CEFTIOFUR
(MONOHYDROCHLORIDE and SODIUM SALTS)

First draft prepared by
Dr. J.D. MacNeil
Agriculture & Agri-Food Canada
Health of Animals Laboratory
Saskatoon, Canada

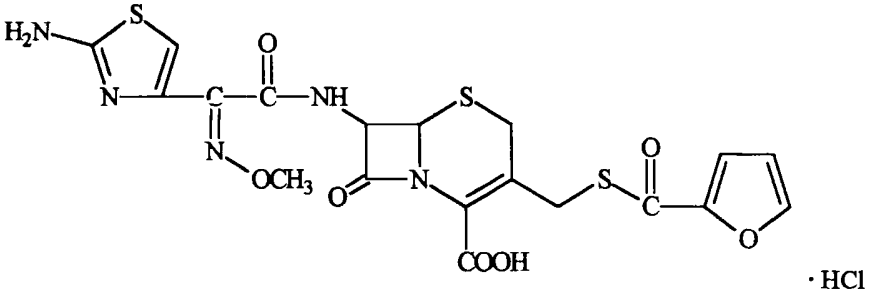
ADDENDUM
to the Ceftiofur sodium monograph prepared by the forty-fifth meeting of the Committee and published in FAO Food and Nutrition Paper 41/8, Rome 1996

IDENTITY

Chemical name: Ceftiofur monohydrochloride; (6R-7R)-7-[[(2-amino-4-thiazolyl)-Z-(methoxyimino)acetyl]amino]-3-[[(2-furanylcarbonyl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monohydrochloride.

Synonyms: Ceftiofur monohydrochloride, PNU-64,279A

Structural formula:



Ceftiofur monohydrochloride

Molecular formula: $C_{19}H_{17}N_5O_7S_3 \cdot HCl$

Molecular weight: 560.03

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Ceftiofur hydrochloride is a broad spectrum cephalosporin which is formulated as a sterile suspension for administration by injection. The formulation is ready for use and contains 50 mg ceftiofur equivalents/mL. The product is similar in intended use to the sodium salt which was previously reviewed at the 45th meeting of the Committee in 1995.

Dosage

The typical maximum dosage is 2.2 mg/kg BW on 5 successive days in cattle and 3 mg/kg on 3 successive days in swine. Higher dosages have been tested.

METABOLISM AND PHARMACOKINETICS

Cattle

Six calves (average BW 376 kg at slaughter) each received 2.2 mg ceftiofur free acid equivalents (CFAE)/kg BW by intramuscular injection (IM) of ^{14}C-ceftiofur hydrochloride on 5 successive days (Johnson *et al*, 1987). Blood samples were collected and analyzed after each treatment. Maximum ceftiofur residues of 2.56 to 6.39 mg/L were found in blood samples collected at 2 to 4 hours post-treatment, declining to 0.42 to 1.46 mg/L 24 hours after each treatment. Depletion curves showed a small bioaccumulation effect and had an area under the curve (AUC) similar to that observed for ceftiofur sodium, indicating bioequivalence for the two salts. Average excretion at 12-hour withdrawal was 78.9 ± 5.3%. The amount excreted in the urine was approximately three times that found in feces, again similar to observations with ceftiofur sodium. All animals were slaughtered at 12-hour withdrawal and assayed for total residues. Average residues found were: kidney, 4.89 ± 1.62 mg/kg; liver, 1.41 ± 0.47 mg/kg; muscle, 0.19 ± 0.05 mg/kg; and fat, 0.86 ± 0.30 mg/kg. The tissue residue results are similar to those previously reviewed for ceftiofur sodium.

A study was also presented in which six cattle (3 male, 3 female) received an average daily dose of 2.45 mg/kg BW per day on 5 successive days, administered by intramuscular injection of ^{14}C-ceftiofur hydrochloride (Beconi-Barker *et al.*, 1996). In total, 88.9% of the administered drug was accounted for, with 55.2% excreted in urine and 31.1% in feces. Highest residue concentrations (29.9 ± 12.9 mg/kg) were found in the injection sites for the final injection, which was administered 12 hours prior to slaughter. Data on urine and plasma metabolite profiles were collected in this study, but were not available at the time of preparation of this report. Residue results are discussed in the next section.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Cattle

Six cattle (3 male, 3 female) each received an average IM dose of 2.45 mg CFAE/kg BW, administered as ^{14}C-ceftifofur hydrochloride on 5 successive days, and were slaughtered at 12 hours after the final injection (Beconi-Barker *et al*, 1996). In females, average total residues measured as total radioactivity found in edible tissues at slaughter were: kidney, 7.91 mg/kg; liver, 1.60 mg/kg; muscle, 0.28 mg/kg; and fat, 0.42 mg/kg. In males, average residues in edible tissues collected at slaughter were: kidney, 6.82 mg/kg; liver, 1.43 mg/kg; muscle, 0.21 mg/kg; and fat, 0.40 mg/kg. Average residues in injection sites in females varied from 35.41 ± 12.03 mg/kg in the day 5 site to 0.85 ± 0.22 mg/kg in the day 1 injection site, while in males the equivalent findings were 24.45 ± 13.54 for day 5 and 1.97 ± 0.88 mg/kg for day 1. The dose administered is approximately 11% in excess of the recommended dose of 2.2 mg/kg BW, so residues are slightly elevated above those expected at the label dose. Residue concentrations reported in this study are total residues, not marker residue determined as desfuroylceftiofur (DFC).

The tissues assayed for total residues of ^{14}C-ceftiofur were also assayed for the marker residue, DFC, by HPLC (Beconi-Barker, 1996). The findings are summarized in Table 1.

Table 1. **Comparison of total residues of ceftiofur, determined as ^{14}C-ceftiofur equivalents, with ceftiofur and metabolites containing an intact β-lactam ring, determined as desfuroylceftiofur by HPLC. Tissues were collected at sacrifice 12 hours after final treatment of cattle (n = 6) with 2.45 mg CFAE/kg BW (IM administration, treatments on 5 successive days).**

Tissue	Total Residues as ^{14}C-ceftiofur equivalents (mg/kg)	Marker Residue DFC by HPLC (mg/kg)	Ratio marker residue/total residue
Kidney	7.37	3.35	0.45
Liver	1.52	0.68	0.45
Muscle	0.24	0.12	0.50
Fat	0.41	0.22	0.54

Swine

Tissues collected from swine treated with ^{14}C-ceftiofur hydrochloride were analyzed for both total residues, based on the determination of total radioactivity, and intact β-lactam ring-containing residues, determined by HPLC as DFC (Beconi-Barker *et al*, 1995). In the first phase of the study, twelve swine (6 male, 6 female) were randomly divided into 3 sub-groups of 2 males and 2 females. Within each sub-group, 1 male and 1 female each received IM 5.0 mg CFAE/kg BW at 24-hour intervals on three successive days, while the remaining pair in each sub-group received 7.5 mg CFAE/kg BW, following the same treatment regimen. Animals were slaughtered 12 hours after administration of the last injection of ceftiofur and tissues were collected and analyzed. In the second phase of the study, 6 males and 6 females were randomly assigned to two sub-groups (3 males, 3 females per sub-group). All animals received IM 3.0 mg CFAE/kg BW on three successive days. One male and one female from each sub-group were slaughtered at each of 12, 72 and 120 hours after the last injection with ceftiofur. Tissues were collected and analyzed. Results showing the total residues as determined by combustion and the marker residue as determined by HPLC are given in Table 2.

Table 2. **Marker residue (DFC) and total residues (TR as ^{14}C-ceftiofur equivalents) found in swine which received ^{14}C-ceftiofur hydrochloride at 3.0, 5.0 or 7.5 mg CFAE/kg BW by IM administration on 3 successive days**

WT (h)	Dose CFAE/ kg BW	Muscle (mg/kg)		Liver (mg/kg)		Kidney (mg/kg)		Fat (mg/kg)	
		DFC	TR	DFC	TR	DFC	TR	DFC	TR
12	7.5	0.70	1.07	1.29	2.64	4.16	10.68	1.00	2.45
	5.0	0.52	0.80	0.94	1.79	3.20	6.33	0.75	1.35
	3.0	0.29	0.44	0.64	1.06	1.10	3.62	0.50	1.05
72	3.0	ND	0.06	ND	0.36	ND	1.00	ND	0.17
120	3.0	ND	0.10	ND	0.35	ND	1.65	ND	0.12

WT = withdrawal time; ND = not detected (<0.05 mg/kg)

Other Residue Depletion Studies (with unlabelled drug)

Cattle

Fourteen cattle (7 steers, 7 heifers, BW 305 to 455 kg) were used in a study in which 12 animals each received 2.2 mg/kg BW ceftiofur hydrochloride by subcutaneous (SC) injection on 5 successive days, with slaughter at 12 hours after the final treatment (Brown *et al, 1995)*. Two animals served as controls. Edible tissues were assayed for ceftiofur and metabolites with an intact β-lactam ring as desfuroylceftiofur acetamide (DFC) by HPLC. Residues were also determined in kidneys using an analytical procedure that isolates the bound residues, then liberates them for analysis as DFC by HPLC. Residues found were as follows: kidney (total marker as DFC), 4.06 ± 0.61 mg/kg; kidney (bound residue as DFC), 1.49 ± 0.36 mg/kg; liver, 0.99 ± 0.25 mg/kg; and muscle, 0.20 ± 0.04 mg/kg.

A study was also reported in which 30 male animals per location were treated with ceftiofur sodium at each of two test locations (Ibayashi *et al*, 1996a). At each location, the animals were divided into two groups of 15, with one group receiving 5 daily IM treatments of 2.0 mg CFAE/kg BW and the second group receiving 4.0 mg CFAE/kg BW according to the same regimen. Sub-groups of 3 animals from each treatment group were slaughtered at 1, 3, 15, 20 and 25 days after the last injection. Tissue samples were collected at slaughter and analyzed by HPLC, the results of which are given in Table 3 for samples collected at 1 to 15 days withdrawal. No detectable residues were found in any samples collected at 20 or 25 days. Highest residues were in kidneys at day 1, but at days 3 and 15 residues were higher in liver than in kidney. Significant residues were found in injection sites at day 1, but residues rapidly depleted over the subsequent sampling dates.

Table 3. Residues of ceftiofur, determined by liquid chromatography as desfuroylceftiofur acetamide (LOD 0.05 mg/kg) following IM injection of ceftiofur sodium at a dose of 2 mg CFAE/kg BW or 4 mg CFAE/kg BW on five successive days in male cattle.

Withdrawal time (days)	Treatment (mg CFAE/kg BW)	Residues measured as DFC (mg/kg)				
		Kidney	Liver	Muscle	Fat	Injection site
1	2	0.49	0.44	<0.05	0.10	4.44
	4	0.84	0.54	0.07	0.18	22.2
3	2	0.11[1]	0.30[1]	<0.05	0.08[1]	0.18
	4	0.16[1]	0.41[1]	<0.05	0.07[1]	0.18[1]
15	2	0.05[2]	<0.05	---[3]	<0.05	<0.05
	4	<0.05	0.11[1]	<0.05	<0.05	<0.05

[1] Samples below LOD not included in mean; [2] Only one sample was >LOD; [3] Not analyzed.

Swine

Studies were conducted with ceftiofur sodium at two different geographical locations using 30 castrated swine at each site (BW 22.6 to 33.9 kg), with two treatment groups duplicated per site (Ibayashi *et al*, 1996b). At each site, 15 animals received 3.0 mg ceftiofur free acid equivalents (CFAE)/kg BW by IM injection on 3 successive days, while the remaining 15 animals received 6.0 mg CFAE/kg BW by IM injection on 3 successive days. Groups of 3 animals were randomly selected for slaughter at 1, 3, 7, 10 and 15 days after the third injection. A control animal was also slaughtered on Day 1 in each treatment group. Samples of plasma, fat, muscle, kidney, small intestine, liver, injection site muscle and tissue surrounding the injection site were collected from each animal at slaughter. All

samples were analyzed by liquid chromatography for the marker residue, desfuroylceftiofur acetamide (DFC), which includes all remaining residues with an intact β-lactam ring. No residues were detected in the control animals. Results of the analyses of the edible tissues and the injection site are given in Table 4.

Table 4. Residues of ceftiofur in swine, determined by liquid chromatography as desfuroylceftiofur acetamide (LOD 0.05 mg/kg) following IM injection of ceftiofur sodium at a dose of 3 mg CFAE/kg BW on three successive days or 6 mg CFAE/kg BW on three successive days

Withdrawal time (days)	Treatment (mg CFAE/kg BW)	Residues measured as DFC (mg/kg)				
		Kidney	Liver	Muscle	Fat	Injection site
1	3	0.40	0.15	0.16	0.20	0.26
	6	0.68	0.24	0.23	0.29	0.57
3	3	0.07	<0.05	<0.05	0.08	<0.05
	6	0.08	0.06	<0.05	0.07	0.08
7	3	<0.05	<0.05	<0.05	<0.05	<0.05
	6	<0.05	<0.05	<0.05	<0.05	<0.05

Residues depleted rapidly in all tissues, with no detectable residues in any tissues at 7 days following the final treatment. For the 3 mg CFAE/kg BW treatment, only kidneys and fat contained detectable residues at 3 days, while residues were detectable in all tissues except muscle at the higher treatment rate for this period from final treatment to slaughter. Once samples of any tissue contained no detectable residues for two successive sampling dates, these tissues were not tested for the remaining sampling dates. None of the samples collected at 15 days were tested and only kidneys were tested at 10 days withdrawal. As in previous studies, highest and most persistent residues were found in the kidneys.

APPRAISAL

At the 45th JECFA meeting, an ADI of 0 - 50 mg/kg bw was established for ceftiofur, based on a microbiological end-point. MRLs were recommended for ceftiofur as follows, for both cattle and pigs: muscle, 200 µg/kg; liver, 2000 µg/kg; kidney, 4000 µg/kg; and fat, 600 µg/kg; and, for cows' milk, 100 µg/l; determined as desfuroylceftiofur acetamide (DFC). These MRLs accounted for a maximum daily intake of 640 µg, while the ADI set by the Committee allowed for a maximum daily intake of 3000 µg for a 60-kg person. The Committee agreed to consider additional information on the depletion of ceftiofur, if such information should become available in the future, particularly with respect to the depletion of the marker residue in treated animals 12-72 hours after treatment. The Committee received the following new studies for review, all conducted according to appropriate standards for study protocol and conduct.

Cattle. Six cattle each received an average IM dose of 2.45 mg Ceftiofur/kg bw, administered as ^{14}C-ceftiofur monohydrochloride, on 5 successive days and were slaughtered 12 hours after the final injection. Total ^{14}C-residues at slaughter in edible tissues were: muscle, 0.15 - 0.29 mg/kg; kidney, 6.0 - 8.3 mg/kg; liver, 1.2 - 1.8 mg/kg; and fat, 0.35 - 0.46 mg/kg. Residues in day 1 (initial) injection sites ranged from 0.6 - 2.7 mg/kg, while the range for day 5 (final) injection sites was 12.6 - 48.4 mg/kg. Marker residue accounted for approximately one-half the total residue present in all edible tissues at slaughter 12 hours following the final administration of the drug.

Twelve cattle (bw 305 to 455 kg) each received 2.2 mg/kg bw ceftiofur hydrochloride by subcutaneous (SC) injection on 5 successive days, with slaughter 12 hours after the final treatment. Residues in edible tissues,

determined as DFC, were: muscle, 0.12 - 0.29 mg/kg; kidney, 3.7 - 5.6 mg/kg; and liver, 0.77 - 1.8 mg/kg. Results were consistent with those observed in studies where the drug was administered by IM injection.

Sixty male calves (98.7 to 168.1 kg bw) were used in a trial conducted at two separate locations. At each location, 15 cattle received 2.0 mg/kg Ceftiofur/kg bw per day as ceftiofur sodium by IM on each of five successive days and the remaining 15 cattle received twice that dose. Edible tissues and injection site tissue were collected from each of three animals slaughtered at 1, 3, 15, 20 or 25 days after the final injection and analyzed for DFC by HPLC. For cattle treated at 2.0 mg/kg bw, no DFC residues were found in muscle at any sampling date. In kidney, residues ranged from 0.27 - 0.70 mg/kg at day 1, declining to <0.05 - 0.17 mg/kg at day 3 and were not detected in subsequent samples. Residues in liver were more persistent, declining from 0.10 - 1.40 mg/kg at day 1, to <0.05 - 0.46 mg/kg at day 3, and <0.05 mg/kg by day 15. In fat, residues ranged from 0.07 - 0.15 mg/kg at day 1, <0.05 - 0.10 mg/kg at day 3, and <0.05 mg/kg at day 15. At the 4.0 mg/kg bw dose, residues in muscle were <0.05 - 0.08 mg/kg at day 1, but <0.05 mg/kg on subsequent sampling dates. Residues in kidney, liver and fat were generally higher than at the lower dose, but had depleted in all tissues by day 15, except for liver (<0.05 - 0.16 mg/kg). Residues in injection sites for the high dose group were 4.2 - 90 mg/kg at day 1, but had declined to <0.05 - 0.38 mg/kg at 3 days following final treatment. For the 2.0 mg/kg injection sites, residues ranged from 0.36 - 9.1 mg/kg at day 1, but were 0.05 - 0.32 mg/kg at 3 days following final treatment.

Pigs. In the first phase of a study using ^{14}C-ceftiofur monohydrochloride in which tissues were analyzed for both total ^{14}C- residues and DFC, six pigs each received three IM doses of 5.0 mg Ceftiofur/kg bw at 24-hour intervals, while another six pigs each received 7.5 mg Ceftiofur/kg bw, following the same treatment regimen. Animals were slaughtered 12 hours after administration of the last injection of ceftiofur and tissues were collected and analyzed. In the second phase of the study, 12 pigs each received IM 3.0 mg Ceftiofur/kg bw on three successive days. Four pigs were slaughtered at each of 12, 72 and 120 hours after the last injection with ceftiofur. Residues increased with increasing dosage, but marker residue was found only in the samples collected from animals slaughtered 12 hours after the final treatment, with the exception of kidney from one pig in the 72-hour group which contained detectable residues. At 12 hours, the DFC residues detected were:

- 3.0 mg Ceftiofur/kg bw: muscle, 0.20-0.35 mg/kg; kidney, <0.05-1.76 mg/kg; liver, 0.55-0.74 mg/kg; and fat, 0.43-0.57 mg/kg.
- 5.0 mg Ceftiofur/kg bw: muscle, 0.40-0.72 mg/kg; kidney, 2.54-3.64 mg/kg; liver, 0.68-1.18 mg/kg; and fat, 0.29-1.29 mg/kg.
- 7.5 mg Ceftiofur/kg bw: muscle, 0.55-0.87 mg/kg; kidney, 3.24-4.98 mg/kg; liver, 1.04-1.59 mg/kg; and fat, 0.62-1.88 mg/kg.

Ratios of total residues, determined as DFC to ^{14}C-ceftiofur,were, for the various edible tissues, as follows: muscle, 67; kidney, 33; liver, 50; and fat, 40%.

Studies were conducted at two different geographical locations using 30 castrated pigs at each location (bw 22.6 to 33.9 kg), with two treatment groups of 15 animals per site, which received, respectively, 3.0 or 6.0 mg Ceftiofur/kg bw by IM injection on 3 successive days. Samples of muscle, kidney, liver, fat and injection site muscle were collected from each of three animals at each dose rate slaughtered at 1, 3, 7, 10 or 15 days after the third injection and analyzed by HPLC for the marker residue, DFC. At day 1, for the 3 mg Ceftiofur/kg bw treatment, residues were: muscle, 0.12-0.22 mg/kg; kidney, 0.34-0.68 mg/kg; liver, 0.12-0.20 mg/kg; fat, 0.20-0.32 mg/kg; and injection site muscle, 0.09-0.42 mg/kg. At the 6 mg/kg bw treatment rate, residues were: muscle, 0.17-0.28 mg/kg; kidney, 0.48-0.75 mg/kg; liver, 0.18-0.31 mg/kg; fat, 0.26-0.37 mg/kg; and injection site, 0.43-0.78 mg/kg. For the 3 mg Ceftiofur/kg bw treatment, only kidney (0.07 mg/kg) and fat (0.08 mg/kg) from one pig contained detectable residues at 3 days, while residues were detectable in all tissues except muscle at the 6 mg Ceftiofur/kg bw treatment rate (kidney, 0.06-0.10 mg/kg; liver, <0.05-0.06 mg/kg; and fat, <0.05-0.10 mg/kg). There were no detectable residues in any tissues at either dosing level at 7 days following the final treatment. As in previous studies with pigs, highest and most persistent residues were found in the kidneys. The change in the site of higher residues from kidney to liver at day 1 and at subsequent sampling dates observed in cattle was not seen in pigs.

Maximum Residue Limits

In reaching its decision on the MRLs for ceftiofur, the Committee took into account the following:

- The ADI for ceftiofur established by the 45th JECFA based on a microbiological end-point is 0-50 μg/kg of body weight.
- A significant proportion of the total residue is considered to be inactive because the β-lactam ring is no longer intact. MRLs are expressed as the marker residue, DFC, which accounts for all active residues and only these are used in calculating a theoretical daily exposure.
- Residues measured as DFC are near or below 0.05 mg/kg (LOD) in muscle and fat and <0.5 mg/kg in liver and kidney at 3 days after treatment in both cattle and pigs in studies provided.
- Based on the depletion studies submitted marker residue in excess of the MRLs established for muscle and kidney by the 45th JECFA may be found in some treated pigs and cattle at 12 hours following final administration in accordance with recommended Good Veterinary Practices.
- The MRLs were harmonized for pigs and cattle, on the basis of the depletion studies submitted.
- The drug is used for therapeutic purposes only.
- No new data were submitted on residue levels in milk.

On the basis of the above considerations, the Committee recommended MRLs for ceftiofur, expressed as DFC, of 1000 μg/kg for muscle, 6000 μg/kg for kidney and 2000 μg/kg for fat in both cattle and pigs. The MRLs recommended by the 45th JECFA for ceftiofur of 2000 μg/kg for liver in both cattle and pigs and 100 μg/l for cows' milk were reaffirmed.

The MRLs recommended above would result in a theoretical maximum daily intake of 1050 μg of microbiologically active ceftiofur residues, expressed as desfuroylceftiofur, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5 l of milk.

REFERENCES

Brown, S.A., Beconi-Barker, M.G., Hamlow, P.J., Callahan, J.K., Hubbard, V.L., Roof, R.D., Smith, G.A., and Smith, E.B. (1995). Pivotal tissue residue study of ceftiofur and desfuroylceftiofur metabolites after ceftiofur hydrochloride sterile suspension (EXCENEL™ Sterile Suspension) administered subcutaneously to cattle. Research Report TR-788-7926-94-015, Sponsor submitted.

Beconi-Barker, M.G., Callahan, J.K., Hubbard, V.L., Smith, E.B., Smith, G.A., and Gilbertson, T.J. (1995). Correlation between ceftiofur and related metabolites containing an intact β-lactam ring determined by HPLC as desfuroylceftiofur acetamide and total ceftiofur-related residues determined by combustion in swine tissues. Research Report TR-796-7926-95-001, Sponsor submitted.

Beconi-Barker, M.G. (1996) Ceftiofur hydrochloride in cattle, tissue residue concentrations. Interoffice memo to C.C. Miller dated November 20, 1996, file ID D:\233\233resdc.mgb, Sponsor submitted.

Beconi-Barker, M.G., Arnold, T.S., Smith, E.B., Hornish, R.E., Cox, T.D., Dame, K.J., Flook, T.F., Lewis, V.R., Janose, R.L., and Gilbertson, T.J. (1996). ^{14}C-Ceftiofur hydrochloride residue concentration in tissues, plasma, and excreta from bovine 12 hours after the last of five intramuscular doses at 2.45 mg ceftiofur free acid equivalents/kg body weight. Research Report TR-788-7926-95-008, Sponsor submitted.

Ibayashi, T., Beconi-Barker, M.G., Hibbard, B., and Robb, E.J. (1996a). Disposition of ceftiofur sodium (U-64279E) in cattle 1, 3, 15, 20 and 25 days after the last of five intramuscular injections of 2.0 or 4.0 mg ceftiofur equivalents/kg body weight. Research Report X788-9690-96-003, Sponsor submitted.

Ibayashi, T., Beconi-Barker, M.G., Hibbard, B., and Robb, E.J. (1996b). Disposition of ceftiofur sodium (U-64279E) in swine 1, 3, 7, 10 and 15 days after the last of three daily intramuscular injections of 3.0 or 6.0 mg ceftiofur equivalents/kg body weight. Research Report X796-9690-96-001, Sponsor submitted.

Johnson, D.B., Gatchell, C.L., Kratzer, D.D., Byrd, J.W., Billings, T.J., Santos, A., Johnson, J.S., Whitis, A.K., and Lane, L. (1987). Zero-day withdrawal study of ceftiofur monohydrochloride (U-64,279A) in mixed-breed feeder calves. Research Report TR-788-9760-87-014, Sponsor submitted.