The findings of abomasitis appeared to be unrelated tissue changes with no evidence of being an effect of treatment.

Other histopathologic findings were seen with similar frequencies in all dose groups and were not attributable to treatment.

Frequencies of Various Findings

Organ and Diagnosis	Sex	Group 1	Group 2	Group 3	Group 4
Skeletal Muscle: Examined:	M	5	5	5	5
Myositis Chronic Localized Severe	M	0	0	1	0
Myositis Subacute Localized Mild	M	4	0	0	0
Myositis Subacute Localized Moderate	M	1	2	1	0
Myositis Subacute Localized Severe	M	0	3	3	5

Group 1 Vehicle Control
Group 2 U 64,279, 1 mg/lb/day
Group 3 U 64,279, 3 mg/lb/day
Group 4 U 64,279, 5 mg/lb/day

e. Statistical Analysis A randomized complete block design was used with a pen of four similar animals representing each block. There were five blocks.

Source	Difference	Testing Term
Treatment	3	Error
Blocks	4	---
Error	12	

The hypothesis of no treatment effect associated with the measurements of most interest, RBC count, Hgb, PCV, BUN and serum creatinine, was tested at the .10 level of significance. Planned contrasts of dose groups with control were one-sided tests at the .10 level of significance, since only increases in BUN and serum creatinine and decreases In RBC count, Hgb, and PCV were of concern. The hypothesis of no treatment effect associated with the remainder of the variables was tested at the .05 level. Planned contrasts of dose groups with control were two sided at the .05 level. Organ weights were also analyzed using least squares analysis of variance.

The histopathologic data were analyzed using Fisher's Exact test at the .10 level of significance.

f. Conclusions

No systemic drug related effects were detected following Intramuscular administration of aqueous U 64,279 at dosages of 1, 3 and 5 mg/lb of body weight for 5 days, as was evidenced by the various parameters that were monitored during this study.

Gross and microscopic tissue changes were confined to localized areas of muscle damage at the injection site. This lesion was most pronounced at the highest dose tested (5 mg/lb) which correlated well with the only significantly elevated (P ¾ .05) CPK value.

2. Five Day Tolerance Study in Feeder Calves

   a. Type of Study

      This was a five day tolerance study in feeder calves. Formulated drug was administered intramuscularly up to 25X the highest proposed labeled dose.

   b. Investigator

      Study Director:

      T. J. Kakuk
      Path and Tox Research
      The Upjohn Company
      Kalamazoo, MI 49001

   c. General Design

      Purpose of this tolerance study was to determine the pharmacotoxic effects of formulated freeze-dried ceftiofur following intramuscular injection at an exaggerated daily dosage of 25X the highest labeled dose (1.0 mg/lb body weight) for five consecutive days.

      (i) Animals

      - crossbred beef type animals (steers).
      - starting weight ranged from 182-316 kg and age ranged from 6-10 months
      - eight animals total, four used as controls and four given 25X drug treatment

      (ii) Control was bacteriostatic water for injection (vehicle used as diluent for making sterile solution from the ceftiofur sterile powder) 50 ml per 100 lb body weight.

      (iii) Control and treatment each administered intramuscularly.

      (iv) Dose of 25 mg/lb body weight given in a 50 mg/ml formulation or 50 ml per 100 lb body weight.

      (v) Test period was 15 days, first five as daily treatments of either 25 mg/lb or vehicle.

      (vi) Pertinent Parameters Measured:

      (vii) Toxicological evaluation was predicated on clinical signs including body weight changes, 6 hematological and 17 clinical chemistry measurements, and gross pathologic observations. Tests of statistical significance were made at the $P < 0.05$ level.

d. Results

All of the calves survived. Formulated ceftiofur did not cause any overt signs of toxicity during the five day dosing period or during the 10 day post treatment observation interval. The only changes were the significant elevations in aspartate transaminase (AST) and in creatinine phosphokinase (CPK) in both vehicle and drug treated animals on study day five relative to pretreatment baseline values. These AST and CPK values returned to baseline by day 15 of the study. These transient elevations depict local muscle irritation associated with the formulated drug and the vehicle per se .

e. Statistical Analyses

The hematologic and clinical chemistry parameters were analyzed by the BMDP repeated measure routine to test the hypothesis that there is no difference between the means of dose groups. The adjustments were made for the initial values of the parameters (covariate). Tests of statistical significance were made at the 0.05 level.

A time by treatment t-test supported general findings of the repeated measures analysis.

f. Conclusions

In conclusion, formulated ceftiofur diluted in Bacteriostatic Water for Injection, USP with Benzyl Alcohol 0.945% w/v was well tolerated in feeder calves at 25 times the highest recommended dosage of 1.0 mg/lb/day (25 mg/lb/day) for 5 consecutive days. Results showed that formulated ceftiofur administered intramuscularly had no systemic toxic effects. Local effects of muscle irritation were detected after the last dose (5 consecutive daily doses) as evidenced by significant elevated AST and CPK values. However, these transient elevated values returned to baseline values nine days post treatment.

3. 15 Day Intramuscular Safety/Toxicity Study

a. Description

In this study cattle were administered formulated ceftiofur at daily doses up to 10X the highest proposed doses for 15 days, which is 3X the longest proposed duration of use. Toxicological assessment was based on clinical signs, hematological and clinical chemistry measurements, urinalysis measurements and gross and microscopic evaluations. The study included 5M and 5F, each at daily doses 0, 1, 3, 5 or 10 mg/lb body weight.

b. Study Director:

T. J. Kakuk
The Upjohn Company
Kalamazoo, MI 49001

c. General Design

Purposes of this study were to determine the pharmacotoxic effects of formulated freeze-dried ceftiofur sodium following intramuscular injection at the recommended and exaggerated daily doses given for an exaggerated duration and to measure the potential local muscle irritancy properties of the formulated product.

(i) Animals

- 5 M and 5 F per treatment group
- crossbred beef type
- Initial weights ranged from 180-290 kg
- age at start of trial ranged from 6-12 months

(ii) Dose Form: The product was a reconstituted sterile solution injected intramuscularly. A freeze-dried sterile powder was reconstituted with Bacteriostatic Water for Injection, USD to make the resulting solution.

(iii) Dose Used: Daily doses of 1, 3, 5 or 10 mg drug/lb body weight was given intramuscularly using a sterile solution which contained - 50 mg/drug/ml. Controls were administered ~ 30 ml vehicle per hd/day (based on volume the mg/lb treatment group would receive).

(iv) Route: Intramuscular Injection (no injection site was injected with more than 10 ml; when > 10 ml was indicated multiple sites of the same anatomical area were used).

(v) Test Duration: 15 days; animals were sacrificed on day 16 and necropsied.

(vi) Pertinent Parameters Measured

Toxicological evaluation was based on clinical signs including body weight changes, hematological and clinical chemistry measurements, urinalysis measurements and gross and microscopic evaluation. All tests of statistical significance were made at the $P < 0.05$ level.

d. Results

There were no systemic effects attributable to formulated ceftiofur sodium based on clinical signs, hematological, clinical chemistry and urinalysis parameters and on gross and microscopic determinations.

Local effects of muscle irritation were evidenced by histopathologic evaluation. Lesions were microscopic in size and difficult to find even with the aid of injection site markers.

Percent Calves (of 10) with Findings

Day Post Injection	Findings	Vehicle	Treatment Groups - 1.0 mg/lb	Treatment Groups - 3.0 mg/lb
1	None	10	30	30
1	Acute Myositis	60	40	40
1	Subacute Myositis	30	30	30
1	Chronic Myositis	--	--	--
3	None	0	30	40
3	Acute Myositis	0	20	0
3	Subacute Myositis	100	40	60
3	Chronic Myositis	--	10	--
7	None	0	40	30
7	Chronic Myositis	100	60	70
14	None	100	80	70
14	Chronic Myositis	0	20	30

Statistical Evaluation Data for each sex were analyzed separately. The body weight was analyzed by BMDP repeated measures routine to test the following null hypothesis: (i) there is no difference between the means of dose groups, (ii) the dose groups behave the same over time. The organ weight, ratio organ weight to body weight, blood chemistry and hematology variables were analyzed by one way analysis of variance. Whenever the analysis indicated a dose difference, a Dunnett's t-test was used to compare each active group to the control group. All tests of statistical significance were made at $P<0.05$ level.

e. Conclusions

Formulated ceftiofur is safe when injected intramuscularly into cattle. The formulation is deemed to be a slight muscle irritant.

4. Clinical Trial

The specific design including routes of administration, doses tested, numbers of animals, duration of tests, variables measured, results, statistical procedures and conclusions is addressed in Section IV, EFFECTIVENESS, of this FOI Summary. Please refer to Section IV for additional comments regarding Animal Safety.

C. Corroborative Studies (None)

IV. HUMAN FOOD SAFETY

A. Toxicity Studies

1. Acute Oral Single Dose Study in Rats

   a. Technical Report No. 7263-85-002, June 3, 1985

   b. Starting Date: August 2, 1984

   c. Termination Date: August 16, 1984

   d. Study Director: T. J. Kakuk

   e. Location of Study: The Upjohn Company, Kalamazoo MI

   f. Identification of Substance and Dosage Form: ceftiofur sodium oral

   g. Species and Strain: Sprague-Dawley Rats

   h. Number Animals Per Sex Per Treatment Group: 10 M and 10 F

   i. Drug Levels Tested and Duration of Dosing: 0, 996, 2120, 4276 and 7760 mg/kg body weight one day dosing followed by 15 day observation interval.

   j. Route of Drug Administration: oral

   k. Parameters Tested

   l. Clinical Daily observations were made to determine onset, severity and duration of behavioral changes and to note any evidence of toxicity.

   m. Body weights on days 1 and 15 of study.

   n. Gross necropsy on each animal and ail organs and tissues were examined in situ .

   o. Significant Toxicity Observed

      No deaths occurred at any dose level, nor were there any significant findings at the terminal necropsy. At 24 hours post treatment diarrhea was observed for 25% and 50% of the animals at 4276 and 7760 mg/kg, respectively. No such effects were observed at 996 and 2120 mg/kg. By day 2 all animals appeared clinically normal. A few animals in the two highest dose groups only had transient diarrhea on days 7 and 8, returning to a normal clinical state thereafter. Body weight gains were similar for all animals independent of dose.

p. No Observed Effect Level: the oral single lethal dose value is >7760 mg/kg body weight

q. Statistical Analysis: none

r. Conclusions

The No Observed Effect Level based on the oral single lethal dose in rats is >7760 mg/kg body weight.

2. 30 Day Oral Toxicity Study in Rats

a. Technical Report No. 7263-85-071, December 12, 1985

b. Starting Date: August 20, 1984

c. Termination Date: September 20, 1984

d. Study Director: T. J. Kakuk

e. Location of Study: The Upjohn Company, Kalamazoo, MI

f. Identification of Substance and Dosage Form: ceftiofur sodium in solution

g. Species and Strain: Sprague-Dawley Rats

h. Number Animals Per Sex Per Treatment Group: 15 M and 15 F per group

i. Drug Levels Tested and Duration of Dosing: 0, 1500, 3000 and 6000 mg/kg body weight administered once daily for 30 or 31 days.

j. Route of Drug Administration: oral intragastric intubation

k. Parameters Tested

Clinical signs, weekly body weight and food consumption, seven hematologic, 15 clinical chemistry and eight urinalysis measurements, selected organ weight, and gross and microscopic evaluation.

l. Significant Toxicity Observed

Clinical signs of toxicity observed included diarrhea at 1500 mg/kg or greater, and distended abdomen and hardened stomach contents at 3000 mg/kg or greater. These signs were most prevalent in the 6000 mg/kg group with an onset of 4 to 5 days after ceftiofur dosing was initiated. The incidences of clinical signs in the other treated groups are presented in table form.

Percent Incidence of Various Signs of Toxicity

	Group Number 1	Group Number 2	Group Number 3	Group Number 4
Diarrhea	0	10	33	97
Distended abdomen	0	0	10	89
Stomach hard upon palpation	0	0	20	92

Group 1 0 (Control)
Group 2 1,500 mg/kg
Group 3 3,000 mg/kg
Group 4 6,000 mg/kg

m. No Observed Effect Level <1,500 mg/kg Statistical Analysis

n. The following parameters were examined for significant differences between treated and control groups:

Body Weight Urine Measurements Hemogram Measurements Organ Weights

o. Clinical Chemistry Measurements

Data for each sex were analyzed separately. Treatment group differences were analyzed using analysis of variance on raw data in conjunction with analysis of variance on ranks of data. For statistically significant variables, treatment groups were compared to the vehicle control using the Least Significant Difference method. Tests of statistical significance were made at the 0.05 level.

p. Statistical analysis was conducted on the histopathological findings using the following method:

Data for each sex were analyzed separately. The program used contains methods for the analysis of unadjusted proportions as well as the life table related techniques of Tarone (1975), Cox (1972) and Breslow (1970) for analysis of lesion incidence.

For the unadjusted analysis, an exact test for trend was used to test for an increase (or decrease) in lesion incidence as the dosage administered increased. Additionally, Fisher's exact test was used to compare the proportion exhibiting a particular lesion in each treated group to the proportion exhibiting that same lesion in the vehicle control group. Both tests were two-tailed, using the P value obtained by doubling the one-tailed probability.

Similarly, for the adjusted analysis, tests for trend and treatment groups to vehicle control group comparisons were conducted on the time adjusted proportions.

q. Conclusions

Ceftiofur sodium, administered orally to rats for 30 days, was markedly toxic at 6,000 mg/kg, moderately toxic at 3,000 mg/kg and minimally toxic at 1,500 mg/kg. The target organ was the gastrointestinal tract as evidenced by the clinical signs of diarrhea, distended abdomen, and hardened stomach contents, and grossly by the formation of stomach concretions of the drug food mixture at 3,000 mg/kg or greater.

3. 90 Day Oral Toxicity Study in Rats

a. Technical Report No. 7263-85-075, December 17, 1985

b. Starting Date: December 18, 1984

c. Termination Date: March 20, 1985

d. Study Director: T. J. Kakuk

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: ceftiofur sodium oral solution

g. Species and Strain: Sprague-Dawley Rats

h. Number Animals Per Sex Per Treatment Group: 20 M and 20 F

i. Drug Levels Tested and Duration of Dosing: 0, 30, 100, 300, 1,000 and 3,000 mg/kg body weight for 90 or 91 days.

j. Route of Drug Administration: oral intragastric intubation

k. Parameters Tested

Parameters utilized to assess toxicity included clinical signs, weekly body weight and food consumption, body weight gains, hematologic, clinical chemistry and urinalysis measurements, selected organ weights, and gross and microscopic evaluation. Tests of statistical significance on the parameters denoting toxicity per sex per group were made at the .05 level.

l. Significant Toxicity Observed

The target organ was the gastrointestinal tract as evidenced by the clinical signs of diarrhea and hardened stomach contents and grossly by a dose-related increase in stomach content weights at 300 mg/kg/day or greater. In males and females at 3,000 mg/kg/day, the incidence of diarrhea was -5 to 83% and the incidence of hardened stomach contents was 86 to 100%. At 1,000 mg/kg/day diarrhea occurred at an incidence of 6 to 42% in males and females and there was a 5% incidence of hardened stomach contents for the males. Only transient diarrhea (incidence of 5 to 11%) occurred in males at 300 mg/kg/day and in females at 30 and 100 mg/kg/day. This transient diarrhea did not occur in females at 300 mg/kg/day.

At 3,000 mg/kg/day there was formation of gastric concretions of the drug-food mixture at an incidence of 83%. These concretions in the stomach could not pass through the pylorus ("mechanical toxicity"), which led to poor or impaired nutritional status and secondary effects of suppressed body weight gain (-36%) in the males only, gastric erosions and/or ulcers, thickening of the gastric ridge (males only), and death of 10% (4/40) of the rats after 9 to 91 days of treatment. The impaired or poor nutritional condition at 3,000 mg/kg/day was also associated with decreased serum glucose values, electrolyte imbalance, sparseness of fat deposition in the abdominal cavity, and, microscopically, by the lack of hepatocellular glycogen deposition and atrophy of the germinal centers of the spleen, lymph nodes, and thymus. The impaired nutrition and lowered glucose values at 3,000 mg/kg/day, along with a significant increase in food consumption and significant changes in urinary ketones (increase) and pH (decrease) at both 1,000 and 3,000 mg/kg/day, suggest a deficiency in carbohydrate metabolism at 1,000 and 3,000 mg/kg/day. In addition, the lowered urinary pH at 100 mg/kg/day or greater probably reflected the excretion of the compound or its metabolites, including furoic acid. Hence, the lowered pH at 100 mg/kg/day, without any other significant changes, suggested that the compound was being metabolized and excreted as acidic metabolites.

Additionally, based on microscopic findings, the compound caused colitis at an incidence of 25% for males at 1,000 mg/kg/day or greater and in females at an incidence of 10%, 30% and 15% at 300, 1,000 and 3,000 mg/kg/day, respectively. Also, cecitis and/or ileitis occurred at an incidence of 10% (females) to 20% (males) at 3,000 mg/kg/day. Colitis, cecitis, and ileitis were probably associated with the changes in bacterial flora in the gut produced by the antibiotic properties of ceftiofur sodium. Males at 300 mg/kg/day or greater had an inflammatory response (mononuclear cells) located in the renal corticomedullary junction. This change may have been related to the compound favoring the formation of a possible enterotoxin In the gut of these animals. The acid urine (lowered pH) associated with the metabolism of ceftiofur apparently resulted in a significant increase in nephrocalcinosis for females only given ceftiofur sodium at 3,000 mg/kg/day.

m. No Observed Effect Level: 30 mg/kg

n. Statistical Analysis

(i) Statistical Evaluation: The following parameters were examined for significant differences between treated and control groups:

Body Weight Urine Measurements Food Consumption Organ Weights Hemogram Measurements Histopathologic Findings Clinical Chemistry Measurements

(ii) Statistical Method:

Data for each sex were analyzed separately. Treatment group differences were analyzed using analysis of variance on raw data in conjunction with analysis of variance on ranks of data (Conover and

Iman, 1981). For statistically significant variables, treatment groups were compared to the vehicle control using the Least Significant Difference method. Tests of statistical significance were made at the 0.05 level.

(iii) Statistical analysis was conducted on the histopathological findings using the following method:

The computer program of Thomas, Breslow and Gart (1977) was used to analyze the data. This program contains methods for the analysis of unadjusted proportions as well as the life table related techniques of Tarone (1975), Cox (1972), and Breslow (1970) for analysis of lesion incidence.

For the unadjusted analysis, an exact test for trend was used to test for an increase in lesion incidence as the dosage administered increased. Additionally, Fisher's exact test was used to compare the proportion exhibiting a particular lesion in each treated group to the proportion exhibiting that same lesion in the vehicle control group.

Similarly, for the adjusted analysis, tests for trend and treatment groups to vehicle control group comparisons were conducted on the time adjusted proportions.

o. Conclusions

Based on the results, the NOEL was 30 mg ceftiofur sodium per kg of body weight for 90 days. Also, the results indicated that ceftiofur administered orally to the rats for 90 days was markedly toxic at 3,000 mg/kg/day, moderately toxic at 1,000 mg/kg/day and minimally toxic at 300 mg/kg/day.

4. Oral Two-Generation Fertility and Reproductive Study in Rats - Performance of F0 Generation

   a. Technical Report No. 7263-85-082, December 17, 1985

   b. Starting Date: March 27, 1985

   c. Termination Date: August 21, 1985

   d. Study Director: C. J. Price

   e. Location of Study: Research Triangle Institute Research Triangle, North Carolina 27709

   f. Identification of Substance and Dosage Form: ceftiofur sodium dosing solution.

   g. Species and Strain: Sprague-Dawley Rats

   h. Number Animals Per Sex Per Treatment Group: - 0 M and - 0 F per group

   i. Drug Levels Tested and Duration of Dosing: 0, 100, 300 or 1000 mg/kg/day. Males dosed 70 days and females 14 days prior to the breeding stage.

   j. Route of Drug Administration: via gavage

   k. Parameters Tested

      Males (4-47 days of age) were dosed daily via gavage for 70 days and females (5-59 days of age) were dosed daily via gavage for 14 days prior to the cohabitation of FO breeding pairs (1 male and 1 female per cage); daily dosing of males and females was continued throughout the study. Females were singly housed on the day sperm were found in the vaginal lavage or at the end of the 21-day cohabitation period. On the day a litter was cast (pnd 0), the following reproductive parameters were recorded: (1) day of delivery and length of gestation for each dam, (2) the number of live or dead pups in each litter, and (3) the sex and clinical condition of each live pup. The body weight of each live pup was recorded on pnd 0, 4, and 21. On pnd 4, live litters were randomly culled to a maximum size of 10 pups per litter. Postnatal viability of Fl pups was evaluated on pnd 0, during the perinatal period (days 0-4) and during the remainder of lactation (days 4-21). On pnd 21 (day of weaning), 30 male and 30 female pups per group were randomly selected, orally dosed at the prescribed levels and designated for subsequent use of Fl breeders. All remaining Fl pups were sacrificed and necropsied and observations were recorded. Following weaning of F1 litters, F0 rats were sacrificed (79th day of exposure for females; 136th day of exposure for males); each F0 rat was evaluated at necropsy and reproductive organs, gross lesions and/or masses from the control and high-dose groups were evaluated histopathologically. In addition, histopathologic evaluation was performed on reproductive organs, heart, lungs, spleen, kidneys, adrenals or gross lesions and/or masses of animals that died or were moribund during the course of the study.

l. Significant Toxicity Observed

Body weight gain (g) for males was significantly suppressed in a dose-related manner throughout the study (weeks 0-2, 2-6, 6-10, 10-14, 14-18 and 18-necropsy). The effect of ceftiofur upon male body weight gain varied in severity during different phases of the study, suggesting a possible interaction of treatment with length of exposure and/or growth rate during development. Weight gain during the first 2 weeks of exposure was suppressed in the mid and high dose groups. All ceftiofur treated groups were below controls for body weight gain (g) and percent body weight gain for the treatment period as a whole (weeks 0-necropsy). Thus, potential effects of ceftiofur on male reproductive capacity were evaluated within a dose range which produced a statistically significant suppression in male body weight gain.

Female body weight gain was not affected during the first 2 weeks of exposure to ceftiofur (i.e., prior to cohabitation). For confirmed-mated females with litters, body weight gain was significantly suppressed for all ceftiofur-exposed groups during gestational days (gd) 16-20, and weight gain during gestation (days 0-20) was significantly suppressed at the high dose. Body weight gain for females with litters was significantly increased during the first 2 weeks of lactation at the mid and high doses, but was not affected during the third and final week of lactation or for the lactation period as a whole. Thus, female body weight gain was not affected prior to breeding, but dose-related changes in body weight gain were observed during gestation and lactation.

No dose-dependent adverse effects on fertility or reproductive performance were associated with exposure to ceftiofur. Ceftiofur-treated groups were similar to controls for the following endpoints: (1) the proportion of cohabited females with confirmed mating, (2) the proportion of mated females with confirmed pregnancies, (3) the proportion of confirmed pregnancies producing live litters, (4) the length of gestation in confirmed pregnant females, (5) the number of live pups per litter on pnd 0, 4 or 21, and (6) the proportion of live male pups on pnd 0 or 4. Postnatal viability of pups in ceftiofur-treated groups was equivalent to controls on pnd 0, and for pnd 4-21. During the perinatal period (pnd 0-4), 100% of pups in the high-dose group survived, thus contributing to a statistically significant trend toward increased survival; this statistical effect appears to lack biological relevance since survival in all groups was high (avg. % viable pups per litter ranging from 97.57-100%), and since the relevant statistical tests are not optimized when zero variance is observed In any dose group. Live pup body weight per litter was not affected by ceftiofur exposure on pnd 0 or 4; on pnd 21, the low dose group exhibited a significant suppression in body weight relative to the control group (-7.3%), but the absence of an effect at higher exposure levels suggests that this was not a treatment related effect.

Grossly, enlargement of the cecum occurred at an incidence of 59 to 97% in the treated groups compared to 0% in the control. In survivors, no compound related microscopic lesions were observed in the reproductive organs of either sex at the high-dose (1000 mg/kg/day) compared to the

controls. Moreover, there were no compound-related changes in the reproductive organs, heart, lungs, spleen, kidneys or adrenals of animals that died during the course of the study. Documented histopathological findings were considered to be secondary to gavage accidents, unspecified etiologies or background lesions common to rats of this strain and age.

m. No Observed Effect Level: 1000 mg/kg for reproductive performance.

n. Statistical Analysis

Endpoints were statistically evaluated using SAS® software. ANOVA including linear trend analysis and Dunnett's tests for comparison of treatments with controls was used for most variables. Where appropriate non parametric procedures such as Chi Square and Fishers exact test were used.

o. Conclusions

Daily oral administration of ceftiofur sodium at dosages of 0, 100, 300 and 1000 mg/kg/day in Sprague-Dawley rats failed to produce any adverse effects upon fertility or reproductive performance, or any histopathological alterations in the reproductive organs of either sex in the FO generation. Hence, the no observable effect level (NOEL) for reproductive performance and fertility of the FO generation in rats was 1000 mg ceftiofur sodium per kg of body weight.

5. Oral Two Generation Fertility Reproductive Study in Rats - Performance of Fl Generation

a. Technical Report No. 7263-86-031, May 13, 1986

b. Starting Date: July 31, 1985

c. Termination Date: January 7, 1986

d. Study Director: C. J. Price

e. Location of Study: Research Triangle Institute, Research Triangle, North Carolina 27709

f. Identification of Substance and Dosage Form: ceftiofur sodium dosing solution

g. Species and Strain: Sprague-Dawley Rats

h. Number of Animals Per Sex Per Treatment Group: 30 M and 30 F of the F1 generation

i. Drug Levels Tested and Duration of Dosing

0, 100, 300 or 1,000 mg/kg/day. Animals (Fl) were dosed once daily beginning on the day of weaning (postnatal 21) and continuing until the day before scheduled sacrifice (females for an average of 153 days and males an average of 152 days). At 12 to 15 weeks of age the Fl males and

females were cohabited for 21 days. Resulting F2 pups were sacrificed at time of weaning (day 21 of age).

j. Route of Administration: via gavage

k. Variables Tested

In this study, Fl males and females from each respective group were dosed daily via gavage beginning on pnd 21 (weaning) and continuing until the day before scheduled sacrifice. At 12 to 15 weeks of age, the Fl males and females were cohabited in nonsibling breeding pairs for a maximum of 21 days. On the day sperm were found in the vaginal lavage or at the end of the 21-day cohabitation period, females were singly housed and checked at least twice daily thereafter for the presence of pups in the cage. On the day a litter was found (pnd 0), the following reproductive parameters were recorded: (1) day of delivery and length of gestation for each dam, (2) the number of live and dead pups in each litter, and (3) the sex and clinical condition of each live pup. The number of live pups of each sex and the average pup body weight for live pups of each sex per litter were recorded on pnd 0, 4, and 21. On pnd 4, live litters were randomly culled to a maximum size of 10 pups per litter. Postnatal viability of F2 pups was evaluated on pnd 0, during the perinatal period (days 0-4) and during the remainder of lactation (days 4-21). On pnd 21 (day of weaning), all F2 pups were sacrificed, necropsied, and observations were recorded. Following sacrifice of F2 litters, Fl adult animals were sacrificed (146th-160th day of exposure for females; 145th-159th day of exposure for males). Each Fl animal was evaluated at scheduled sacrifice and the gastrointestinal tract, gross lesions and masses from all dose groups were evaluated histopathologically. The reproductive organs of both sexes were evaluated histopathologically for all control and high dose animals. In the absence of any compound related changes in the reproductive organs at the high dose relative to the controls, comparable tissues from the low and mid dose groups were not microscopically examined, as per study protocol. For animals that died or were moribund during the course of the study histopathologic evaluation was performed on reproductive organs, heart, lungs, spleen, kidneys, adrenals, or gross lesions and masses.

l. Significant Toxicity Observed

Except for mechanical effects due to dosing, clinical signs were not related to treatment. Males and females in all ceftiofur sodium groups had an increased tendency to struggle during dosing. Thirty-three (33) rats (18 males and 15 females) died or were moribund over the course of the study. When these data from males and females were combined, the incidence of dead or moribund animals increased in a dose related manner (0/60, 1/60, 11/60, and 21/60 at 0, 100, 300, and 1,000 mg/kg/day, respectively). Ninety-four percent (17/18) of these occurrences in males and 87% (13/15) in females were associated with evidence of accidental causes. For the remaining three (3) (e.g., one male at and 1,000 mg/kg/day, one female at 300 mg/kg/day, and one female at 1,000 mg/kg/day), the cause of death could not be determined.

The potential effects of ceftiofur sodium on Fl male reproductive capacity were evaluated within a dose range which produced a statistically significant increase in male body weight gain (weaning-necropsy) at the low and mid doses, but did not affect weight gain at the high dose. Body weight gain for females was not affected during the first twelve weeks of exposure to ceftiofur sodium (i.e., prior to cohabitation) or during gestation, but was significantly increased at the high dose throughout lactation.

Fertility and reproductive performance of the Fl generation and development of F2 litters through weaning (pnd 21) were not adversely affected by daily ceftiofur sodium exposure. Nor were there any treatment-related gross lesions observed in the F2 pups at scheduled sacrifice. Thus, ceftiofur sodium had no adverse effects on fertility and reproductive performance of the Fl adults or the resulting F2 pups.

Evaluation of F2 animals at scheduled sacrifice revealed an increased incidence of enlargement of the cecum at 300 and 1,000 mg/kg/day, but not at 100 mg/kg/day when compared to the controls. In addition, males in the 300 and 1,000 mg/kg/day groups and females in all ceftiofur sodium dose groups exhibited increased weight of the gastrointestinal tract (i.e., with or without stomach contents) relative to the vehicle control group. At 300 and 1,000 mg/kg/day, there was a greater incidence of degenerative changes in the nonglandular stomach (6 and 92%, respectively) and hypersecretion of mucus in the neck cells of the glandular stomach (12 and 79%, respectively) as compared to the control and 100 mg/kg/day groups.

For F1 animals which survived to scheduled sacrifice, no compound related microscopic findings were observed in the reproductive organs of either sex at the high dose (1,000 ng/kg/day) compared to the vehicle controls. Microscopic changes in the reproductive organs, heart, lungs, spleen, kidneys, or adrenals of animals that died or were moribund during the course of the study were not compound related.

m. No Observed Effect Level: 1,000 mg/kg for reproductive performance and fertility.

n. Statistical Analysis

Endpoints were statistically evaluated using SAS® software. ANOVA including linear trend analysis and Dunnett's tests for comparison of treatments with controls was used for most variables. Where appropriate non parametric procedures such as Chi Square and Fishers exact test were used.

o. Conclusions

Daily oral administration of ceftiofur sodium at dosages of 0, 100, 300, or 1,000 mg/kg/day to Sprague-Dawley rats produced gross enlargement of the cecum and microscopic changes in the stomach at 300 and 1,000 mg/kg/day as compared to the controls. These effects were not observed at 100 mg/kg/day. Ceftiofur sodium failed to produce any adverse effects upon fertility or reproductive performance of the F1 generation.

Likewise, no adverse effects were observed with regard to the growth and viability of F2 litters through weaning (pnd 21). Hence, the no observable effect level (NOEL) for reproductive performance and fertility of the F1 generation in rats was 1,000 mg ceftiofur sodium per kg of body weight.

6. Segment II Teratology Study in Rats

   a. Technical Report No. 7259-85-011,December 12, 1985

   b. Starting Date: February 12, 1985

   c. Termination Date: March 14, 1985

   d. Study Director: T. A. Marks

   e. Location of Study: The Upjohn Company, Kalamazoo MI

   f. Identification of Substance and Dosage Form: ceftiofur sodium powder formulated with sterile filtered deionized water to form a solution.

   g. Species and Strain: Sprague-Dawley Rats

   h. Number Animals Per Sex Per Treatment Group: 24 bred rats per group

   i. Drug Levels Tested and Duration of Dosing: 0, 800, 1,600 and 3,200 mg/kg/day on days 6-15 of gestation

   j. Route of Drug Administration: oral by gastric intubation

   k. Parameters Tested

      All dams were weighed on the day of insemination (day 0 of gestation), throughout the dosing period, and on day 20, the day cesarean sections were performed. Sex, weight, number and location of each dead and resorbed fetus. Live fetuses were evaluated for gross, visceral and skeletal anomalies.

   l. Significant Toxicity Observed

      Ceftiofur administration led to dose related maternal toxicity (soft stools, porphyrin staining of the eye and nares) especially in the high dose group (diarrhea, blood in stools). However, in spite of administration at such high dosages, this cephalosporin did not appear to adversely affect the reproductive capacity of the dams. Although there was a statistically significant ($P < 0.01$) decrease in the proportion of dams in the high dose group that conceived, the fact that the drug was not given until day 6 of gestation, as well as the lack of significant effects on early embryo development (early implant sites and resorptions), suggested that this effect occurred by chance.

      The only statistically significant adverse effect on the embryos was a dose-related decrease in mean fetal body weights. However, the actual decreases did not exceed 7% and no other indication of toxicity toward the

offspring was found. Thus, it was concluded that ceftiofur was not embryotoxic. Since there were no biologically significant increases in the incidences of variations or malformations, it was also concluded that this drug was not teratogenic to the rat.

m. No observed Effect Level: 3,200 mg/kg body weight for teratogenic effects.

n. Statistical Analysis

Several different procedures were used, depending on the end point, ranging from non parametric procedures to Analysis of Variance with LSD Method for pairwise comparisons.

o. Conclusions

Ceftiofur sodium is not teratogenic in the rat at doses up to and including 3,200 mg/kg body weight.

7. 51 Day Oral Toxicity Study in Dogs

a. Technical Report No. 7263-85-077, December 12, 1985

b. Starting Date: September 4, 1984

c. Termination Date: October 26, 1984

d. Study Director: T. A. Jackson

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: ceftiofur sodium in solution

g. Species and Strain: Purebred Beagle Dogs

h. Number Animals Per Sex Per Treatment Group: 4 M and 4 F per group

i. Drug Levels Tested and Duration of Dosing: 0, 300, 1,000 and 3,000 mg ceftiofur/kg body weight administered daily for 51 or 52 days

j. Route of Drug Administration: oral intragastric intubation

k. Parameters Tested

Dogs were evaluated prior to study initiation by physical examination which included ophthalmologic examination and analysis of clinical pathology data. During the study, food consumption data were collected 5 days per week and clinical observations were made daily; body weights were documented on a weekly basis. Blood chemistry and urinalysis data were collected during week 4 and at study termination; ophthalmologic examinations were done at the same period. Hematology data was collected from all dogs at approximately weekly intervals, beginning at week 4. All dogs were necropsied; selected organs were weighed and protocol tissues were fixed in formalin, processed, and evaluated histopathologically.

l. Significant Toxicity Observed

Administration of ceftiofur to dogs at levels of 300 mg/kg/day, 1,000 mg/kg/day, or 3,000 mg/kg/day for a period of up to 51 consecutive days resulted in anemia and thrombocytopenia in both male and female beagles. The two higher dose levels were also associated with the development of elevated neutrophil counts and depression. Two females given 1,000 mg/kg/day and 2 males and 2 females given 3,000 mg/kg/day died prior to the termination of the study due to the clinical syndromes associated with exposure to this compound.

m. No Observed Effect Level: not determined

n. Statistical Analysis

Body weights, food consumption, organ weights, hematology, blood chemistry, urinalysis, and histopathology were evaluated statistically.

o. Conclusions

Under conditions of this study, ceftiofur produced multiple adverse effects on the hematopoietic system of dogs at all dose levels tested.

8. 90 Day Oral Toxicity Study in Dogs

a. Technical Report No. 7263-85-079, December 11, 1985

b. Starting Date: December 20, 1984

c. Termination Date: March 22, 1985

d. Study Director: T. A. Jackson

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: ceftiofur sodium in solution

g. Species and Strain: Purebred Beagle Dogs

h. Number Animals Per Sex Per Treatment Group: 5 M and 5 F per group

i. Drug Levels Tested and Duration of Dosing: 0, 10, 30, 100 and 300 mg/kg body weight given daily for 90 days

j. Route of Drug Administration: oral intragastric intubation

k. Parameters Tested

Dogs were evaluated prior to study initiation by physical examination. During the study, food consumption data were collected 7 days per week and clinical observations were made daily; body weights were documented on a weekly basis. Blood chemistry and urinalysis data were collected prior to study initiation, during week 6, and before study termination; ophthalmologic examinations were done prior to study initiation and at termination of the study. Hematology data were collected from all dogs

prior to study initiation and 6 times during the study (including termination) at approximately 2-week intervals. Coombs' tests were performed on blood from all dogs given 300 mg/kg/day and 3 dogs per sex given vehicle only at day 41; at day 83 blood from all dogs was tested. Examinations of blood smears and differential leukocyte counts were done for all dogs prior to study initiation, at the 6-week bleeding, and at the last bleeding; smears were analyzed from every bleeding for dogs which became anemic and/or Coomb's test positive. A complete necropsy was done on all dogs; selected organs were weighed and protocol tissues were fixed in formalin, processed, and evaluated histopathologically.

l. Significant Toxicity Observed

Administration of 100 mg/kg/day or more of ceftiofur was associated with a non-progressive thrombocytopenia in at least 4 dogs. Multiple dogs treated with 300 mg/kg/day became positive for the Coombs' test indicating the presence of immunoglobulin on the surface of erythrocytes. Two dogs treated at this level became severely anemic and there was no apparent regenerative response by bone marrow until administration of compound ceased (one dog). Major clinical signs were associated with anemia and included pale mucous membranes and depression.

Necropsy observations and major histopathologic findings were related to the process of anemia. A minor inflammatory lesion was noted in the pelvis of kidneys from dogs given 100 mg/kg/day or more of compound; its significance is unknown.

m. No Observed Effect Level: 30 mg/kg

n. Statistical Analysis

Body weights, food consumption, organ weights, hematology, blood chemistry, urinalysis, and histopathology were evaluated statistically.

o. Conclusions

Ceftiofur has obvious effects on the hematopoietic system, causing thrombocytopenia in dogs given 100 mg/kg/day or 300 mg/kg/day and severe anemia in dogs given 300 mg/kg/day. The no observed effect level (NOEL) for ceftiofur in dogs was 30 mg/kg/day in this study.

9. Genotoxicity Study - Ames Assay - Ceftiofur

   a. Technical Report No. 7268-83-019, October 31, 1983

   b. Starting Date: May 4, 1983

   c. Termination Date: May 9, 1983

   d. Study Director: D. H. Swenson

   e. Location of Study: The Upjohn Company, Kalamazoo MI

   f. Identification of Substance and Dosage Form: ceftiofur sodium pulverized powder

   g. Species and Strain: in vitro study ceftiofur dissolved in dimethylsulfoxide at 0.01 mg/ml immediately before use.

   h. Species and Strain: In vitro

   i. Number Animals Per Sex Per Treatment Group: not applicable

   j. Drug Levels Tested and Duration of Dosing: 0.125, 0.25, 0.5 and 1.0 µg/plate plus controls (positive and negative).

   k. Route of Drug Administration: not applicable

   l. Parameters Tested

      The procedures for the Ames assay are the standard ones developed in the laboratory of Dr. B. N. Ames. Briefly, histidine auxotrophs of Salmonella typhimurium are mixed with the test compound in 0.1 ml dimethylsulfoxide, the 9,000 x g supernatant of liver homogenates (or saline) in molten (45°C) agar. The molten agar mix is poured onto a Petri plate containing a histidine-deficient base agar. Revertants to histidine prototrophy are scored as colonies, after incubation at 37°C for 22 days. A single plate is used for each dose level and the experiment is repeated. Vehicle controls are run in triplicate for each strain in each experiment and reported as an average of the three values.

   m. Significant Toxicity Observed

      At no dose level was an appreciable increase in colonies per plate observed, while the positive controls 2-acetyl-aminofluorene (TA98, TA100, and TA1538), cyclophosphamide (TA1535) and 9-aminoacridine (TA1537) showed positive mutagenicity.

n. No Observed Effect Level: not applicable

o. Statistical Analysis: not applicable

p. Conclusions

The results of this study show that ceftiofur was not a mutagen for S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 with or without metabolic activation.

10. Genotoxicity Study - The V79 Mammalian Cell Mutation Assay - Ceftiofur

a. Technical Report No. 7268-83-030, November 29, 1983

b. Starting Date: June 14, 1983

c. Termination Date: August 2, 1983

d. Study Director: D. H. Swenson

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: ceftiofur in solution

g. Species and Strain: not applicable

h. Number Animals Per Sex Per Treatment Group: not applicable

i. Drug Levels Tested and Duration of Dosing: 1, 2 and 4 mg/ml plus control (positive and negative)

j. Route of Drug Administration: not applicable in vitro test

k. Parameters Tested

The mammalian cell mutagenesis assay is an accepted in vitro short term testing procedure for detecting potential mutagens and carcinogens. This assay uses V79 (Chinese hamster) cells and measures the production of mutants resistant to 6-thioguanine (TG) as a result of exposure to the test compound. Mutant cells without HGPRT activity cannot metabolize TG, and thus escape its lethal effect.

Some compounds are negative in this assay unless they are metabolized to reactive forms. Because the V79 cell is incapable of such metabolic activation, an exogenous activation system is required. The rat liver S9 system is a widely used activation system for bacterial and mammalian cell mutagenesis. Thus, ceftiofur was tested with and without an S9 activation system.

l. Significant Toxicity Observed

There were no significant differences between the mutation frequencies of cultures treated with ceftiofur and control cultures. All the treatment/control ratios were very close to 1.0, and there was no

significant linear dose response. Thus, ceftiofur with or without S9 activation, was not mutagenic in this study.

m. No Observed Effect Level: not applicable

n. Statistical Analysis

The raw data was entered into a computer program which calculated the mutant frequencies and generated summary tables and a statistical analysis. The mutation frequencies of the cultures were transformed to (1 + mutants/10e6)(raised to 0.15 power). The mean response at each dose was compared to the mean control (no treatment) response using the one-sided t-test. The mean square error from a two-way analysis of variance (AOV) was used for a variance estimate. In addition, a one-sided t-test was used to test for an increasing linear relationship between log dose and the transformed response. The mean square error from a covariance model with assays as the grouping variable was used for the variance estimate. To increase the sensitivity of the latter test, the control vehicle responses were incorporated into the analysis by assigning them dose values equal to R times the smallest dose where R is the ratio of two successive doses.

The alpha levels of the statistical tests were adjusted so that the experimental alpha level was not greater than .1. Thus, the alpha levels were set at .025 for the four-dose assays.

o. Conclusions: Ceftiofur was not mutagenic in this study.

11. Genotoxicity Study - Unscheduled DNA Synthesis (UD) Assay - Ceftiofur

a. Technical Report No. 7268-84-018, May 24, 1984

b. Starting Date: May 24, 1983

c. Termination Date: May 31, 1983

d. Study Director: D. H. Swenson

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: A solution of ceftiofur at 10 mg/ml in dimethylsulfoxide (DMSO)

g. Species and Strain: not applicable

h. Number Animals Per Sex Per Treatment Group: not applicable

i. Drug Levels Tested and Duration of Dosing

0.03, 0.1, 0.3 and 1 mg ceftiofur/ml with a solvent control and UV light as a positive control.

j. Parameters Tested

Genotoxins are agents that can damage DNA or alter chromosome structure and with very few exceptions are generally mutagenic or

carcinogenic. As a class of compounds, genotoxins have wide structural diversity. Some are active per se but most require metabolic activation before they are capable of exerting their biological effects. Not all types of DNA damage lead to mutagenic or carcinogenic endpoints. Some types of DNA damage are repaired by the cellular repair system before the deleterious consequences are manifest. DNA repair, therefore, implies DNA damage and as such can be used as an endpoint for assaying genotoxic action. Cells vary greatly in their ability to repair genotoxic lesions and depending on the type of lesion formed can be repaired in a variety of ways. A brief description of DNA excision repair serves to illustrate the repair process. Briefly, a genotoxic lesion is recognized and excised from the DNA strand by repair endonucleases, along with several nucleotides on either side of the lesion. The resulting gap is patched with nucleotides, using the opposite strand as a template. This repair is known as unscheduled DNA synthesis or UDS and can be measured by a variety of methods. The most common method used to monitor unscheduled DNA synthesis is by observing increased incorporation of [3H] thymidine into the repair patches by autoradiography.

Primary rat hepatocytes have three characteristics that make them useful for detecting UDS. First, hepatocytes are nondividing, therefore, a UDS response is not confused with cells undergoing semi-conservative replication (scheduled DNA synthesis). Second, hepatocytes have good capabilities for metabolic activation of genotoxins. Third, hepatocytes have excellent DNA repair capabilities.

For this test an increased frequency of nuclear grains indicates DNA repair and implies that the agent responsible for the increase may be carcinogenic and mutagenic.

k. Significant Toxicity Observed

UV light (1.5 joules/sq m) was used as a positive control and resulted in a 4-fold increase of nuclear grains as compared to controls.

Ceftiofur did not cause a dose-related increase of UDS nor did it cause a doubling of UDS relative to untreated control cultures that received equivalent volumes of vehicle (dimethylsulfoxide).

l. No Observed Effect Level: not applicable

m. Conclusions: Under the conditions employed for this study, ceftiofur did not act as a DNA damaging agent.

12. Genotoxicity Study - The Micronucleus Test - Ceftiofur

a. Technical Report No. 7268-84-011, March 16, 1984

b. Starting Date: June 1, 1983

c. Termination Date: June 3, 1983

d. Study Director: D. H. Swenson

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: Ceftiofur dissolved in sterile water to make 100 mg drug/ml solution.

g. Species and Strain: Male Sprague-Dawley Rats

h. Number Animals Per Sex Per Treatment Group: 10 M per group

i. Drug Levels Tested and Duration of Dosing

Ceftiofur at 250, 500 and 1,000 mg/kg body weight given in two equal doses 24h apart intraperitoneally (ip), water controls and cyclophosphamide as a positive control at 1 ml/kg.

j. Route of Administration: intraperitoneal

k. Parameters Tested

The micronucleus test is an in vivo procedure used for the detection of compounds that cause chromosome damage or nondisjunction of chromosomes (as induced by spindle poisons).

Micronuclei are chromosome fragments (as a result of clastogenic events) or whole chromosomes (a result of nondisjunctional events) that are left behind after expulsion of the nucleus during maturation of erythroblasts to erythrocytes in the bone marrow of mammals. For this test an increased frequency of micronucleated polychromatophilic erythrocytes indicates chromosome damage, nondisjunction, or chromosomal deletions from the genome.

At 30 and 48 hours after the first dose (i.e., 6 and 24 hours after the second dose), the rats were sacrificed by cervical dislocation. The long bones of the hind quarters were exposed and the epiphyseal ends were snipped off to expose the marrow. A 20 gauge needle, on a 1-ml syringe containing 0.2-0.3 ml fetal calf serum, was inserted into the marrow cavity while simultaneously providing aspiration with the syringe plunger. The marrow plugs of the long bones of the hind quarters were dispersed in a disposable test tube, after which the cell suspension was placed on a

microscope slip and spun at maximum speed for 10 seconds in a Perkin-Elmer slide spinner. The slides were air dried overnight at room temperature.

l. Significant Toxicity Observed

Cyclophosphamide, a mutagen that requires metabolic activation, was used as a positive control and significantly increased the incidence of micronucleated PCEs. None of the ceftiofur treatment groups exhibited a statistically significant increase in the rate of micronucleated PCEs over that of the control group, nor was the test for a positive dose response relationship statistically significant. These results indicate that ceftiofur did not have clastogenic or nondisjunctional effects on the chromosomes of rat bone marrow cells.

m. No Observed Effect Level: not applicable

n. Statistical Analysis

Summary statistics were calculated for each treatment group. Each ceftiofur treatment group was compared with the vehicle control group using the rank transformation of the one sided t-test. The existence of a positive dose response relationship was tested using the rank transformation of the one-sided t-test for a positive slope.

o. Conclusions: Under conditions of this study, ceftiofur did not act as a clastogen or chromosomal mutagen.

13. Genotoxicity Study - Ames Assay - Furoic Acid

a. Technical Report No. 7268-85-021, December 11, 1985

b. Starting Date: August 7, 1985

c. Termination Date: August 15, 1985

d. Study Director: D. M. Zimmer

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: Furoic acid dissolved in dimethylsulfoxide

g. Species and Strain: in vitro

h. Number Animals Per Sex Per Treatment Group: not applicable

i. Drug Levels Tested and Duration of Dosing: 250, 500, 1000 and 2000 µg/plate plus positive and negative controls

j. Route of Administration: not applicable

k. Parameters Tested

The procedures for the Ames assay are the standard ones developed in the laboratory of Dr. B. N. Ames. Briefly, histidine auxotrophs of Salmonella typhimurium are mixed with the test compound (in 0.1 ml dimethylsulfoxide) and the 9000 x g supernatant of liver homogenates (or saline) in molten (45°C) agar. The molten agar mix is poured onto a Petri plate containing a histidine-deficient base agar. Revertants to histidine prototrophy are scored as colonies, after incubation at 37°C for 2 days. A single plate is used for each dose level and the experiment is repeated. Vehicle controls are run in triplicate for each strain in each experiment and reported as an average of the three values.

l. Significant Toxicity Observed

At no dose level was an appreciable increase in colonies per plate observed, while the positive control 2-aminoanthracene was mutagenic in all strains (where S-9 was present).

Toxicity, as indicated by a lightening of the bacterial lawn or by a reduction in reversion rate with increasing dose, was not observed in this study.

The results of this study show that furoic acid was not a mutagen for S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 with or without metabolic activation.

m. No Observed Effect Level: not applicable.

n. Statistical Analysis: none.

o. Conclusions

Although the results of the Salmonella /microsome test do not give an absolute answer about the carcinogenic or mutagenic hazard to man, they serve as a general indicator of potential hazard. In this case no mutagenic or carcinogenic hazard was suggested for furoic acid.

14. Genotoxicity Study - Ames Assay - Furoic Acid

a. Technical Report No. 7268-87-001, May 12, 1987

b. Starting Date: December 16, 1986

c. Termination Date: January 11, 1987

d. Study Director: C. S. Aaron

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: Furoic acid dissolved in dimethylsulfoxide

g. Species and Strain: in vitro

h. Number Animals Per Sex Per Treatment Group: not applicable

i. Drug Levels Tested and Duration of Dosing: 1,250, 2,500, 5,000 and 10,000 µg/plate plus positive and negative controls

j. Route of Administration: not applicable

k. Parameters Tested

The plate incorporation procedures used at TUCO for conduct of the Ames test are generally those of Ames and co-workers. Histidine auxotrophs (Strains TA-98, TA-100, TA-1535, TA-102 and TA-1537) of Salmonella typhimurium were thawed from frozen permanents and grown in Oxoid broth No. 2 for 12 hours. The bacteria (108) were combined with top agar (45°C), and then the test agent or solvent control (DMSO) was added either with or without metabolic activation S-9 mix to give a total volume of 2.5 ml. The molten mixture was then poured onto a petri plate (each plate results from an independent treatment tube) containing histidine deficient base agar and was incubated at 37°C for 2 days. The plates were examined for evidence of toxicity as shown by a diminished or absent background lawn. After incubation the colonies were counted using an automatic colony counter (Artek Inc.).

Criteria for judging an Ames Salmonella /microsome assay The criteria for judging any in vitro assay fall into two categories, namely, those which affect the acceptability of the assay and those which affect the

interpretation of the result of the assay. The data presented here relate only to the criteria for the acceptability of the test as valid.

Criteria for judging an Ames Salmonella /microsome assay

The criteria for judging any in vitro assay fall into two categories, namely, those which affect the acceptability of the assay and those which affect the interpretation of the result of the assay. The data presented here relate only to the criteria for the acceptability of the test as valid.

Criteria for the acceptability of an Ames Salmonella / microsome assay

The following conditions define an acceptable Ames Salmonella /microsome assay -

(1) Negative control frequencies should fall within the acceptable range for each strain and the negative control plates should display normal growth (as shown by the presence of a background lawn of microcolonies). The mean spontaneous frequency of revertants is typically higher in the presence than in the absence of S-9. Individual strains outside the acceptable range should be excluded from data interpretation.
(2) Positive control frequencies should fall within the acceptable ranges or within the expectations established with range finding experiments if other positive controls are used. Failure of a positive control to perform as expected is interpreted to indicate any of several problems and data for that particular strain/activation combination should not be the subject of further interpretation.

Experiments in which the negative controls and positive controls are acceptable may be further interpreted.

Criteria for a positive or negative response in an Ames Salmonella /microsome assay -

The following conditions define a positive response in the Ames Salmonella /microsome assay -

(1) A reproducible dose response, i.e. systematic increase in revertants per plate as a function of dose or
(2) A reproducible elevation of mutant colonies per plate at a single dose which amount to a tripling of the spontaneous frequency of revertants in any strain.

The following conditions will define a negative response in the Ames test -

(1) No evidence of systematic reproducible elevation of mutation frequencies is observed in any strain/activation group and,
(2) No dose level produces a reproducible doubling of the background frequency of revertants in any strain.

Cases which do not clearly fit into the positive or negative category, i.e. between a doubling and a tripling of the background frequency of revertants, may be judged equivocal. These cases will require further

testing in other test systems to resolve the question of potential mutagenicity of the test agent.

l. Significant Toxicity Observed

At no dose level was an appreciable increase in colonies per plate observed, while the strain specific positive controls worked properly in all cases. The concentrations used in the study were chosen on the basis of preliminary range-finding experiments.

m. No Observed Effect Level: not applicable.

n. Statistical Analysis: none.

o. Conclusions

The Ames Salmonella /microsome assay is a qualitative assay for mutagenicity and as such has gained considerable standing (Haworth et al, 1984, Dunkel et al, 1985) in the evaluation of a variety of agents for mutagenic potential. The simplicity of conduct and ease of performance have led to the adoption of its use by government, industry and academic laboratories as a tool in investigation of mechanisms, discovering hazardous materials and regulatory decision making. The results of the study of 2-furoic acid failed to demonstrate any tendency to cause mutations in bacteria. Therefore, 2-furoic acid is judged to be non-mutagenic under the conditions of this test. These results confirm the previously obtained negative results with furoic acid.

15. Genotoxicity Study - Unscheduled DNA Synthesis (UDS) Assay - Furoic Acid

a. Technical Report No. 7268-85-023, December 11, 1985

b. Starting Date: August 14, 1985

c. Termination Date: October 16, 1985

d. Study Director: C. S. Aaron

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: Furoic acid in solution

g. Species and Strain: in vitro

h. Number Animals Per Sex Per Treatment Group: not applicable

i. Drug Levels Tested and Duration of Dosing

1, 3, 10, 30, 100, 300 and 1,000 µg/ml plus two positive controls (DMN and 2-AAF) and a negative (solvent) control

j. Route of Administration: not applicable

k. Parameters Tested

This DNA repair assay, as developed by G. M. Williams measures unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes treated with the test compound in vitro . This assay is useful as a genotoxicity screen because it measures the repair of DNA damage induced by many classes of mutagens and carcinogens. Genotoxic compounds or their metabolites react with DNA to form adducts which are repaired by an enzymatic process in which the adduct is excised, and the DNA strand is polymerized and ligated. This process, referred to as unscheduled DNA synthesis, can be quantitated by measuring the incorporation of labeled thymidene into the nuclear DNA of cells not in S phase. Thus, compounds that induce repairable DNA damage without inhibiting that repair can be detected by measuring UDS.

Primary hepatocytes are particularly useful for this assay because of their high capacity to metabolize procarcinogens to a genotoxic form. In addition, the cultures are essentially non-dividing, so that normal replicative DNA synthesis is too low to interfere with UDS detection.

A test compound is considered a positive if the UDS net grain count of any tested concentration is > = 5 NG in both the preliminary and replicate assays and if the percentage of cells in repaid (% IR) Is > = 10. The results are considered as either inconclusive or a potentially weak positive response if the highest UDS net grain count for any tested concentration is between 0 and 5 NG. The results are considered negative if all tested concentrations of the test article have a UDS need grain count of < = 0 NG, and if testing was performed to the limits of solubility or cytotoxicity, or at 3.0 mg/ml.

l. Significant Toxicity Observed

Cytotoxicity was observed at 1,000 µg/ml. The net grain counts were negative for each concentration of furoic acid, and for the negative controls. There was no significant difference in net grain counts between furoic acid and the negative controls. DMN and 2-AAF each induced a strong positive response.

m. No Observed Effect Level: not applicable

n. Statistical Analysis

The mean and cell to cell standard deviation (SD) for the nuclear, cytoplasmic and net grain counts were calculated for each slide. The NG mean, slide to slide standard error (SE), and frequency distribution were calculated for each dose.

o. Conclusions

Furoic acid did not induce a significant increase in UDS over the negative controls. Therefore, furoic acid was not a genotoxic agent under the conditions of this in vitro rat hepatocyte DNA repair assay.

16. The V79 Mammalian Cell mutation Assay - Furoic Acid

a. Technical Report No. 7268-85-027, December 17, 1985

b. Starting Date: July 15, 1985

c. Termination Date: September 24, 1985

d. Study Director: D. M. Zimmer

e. Location of Study: The Upjohn Company, Kalamazoo MI

f. Identification of Substance and Dosage Form: Furoic acid in solution

g. Species and Strain: not applicable

h. Number Animals Per Sex Per Treatment Group: not applicable

i. Drug Levels Tested and Duration of Dosing: 250, 500, 1000 and 1500 µg/ml plus controls (positive and negative)

j. Route of Administration: in vitro test - not applicable

k. Parameters Tested

The V79/HGPRT Mammalian Cell Mutation Assay is one component of a battery of tests used at The Upjohn Company to evaluate chemicals for their ability to cause genotoxic effects. The V79 assay is widely used and accepted in the field of genetic toxicology as a valid means of estimating the potential of chemicals to cause gene mutations in mammalian cells in culture. We report here the results of furoic acid in the V79/HGPRT Mammalian Cell Mutation Assay.

The V79/HGPRT assay measures the ability of a test compound to induce point mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster V79 cells. Cells which are unable to synthesize the enzyme HGPRT (presumptive mutants) cannot metabolize the purine analog 6-thioguanine (6-TG) to its toxic form and are therefore resistant to it. The V79/HGPRT Assay has been described and reviewed in the literature, and is a widely accepted means of estimating the ability of chemicals to induce forward point mutations in mammalian cells.

l. Significant Toxicity Observed

According to criteria for positive response (either a dose related or statistically significant increase in mutation frequency compared to vehicle controls), furoic acid was nonmutagenic in the V79/HGPRT Mammalian Cell Mutation Assay either with or without metabolic activation. Positive controls (DMBA or MNU) induced mutation frequencies similar to expected values.

m. No Observed Effect Level: not applicable

n. Statistical Analysis

Raw data (plate counts) were used to generate mean mutation counts per treatment group and each furoic acid group compared with controls.

o. Conclusions: Furoic acid was not mutagenic in this test system.

B. Hypersensitivity Studies

1. Passive Cutaneous Anaphylaxis Study in Guinea Pigs

a. Technical Report No. 7263/87/077

b. Starting Date - January 28, 1987

c. Termination Date - November 2, 1987

d. Study Director - Terry A. Jackson

e. Location of Study - The Upjohn Company, Kalamazoo, MI

f. Identification of Substance and Dosage Form - Ceftiofur (U-64,279E) was tested in four forms

Parent compound (bulk drug)
Parent compound conjugated to hen egg albumin as the carrier protein
Metabolite II
In addition to the 4 forms of ceftiofur, 2 additional conjugates with hen egg albumin were tested -
Compound A - structurally similar to Metabolite VII Compound B - common to parent compound and all metabolites

g. Extract of residue from kidney and injection site muscle of treated animals.

h. Species and Strain - Hartley Albino Guinea Pigs

i. Number Animals Per Sex Per Treatment Group - Females only, variable number per group (2 - 12), depending on challenge material and level.

j. Route of Drug Administration - IV and Oral

k. Drug Levels Tested and Duration of Dosing

Single challenge doses were given to animals passively sensitized with antibody to benzylpenicillin G and/or ceftiofur. The challenge levels tested were based on anticipated maximum human exposure level (approximated 0.083 mg/kg), varied with the form of the drug and were usually given in 10-fold increments. Routinely, oral challenges were at levels 10X greater than those given IV:

Parent compound (bulk drug)
IV: 0.076,0.76, 7.6 mg/kg
Oral: 0.76, 7.6, 76 mg/kg

Ceftiofur conjugate (ceftiofur to hen egg albumin)
IV: 0.01, 0.10, 1.0 mg/kg
Oral: 0.10, 1.0, 10.0 mg/kg

Metabolite II
IV: 0.076, 0.76, 7.6, 76, 760, 7600 µg/kg
Oral: 0.76, 7.6, 76, 760, 7600 µg/kg

Compound A conjugate (to hen egg albumin)
IV: 0.0083, 0.083, 0.83 mg/kg (actual content of Compound A not determined) IV: 0.0083, 0.083, 0.83 mg/kg (actual content of Compound A not determined)

Compound B conjugate (to hen egg albumin)

IV: 0.83 mg/kg (actual content of Compound B not determined)

Extract of drug residue from injection-site muscle and kidney

Oral: 830 µg/kg

Passive Cutaneous Anaphylaxis (PCA) following IV and/or oral challenge. Briefly, skin sites on guinea pigs were passively sensitized with antibody of desired specificity and five days later the sensitized animals were given Evans blue dye and challenged by either the IV or oral route with appropriate test material. Sensitized skin sites were subsequently examined for evidence of PCA reactions as indicated by the leakage of Evans blue dye from the vascular system into the skin.

l. Significant Findings

Regardless of dose, there were no positive reactions of passive cutaneous anaphylaxis (PCA) when guinea pigs sensitized with penicillin antibody were challenged by either route with any form of ceftiofur.

Guinea pigs sensitized with ceftiofur antibody did not respond to challenges with parent compound by either route. However, positive PCA reactions occurred following challenge with protein conjugate by both routes. While an IV challenge of 0.01 mg/kg produced positive reactions, oral exposure resulted in PCA activity only at a challenge level of 10 mg/kg.

Metabolite II caused PCA reactions over a wide range of dose levels when given by either the IV or oral route to guinea pigs sensitized with ceftiofur antibody. While as little as 0.076 µg/kg produced positive responses following IV challenge, levels of at least 76 µg/kg were required to induce positive PCA activity as a sequel to oral exposure.

Guinea pigs sensitized with ceftiofur antibody then challenged IV with conjugates of Compound A or Compound B responded to the challenge. These positive responses confirm that the model is able to recognize all metabolites in the extract of kidney or muscle.

Guinea pigs sensitized with ceftiofur antibody and challenged orally at a level of 0.83 mg/kg with acetic acid extract of drug (ceftiofur) residue from kidney and injection-site muscle were uniformly negative.

m. No Observed Effect Level - 0.83 mg/kg. The relevant NOEL is for oral challenge of sensitized animals with the acetic acid extract of ceftiofur residue from kidney and injection site muscle.

n. Statistical Analysis - Not applicable.

o. Conclusions

Antibody to benzylpenicillin G did not react with any form of ceftiofur tested, regardless of route or dose. Data from the IV challenges indicate that ceftiofur antibody used for this study was capable of detecting the drug as a conjugated hapten in challenge material given at a level as low as .01 mg/kg body weight (lowest level tested). Positive PCA reactions also occurred with IV challenges of Metabolite II at levels as low as .076 µg/kg body weight (lowest level tested). The route was of considerable importance for both the protein conjugate and Metabolite II because of the differences in the level of challenge necessary to elicit a positive PCA reaction. With both materials, the oral challenge level necessary to produce a positive response was approximately 1,000X the minimum level required for a PCA reaction following IV challenge. This suggests that the gastrointestinal tract may play an important role in modulating the effect of potential hypersensitive reactions with ceftiofur.

Further, the data suggest that ceftiofur does not exist in residue from injection site muscle or kidney in a form or concentration such that PCA reactions occur following oral exposure of animals sensitized with ceftiofur antibody and challenged with extract of the residue. Levels of extract tested were at least 10X the maximum anticipated human exposure level, based on consumption of injection site muscle at the time of maximum residue concentration.

2. Radioallergosorbent Test (RAST) Inhibition with Human Serum

a. Study Director - Timothy J. Sullivan, M.D.

b. Location of Study

Department of Internal Medicine
University of Texas Health Sciences Center
Dallas, Texas

c. Substance and Dosage Form - Not applicable

d. Species and Strain - in vitro

e. Number Animals Per Sex Per Treatment Group - Not applicable

f. Drug Levels Tested and Duration of Dosing - Not applicable

g. Route of Administration - Not applicable

h. Parameters Tested

The procedures for the RAST assay are the ones routinely used. Briefly, subjects were skin tested with a full battery of penicillin determinants and serum from positive reactors were subsequently studied using a penicilloyl RAST system. Serum samples from individuals with negative reactions were used as controls.

PENICILLOYL RAST: Benzylpenicillin G was conjugated to human serum albumin in the penicilloyl configuration by the pH method. This material was then conjugated to cyanogen bromide activated cellulose disks for use in RAST assays. Studies with positive and negative sera validated the assay. Serum giving apparently positive tests were further evaluated by preincubating the serum in the presence of 100 µg/ml of penicilloylbenzylamine (BPO-BA) as a positive inhibition control. Buffer alone served as a negative control. These preparations were then studied for their ability to bind in the standard RAST assay.

CEFTIOFUR INHIBITION: ceftiofur was conjugated to bovine gamma globulin (BGG) using the pH method, resulting in a binding ratio of 16 ceftiofur determinants per BGG molecule. The resultant BGG-ceftiofur molecule was then used as a potential inhibitor, as was done with BPO-BA.

i. Significant Results

The BPO-BA molecule was able to significantly inhibit the binding of serum antibody from 17 patients to the penicilloyl molecule. However, in no case was the preincubation of BGG-ceftiofur with serum from any of the patients able to significantly inhibit the binding of antibody to the penicilloyl molecule.

j. No Observed Effect Level - Not applicable

k. Conclusions

Ceftiofur-derived determinants do not react with IgE to penicillin in the RAST system and therefore, individuals allergic to penicillin are not at risk if exposed to ceftiofur.

C. Safe Concentrations of Total Residues

1. The lowest No-Observed-Effect Level was 30 mg/kg which was from both the 90 day oral feeding study in the rat and from the 90 day oral feeding study in the dog.

   a. Safe Concentration calculations are as follows:

      (i) No Observable Effect Level

      lowest NOEL is 30 mg/kg based on 90 day oral studies in dogs and in rats based on data included in this NADA.

      (ii) Safe Concentration = Allowable Daily Intake x Human Weight / Daily Consumption of Meat

      where

      Human Wt = 60 kg

      Daily Meat Consumption = 500 g

      (iii) ADl = NOEL/Safety Factor SF = 1000 because 90 day feeding study data are used therefore, ADl = 30 mg/kg ÷ 1,000 or 0.03 mg/kg and,

      SC = .03 mg/kg * 60 kg / 500g = 3.6 mg/kg or 3.6 ppm

      Present FDA policy limits the S.C. in muscle to no more than 3.0 ppm unless chronic toxicity data exist. Therefore, the S.C. on total residues is 3.0 ppm.

2. This compound is a Category A compound as derived from the Threshold Assessment considerations. Based on Structural Activity Assessment it was assigned to Category C (non carcinogen). Subsequent to this the 90 day feeding studies allowed it to be classified as Category A. Because of the therapeutic use on specific animals it is considered a Low Use Drug. Accordingly, Chronic studies were not required, and based on 90 day studies, a Safety Factor (SF) of 1000 is used in the Safe Concentration (SC) calculations.

3. Considering the hypersensitivity studies and the toxicity data from oral feeding studies in the rat and dog, the Safe Concentration of total residues is 3.0 ppm in muscle tissue.

D. Total Residue and Metabolism Studies

1. Investigator: D. B. Johnson, The Upjohn Company, Kalamazoo, MI.

2. Animals used: 3 heifers, 2 steers and 1 bull calf ranging in weight from 274 lb to 421 lb.

3. Drug was administered intramuscularly at 1.0 mg/lb (2X label dosage).

4. Ceftiofur sodium was administered once a day for five consecutive days at 24 h interval.

5. Animals were slaughtered at 8 hours after last injection.

6. 14-C labeled ceftiofur sodium was used for this study. The 14-C label was demonstrated to be stable. All of the urine metabolites were shown to include a common structure which included the 14-C label. Likewise tissue metabolites were identified and more than 90% are known to be of a common structure which includes the 14-C label.

Total Residues (ppm) in Edible Tissues (6 animals):

	Beef Consumpton Factors	Calculated Safe Concentrations (1)	Mean	Std. Dev.
Muscle	1X	3.0 ppm	.23	.05
Fat	4X muscle	12.0	.56	.34
Liver	2X muscle	6.0	1.35	.24
Kidney	3X muscle	9.0	5.54	1.30
Injection Site	10X muscle	30.0	6.38	3.05

(1) Refer to Part C. of this Section.

E. Comparative Metabolism Study Results

Including the parent compound, ceftiofur, there are eight known metabolites of ceftiofur. These are identified as I (ceftiofur), II, IV, V, VII, VIII, IX and furoic acid. A polar metabolite U-3 also occurs in small amounts in some rats and in cattle.

Rats were orally dosed 14-C labeled ceftiofur sodium at 800 mg/kg body weight for five consecutive days then sacrificed 8 h post last feeding. The dose

approximates the highest NOEL (1,000 mg/kg) observed in any of the oral rat feeding studies. Cattle were intramuscularly administered 14-C labeled formulated ceftiofur sodium at 1 mg/lb for five consecutive days at a 24 h interval then sacrificed eight hours post last treatment. Metabolic profiles were compared between the rat and cattle in urine, kidney and liver tissues. Kidney is the limiting tissue because observed concentrations of total residues are highest in this tissue relative to the calculated safe concentration.

1. Urinary Metabolites

   In both species, the initial metabolite is II. Further metabolism of II in rats from oral treatment gave rise to IX which was not present in any significant amounts from IM treatment of cattle with ceftiofur.

   The major metabolite V in the urine of cattle was also present in the rat urine from oral treatment. Most of the metabolite V may not be a true metabolite but is an artifact which forms from II.

   A similar situation applies to the metabolite VII. When fresh samples of urine were analyzed from treatment of rats with 14-C ceftiofur, very high concentrations of II were found and very small amounts of VII or the U-3 polar metabolites were present. It appears that when II is not analyzed within a few hours, VII and U-3 are present in higher amounts. This finding supports the view that these compounds are formed from II.

   Metabolite IV is a product of II and its formation has been observed in rats and in cattle. Similarly VIII is formed from VII and is present in both rats and cattle.

   Metabolite IX was present in the rat urine but not present in bovine urine. There were no major metabolites present in bovine urine but absent from rat urine.

2. Kidney and Liver Metabolites

   Free metabolites in kidney and liver of rats and cattle are similar. Polar metabolite U-3 was present in both tissues of both species. Metabolites VII/VIII are major metabolites in both tissues of both species while metabolites II/IV are present in lesser amounts in both tissues of both species. The majority of the metabolites present in both rat and bovine liver and kidney tissues could be converted to a common derivative (compound X). This shows that liver and kidney tissues in rats and cattle all had related structures. Bound residue was metabolite II linked to macromolecules in both tissues of both species. There are no major metabolites present in either tissue of cattle but absent from rats. Likewise, there are no major metabolites present in the rat but not present in corresponding bovine tissues.

These data support the contention that the rat was autoexposed to metabolites to which humans would be exposed from consuming edible tissues of ceftiofur-treated cattle. Therefore, the toxicology studies in the rat accurately reflect the toxicity of these metabolites to which man would be exposed.

F. Tolerance for the Marker Residue

Total residues at zero day withdrawal are substantially lower than corresponding calculated safe concentrations for edible tissues, therefore neither a marker compound nor a tolerance for a marker compound is required. The permitted safe concentration for total residues in edible tissues is as follows:

- muscle 3.0ppm
- kidney 9.0 ppm
- liver 6.0 ppm
- fat 12.0 ppm

G. Study Establishing Withdrawal Period

Based on the toxicology data, and using the lowest No-Observed-Effect-Level of 30 mg/kg, a permitted Safe Concentration (SC) of 3 ppm in muscle and 9 ppm in kidney was established (Part C. of this section). Since the hypersensitivity studies demonstrated the risk of a hypersensitivity reaction due to ingestion of ceftiofur residues to be insignificant, the SC based on the toxicology data is applied.

Data presented in Part D. of this section showed that the mean concentrations of total ceftiofur residues at zero day withdrawal following the administration of 1.0 mg/lb (2X label dosage) were well below the permitted Safe Concentration. Therefore, a zero day pre-slaughter drug withdrawal has been established and no residue decline studies were required.

H. Regulatory Method

A regulatory method is not required; the residue and toxicology data support a zero withdrawal period (see Part G. of this Section).

1. Human Safety Considerations Other Than Food Safety

   a. Acute 4 Hour Dust Inhalation Study

      (i) Technical Report No. 7277-85-018, November 15, 1985

      (ii) Starting Date - April 11, 1985

      (iii) Termination Date - April 25, 1985

      (iv) Study Director - Basil K. J. Leong

      (v) Location of Study - The Upjohn Company, Kalamazoo, MI

      (vi) Identification of Substance and Dosage Form - Ceftiofur sodium - inhalation

      (vii) Species and Strain - Sprague-Dawley Rats

      (viii) Number Animals Per Sex Per Treatment Group - 5 M and 5 F

      (ix) Drug Levels Tested and Duration of Dosing - 8 mg/l for 4 h

      (x) Route of Drug Administration - Inhalation

(xi) Parameters Tested - Pharmacologic signs, body weights, mortality, gross necropsy exam and histology

(xii) Significant Toxicity Observed

During the first hour of exposure, most of the rats exhibited salivation and/or nasal discharge. By the end of the 4-hour exposure all the rats exhibited slight dyspnea. However, when the rats were removed from the chamber approximately 1 hour post exposure, all but 1 of the rats appeared normal. Following exposure, diarrhea was observed in 6 rats for 1 to 2 days between days 2 and 5 post exposure. By day 6 post inhalation exposure no significant compound related pharmacotoxic signs were observed except for 1 male appearing unkempt between post exposure days 4 and 10, and 1 female showing a red material encrusted around the nares from days 10 to 12 post exposure. Gross necropsy examination of the rats, which were sacrificed at the end of the 14 day study period, revealed no compound related gross lesions. Histopathological examination of the lungs, liver, kidney, trachea and nasal turbinates revealed no significant changes.

(xiii) No Observed Effect Level - No effect at 8 mg/l

(xiv) Statistical Analysis - Not applicable to data generated from this dose study

(xv) Conclusions

The acute 4-hour median lethal concentration (LC50) for rats is greater than 8.3 mg/l. Thus, ceftiofur sodium dust is unlikely to cause any harmful effects to humans at concentrations which may be encountered during therapeutic or industrial handling of the bulk drug.

b. Primary Eye Irritation Study in Albino Rabbits

(i) Technical Report No. 7277-85-008, December 9, 1985

(ii) Starting Date - May 30, 1985

(iii) Termination Date - June 13, 1985

(iv) Study Director - Basil K. J. Leong

(v) Location of Study - The Upjohn Company, Kalamazoo MI

(vi) Identification of Substance and Dosage Form - Ceftiofur sodium pulverized powder

(vii) Species and Strain - New Zealand White Rabbits

(viii) Number Animals Per Sex Per Treatment Group - 6 M and 6 F

(ix) Drug Levels Tested and Duration of Dosing - 100 mg instilled once into the conjunctival sac of the right eye of each rabbit; left eye used as control.

(x) Route of Drug Administration - Ocular exposure

(xi) Parameters Tested

Clinical - treated and non treated eyes were examined and compared at 1, 24, 48 and 72 h and at 4, 7 and 14 days after treatment. Grading and scoring of irritation were performed in accordance with Draize's procedure.

Pathology - complete gross necropsy was performed on all rabbits that died during the study.

Histopathology - tissues and organs obtained from dead animals of the first 6 tested rabbits included lungs, kidney, heart, urinary bladder, liver, cecum, eyes, adrenal, pancreas, mesenteric lymph node, duodenum, rectum, thymus and from the second 6 tested rabbits included eyes, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, lung, heart, liver, kidney, adrenal and spleen.

(xii) Significant Toxicity Observed

In the first experiment, a single 100 mg dose of ceftiofur sodium bulk drug powder was instilled into the conjunctival sac of the right eyes of 3 male and 3 female albino rabbits. The left eyes were untreated and served as controls. At one hour post dosing all the treated eyes exhibited slight conjunctival redness and/or swelling. In addition the treated eyes of 2 male and 3 female rabbits also exhibited slight to moderate discharge. By 24 hours post treatment, the treated eyes of all 6 rabbits appeared normal. However, on day 3 post instillation, 2 of the 6 rabbits were found dead. One had died from severe pulmonary edema, and the other from severe necrotizing cecitis. The remaining 4 rabbits appeared normal for the remainder of the 14 day study period.

Because of the unexpected deaths, the eye irritation experiment was repeated on another group of 3 male and 3 female rabbits. Added to the experimental procedure were daily observations for pharmacotoxic signs and terminal necropsy and histopathologic examination on all animals. The results indicated that the eye irritation was minimal, as it was in the first experiment. However, 5 of the 6 rabbits exhibited diarrhea and body weight loss beginning 2 to 3 days post treatment and 2 of the 5 affected rabbits were found dead in 5 and 13 days post treatment, respectively. The cause of death was apparently enteritis in one rabbit and mucoid enteropathy in the other. It was concluded that the cause of death in the Experiment 1 rabbit with cecitis and the Experiment 2 rabbit with enteritis may have been enterotoxemia, which may have been related to ceftiofur.

(xiii) No Observed Effect Level - Not applicable

(xiv) Statistical Analysis - None

(xv) Conclusions

The experimental results indicated that ceftiofur sodium powder is minimally irritating to rabbit eyes. However, the drug was apparently absorbable via the ocular route and caused detrimental systemic effects in some rabbits.

For industrial hygiene purposes, avoid gross eye exposure to the bulk drug powder. Should eye exposure occur, promptly rinse the contaminated eye(s) with water and obtain medical attention.

V. AGENCY CONCLUSIONS

Under the Agency's proposed supplemental approval policy (42 FR 64367, December 23, 1977), this is a Category II change. This supplemental application for Naxcel Sterile Powder provides for a change in dosage from 0.5 mg/kg BW to a range of 0.5 to 1.0 mg/lb BW. All other aspects of the original NADA approval remain unchanged. Human food safety, target animal safety and environmental safety data in the original NADA were generated using a dose of 1.0 mg/lb, therefore, no new data were necessary to determine the safety and efficacy of this product at the 1.0 mg/lb dose level.

A reevaluation of the original data supports the conclusion that in certain outbreaks of bovine respiratory disease or "shipping fever" the use of 1.0 mg/lb is advantageous.

The clinical condition due to bovine respiratory disease (shipping fever) varies considerably depending on the organism (species and number of bacteria, resistance), the nutritional condition of the cattle, the weather, age of animals, and husbandry practices.

The dose range allows the veterinarian to treat according to his or her professional judgment as to the clinical severity of the outbreak. The range also provides for the safe and efficacious use of Naxcel within an acceptable window for target animal and human safety and within FDA approved labeling.

The product remains a prescription drug. No preslaughter withdrawal time is needed because tissue residue levels are below the safe concentration in all edible tissues 8 hrs after administration of the product at 1 mg/lb body weight. Naxcel causes an immediate and transient local pain at the intramuscular injection site which is not significantly more severe at the 1 mg/lb level. The environmental assessment in the original NADA was conducted at both dose levels and a finding of no significant impact (FONSI) was prepared.

The Agency concludes that Naxcel Sterile Powder is safe and effective when used for the treatment of bovine respiratory disease in beef and non-lactating dairy cattle at the dosage range of 0.5 to 1.0 mg/lb of body weight.

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