California Department of Food and Agriculture
Protocol for Diversion of Confirmed Positive Bulk Raw Milk Tankers to Calf Ranches – Beta Lactams Only
Revised 7/19/02

Protocol: All bulk raw milk tankers confirmed positive for beta lactam intended for diversion to calf feed shall be cleared for such use by a negative test obtained from a 1:100 dilution of the tanker milk sample as an additional test step by the M-a-85 screening test used for the Screening Test Positive (Load Confirmation) procedure. The sample shall be diluted with milk confirmed negative for beta lactams.

Loads testing negative on a 1:100 dilution may be diverted to animal feed provided the shipping invoice includes: the negative test result, dilution, test used and recorded test reading, certified laboratory identification, signature of certified analyst and load identification verification plus a clear statement “MEDICATED ANIMAL FEED –WITHDRAW 45 DAYS BEFORE SLAUGHTER”

Loads testing positive with 1:100 dilution must be disposed by an approved manner other than animal feed use.

The table below represents the maximum expected beta lactam drug residue present in a negative 1:100 diluted sample tested by Charm SL. 3rd Quarter 2001, 25 of 36 tankers confirmed by Charm SL or 70% of tankers. (See Farad Report below)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tolerance or Safe concentration</th>
<th>CDFA Proposal</th>
<th>Charm SL Performance Standard*</th>
<th>Charm SL Performance Standard* @ 1:100</th>
<th>FARAD* Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>5 ppb</td>
<td>500 ppb</td>
<td>3.6 ppb</td>
<td>360</td>
<td>14,000 ppb</td>
</tr>
<tr>
<td>Cephapirin</td>
<td>20 ppb</td>
<td>2000 ppb</td>
<td>13.7 ppb</td>
<td>1370</td>
<td>14,000 ppb</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>10 ppb</td>
<td>1000 ppb</td>
<td>5.6 ppb</td>
<td>560</td>
<td>2000 ppb</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10 ppb</td>
<td>1000 ppb</td>
<td>8.5 ppb</td>
<td>850</td>
<td>2000 ppb</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>10 ppb</td>
<td>1000 ppb</td>
<td>50.0 ppb</td>
<td>5000</td>
<td>6000 ppb</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>100 ppb</td>
<td>5000 ppb</td>
<td>46.2 ppb</td>
<td>4600</td>
<td>6000 ppb</td>
</tr>
</tbody>
</table>

* Performance Standard in this table refers to the point where we are 95% confident that 90% of the time the kit will give a correct answer for a positive sample.

FARAD REPORT

EXECUTIVE SUMMARY
From the California Dept. of Food & Ag: “What are the maximum amount of penicillin G, cephapirin, amoxicillin, ampicillin, cloxacillin, and ceftiofur which can be fed to calves which following a 45 day withdrawal which would not result in tissue residues?” This is a follow-up question to FARAD consultation CA-012201-8736. The proposed residue limits are tabulated in the “FARAD RESPONSE” section below.

FARAD REQUEST
FARAD had previously performed an assessment regarding the proposed waste milk diversion (see FARAD call record CA-012201-8736). Specifically CDFA proposed allowing the feeding of milk containing penicillin G, cephapirin, amoxicillin, ampicillin, cloxacillin, and ceftiofur at up to 50, 200, 100, 100, 100 and 500 ppb respectively. The CDFA proposal is based in part on a protocol developed by a Texas feeding company which received a letter of “non-objection” from the federal Food and Drug
Administration (FDA). Based on published and unpublished data available at that time the Texas feeding company’s 45-day withdrawal period was virtually certain to prevent violative tissue residues in calves fed milk containing antibiotic residues at levels proposed by CDFA. While some data gaps existed, it was unlikely that the assumptions made in assessment would prove problematic.

CDFA contacted FARAD subsequent to the first assessment. The State is reviewing its new policy on diversion of waste milk for calf feeding. As the milk diversion protocols where being fine-tuned CDFA required an estimate what level of beta lactams would be necessary to see organ/tissue residues in calves after a 45-day withdrawal. The concern was that the levels originally proposed by CDFA were to conservative relative to the magnitude of many of the tanker contaminations occurring every year.

The question put to FARAD: “What are the maximum amount of penicillin G, cephapirin, amoxicillin, ampicillin, cloxacillin, and ceftiofur which can be fed to calves which following a 45 day withdrawal would not result in tissue residues?” The initial assessment and studies published subsequent to the initial assessment were reviewed.

**FARAD RESPONSE**

CDFA requests the maximum amount of antibiotics that could be fed to calves and not result in tissue residues following a 45-day withdrawal. Below is a chart which compares the current tolerance (or safe concentration), CDFA’s original proposed contamination limits, the Charm SL detection levels (the assay used in about 70% of California’s testing sites) and FARAD’s recommendation for maximum contamination assuming a 45-day slaughter withdrawal interval (WDI). The pivotal reference(s) upon which the FARAD recommendation was made is also footnoted.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tolerance or Safe concentration</th>
<th>Previous CDFA proposal</th>
<th>Charm SL LOD</th>
<th>FARAD Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>5 ppb</td>
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<td>Cloxacillin</td>
<td>10 ppb</td>
<td>100 ppb</td>
<td>50.0 ppb</td>
<td>6000 ppb</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>100 ppb</td>
<td>500 ppb</td>
<td>46.2 ppb</td>
<td>6000 ppb</td>
</tr>
</tbody>
</table>

**Footnotes:**

A FARAD Database  
B Charm SL detection levels (M-a-85 revision #8)  
C Penicillin Assessment: Pivotal tissue residue depletion study (JAVMA 219:3) modeled a 21-hour WDI for 3,333 ppb and 27-hour WDI for 14,665 ppb. A separate study (9005522) found no kidney/muscle residues @ 24 hrs after feeding 6,600 & 13,200 ppb  
D Cephapirin Assessment: Models using reported serum T ½ and tissue residues following IM treatment of 8.5-30 mg/kg (equivalent to milk containing up to 44,625 - 360,000 ppb) would fall below tolerance in 6.5 – 10 days (9001522, 9000360, European Committee For Veterinary Medical Products-EMEA/MRL/0128/96). The 14,000 suggested limit represent a logistically convenient level.  
E Amoxicillin Assessment: Pivotal study (JDS, 84:126-133) found no serum/urine residues @ 24 hrs after feeding 2,000 ppb Q 12 hours for 3 feedings. Also, label dose of amoxicillin (8.9 mg/kg PO q 12 hours for 5 days) has a 20-day withdrawal and calves would be consuming equivalent of 50,000-200,000 ppb. 24 hours following a single feeding of 7 mg/kg in milk (equivalent to 18,750-75,000 ppb), residues were near tolerance (9001726).  
F Ampicillin Assessment: From previous evaluation (CA-012201-8736): ampicillin has much lower % bioavailability than amoxicillin. Collectively several citations (9003155, 9001725, 9001723, 9001696, 9001467, 9001424, 8004008 and 9000110 indicate that serum, urine, bile and tissue half-lives of ampicillin in calves following oral, IV and/or IM administration is short, on the order of 2-3 hours.  
G Cloxacillin Assessment: A literature search revealed no new pivotal data from previous evaluation (CA-012201-8736) and FARAD’s publication on veal calf residues (JAMVA Vol. 213, No. 1 July 1, 1998).  
H Ceftiofur Assessment: Label IM dose (having a zero withdrawal time) is equivalent to 6,000 ppb.
Note that using the approved Charm SL assay, a milk sample could be diluted 10-fold 2 or 3 times (100 to 1000 - fold dilution).

Each of the contamination levels recommended by FARAD is remarkably conservative. The two primary factors in the selection of these levels are 1) demonstrating to state and federal regulatory authorities that a staggering safety factor exists inherent in the recommendation and 2) the recommendations work logistically with the most common milk assays used in California. In fact, the concentration of antibiotics which could be fed to calves (that would undergo a 45-day slaughter withdrawal) in all cases is likely thousands of folds higher then these recommendations. It is simply that data exists for these proposed limits which are so powerful as to make the assurance of human food safety uncontestable.

RATIONAL
As with the initial consultation request, each antibiotic was evaluated on a individual basis.

Penicillin
Subsequent to first FARAD assessment several relevant articles by Musser and Anderson were published in four prestigious journals (JDS, JAVMA, JVPT, AJVR). Facts pertinent to oral penicillin withdrawal from these articles and FARAD’s previous evaluation (see FARAD citation numbers) appear below.

- 6 calves (2-4 days old) No PenG residues in serum & urine @ 24 hrs post trt 300 & 3000 ppb. (JDS)
- 2 of 6 calves (2-4 days old) had urine Pen G residues @ 24 hrs post trt 5,850 ppb. (JDS)
- 3 hrs after last feeding of pen G at 11,700 ppb, liver (not kidney or muscle) exceeded tolerance. (JDS)
- No statistical differences between tissue concentrations of calves fed 3,333 ng/ml penicillin G at 12% BW PPG for 1 vs 14 days. Very short terminal tissue T1/2s means certainty SS attained. (JAVMA)
- Importantly urine (LOQ 150, LOD 50 ppb) had higher residues then all tissues (LOQ 5, LOD 1.5 ppb) and serum (LOQ 10, LOD 5 ppb) at all times (when LOD allowed detection of differences. (JAVMA)
- Liver had highest residues (tissue T ½ 3 hr), kidney next (T ½ 3.5 hr) muscle never had detectable residues. Last sample (13-hrs post trt) liver was only tissue w/ residue (22 ppb). (JAVMA)
- Using the 95% confidence interval for the 99th percentile of the tissue depletion curve the calculated time for liver residues to decrease below the tolerance at the dose used was 21 hours. (JAVMA)
- The authors used their PK model to estimate a 27-hour slaughter withdrawal for a 40-kg calf receiving 120 mg penicillin G. This is equivalent to 3.0 mg/kg or 4.4 X the dose used in their study. (JAVMA)
- There were no significant differences between oral Na Pen and oral PPG w/in the same age groups but AUC, terminal T1/2, Tmax and Cmax indicated faster elimination at 5 wks then 1 week old for both Na and procaine salt of penicillin G. Thus some maturation of pen excretion ability occurred (AJVR)
- Bioavailability for oral Na pen & PPG at 1and 5 weeks was 10.2 and 7.4%, respectively. (AJVR)
- Kidney or muscle residues in week-old calves were NOT detected by STOP or CAST following 3 days feeding of PPG in milk at 6,600 (n=8) and 13,200 ppb (n=10) and slaughtered 1 day post trt (9005522).
- Even calves injected IM with 66 mg/kg will clear tissue residues by 21 days post-treatment. (9000366)

In the previous evaluation the most significant paper located (FARAD citation 9005522) looked at urine, muscle and kidney tissue following 3 days feeding of 6,600 or 13,200 ppb PPG in milk. All calves were killed 24 hours following the last treatment. Urine at slaughter was positive (3/5 calves at low dose, 8/10 calves at high dose) with LAST, but kidney and muscle tissue were negative with STOP. The positive urine assays in that study are remarkably consistent with the newly examined (JDS 84:126-133) article were urine was negative with 300 and 3000 ppb but 2/6 calves had urine residues 24 hours following feeding with 5850 ppb. This information is also consistent with the finding of Musser (JAVMA 219:3) in which (allowing for differences in LOD) urine was always higher then tissue. Musser (JAVMA 219:3) is also the most instructive study examined to date because it determined (in a classic depletion study) tissue half lives using 3 calves slaughtered at each of 4 time points following the last feeding using 3,333 ppb. Using the 95% confidence interval for the 99th percentile of the tissue depletion curve the calculated time for liver residues to decrease below the tolerance at the dose used was 21 hours. The authors used their PK model to estimate a 27-hour slaughter withdrawal for a 40-kg (88-pound) calf receiving 120 mg penicillin G. This is equivalent to 3.0 mg/kg or 4.4 times the dose used in this study. It would also be equivalent to
14,665 ppb. This publication also supported FARAD’s previous conclusion that it was highly unlikely that significant tissue accumulation would occur following chronic feeding. In the JAVMA study there was no statistical difference between tissue concentrations of calves fed penicillin G for 1 or 14 days. Given the very short terminal tissues T1/2s it is a virtual certainty that steady state is attained by 1-2 days. The last of the newly examined papers (JVPT 24:3) found no significant differences in serum kinetics between the sodium and procaine salts when given orally. This paper also noted that some maturation of penicillin excretion occurred between one and 5 weeks and that bioavailability was 10.2 and 7.4% at 1 and 5 weeks, respectively.

NOTES ON JDS ARTICLE Musser JMB et al. Potential for milk containing penicillin or amoxicillin to cause residues in calves. JDS 84:126-133. 2001.

Bullet Points from this article:
- 6 male calves 2-4 days old were used.
- “The interval between feeding different levels of PPG or amoxicillin was a minimum of 4 days to allow sufficient time for drug elimination form the calf. Urine and serum samples has to test negative prior to the start of a new dosage of drug trials using”… FAST and Charm.
- No Pen G residues in serum & urine @ 24 hours after feeding 300 & 3000 ppb.
- 2 of 6 calves had urine Pen G residues @ 24 hours after feeding 5,850 ppb.
- 3 hours after last feeding of pen G at 11,700 ppb, liver but not kidney or muscle exceeded tolerance.
- Since serum/urine residues were not quantified and tissue samples were from the same time point depletion half-lives cannot be determined from this paper.
- No amoxicillin residues in serum & urine @ 24 hours after feeding 250, 1000, and 2000 ppb.

Experiment #1. Penicillin G at 300, 3000 and 5850 ppb (serum and urine)
Procaine penicillin G added to reconstituted milk replacer to achieve concentrations of 500, 5000 and 10,000 ppb PPG. This is equivalent to 0.293, 2.92 or 5.85 ug/ml or ppm pen G (approximately 300, 3000 and 5850 ppb). Fed to calves (n=6) at 6% body weight every 12 hours for 3 feeding. Serum tested by Charm II and urine by LAST and Charm II. A Charm II LOD for both serum and urine was 0.005 ppm (5 ppb). All serum and urine samples were negative at 0 hours. All but one serum and urine samples were positive at first sampling time of 3 hours post feeding. All samples were negative by 24 hours for the 300 and 3000 ppb feeding levels. For the 5850 ppb level 2 of six urine samples were still detectable by 24 hours.

Experiment #2. Amoxicillin at 250, 1000 and 2000 ppb (serum and urine)
Amoxicillin added to reconstituted milk replacer to achieve concentrations 0.25, 1.0 and 2.0 ug/ml (ppm) pen G (250, 1000 and 2000 ppb). Calves fed at 6% body weight every 12 hours for 3 feedings. Serum tested by Charm II and urine by LAST and Charm II. A Charm II LOD for serum and urine was 0.006 and 0.013 ppm respectively (6 and 13 ppb). All serum and urine samples were negative at 0 hours. All but one serum and urine samples were positive at first sampling time of 3 hours post feeding. All samples were negative by 24 hours for the 300 and 3000 ppb feeding levels. For the 5850 ppb level 2 of six urine samples were still detectable by 24 hours.

Experiment #3. Penicillin G at 11,700 ppb (serum, urine and tissues)
Procaine penicillin G added to reconstituted milk replacer to achieve concentrations of 11.7 ppm equivalent 11,700 ppb Pen G or 20 ppm PPG) Fed to calves (n=6) at 6% body weight every 12 hours for 5 feeding. Serum tested by Charm II and urine by LAST and Charm II. A Charm II LOD for serum and urine was 0.006 and 0.013 ppm respectively (6 and 13 ppb). All serum and urine samples were negative at 0 hours. All but one serum and urine samples were positive at first sampling time of 3 hours post feeding. All samples were negative by 24 hours following the final feeding.

Tissue residues in 6 calves fed 6% BW milk replacer Q 12 hours containing 11,700 ppb penicillin and slaughtered at 3 hours after the final feeding.

<table>
<thead>
<tr>
<th>Tissue*</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.409</td>
<td>0.167</td>
<td>0.64-0.239</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.031</td>
<td>0.012</td>
<td>0.046-0.013</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.008 (&lt;LOD)**</td>
<td>0.002</td>
<td>0.011-LOD</td>
</tr>
</tbody>
</table>
“In previous studies with microbial growth inhibition tests drug residues were not detected (Duby 1984 & Prange 1984) However kidney and muscle were the only tissues examined. Slaughter times were 14 to 18 hours (Duby 1984) and 16 hours after feeding diluted milk with PenG (Prange 1984). The greater time period between drinking spiked milk replacer to slaughter and the fact that the liver was not tested for violative drug residues may have contributed to residues not being detected in (these) other studies. In a study on the depletion of pen G residues in yearling steers after parenteral administration the liver had greater concentrations of pen G than did the kidney (Korsrud 1993); this finding was also reflected in our study. Neither kidney nor muscle had pen G concentrations above the established tolerance level, although kidney concentrations were very close to the tolerance level. Our results indicating the highest concentrations of pen G were in liver with muscle concentrations approximately ¼ to ½ of serum concentrations were similar to previous studies (Korsrud 1993).” The authors concluded that more work was indicated which would examine prolonged feeding of waste milk. The add however “Until more definitive studies are completed, this study suggests it would be prudent to refrain from marketing calves recently fed milk form cows treated with beta lactam antibiotics for at least 24 hours”. The authors suggest that FARAD’s recommendations may be useful in determining appropriate slaughter withholding periods necessary to prevent residues in calves fed milk containing penicillin or amoxicillin.

NOTES ON JAVMA ARTICLE
Musser JMB et al. Tissue disposition and depletion of penicillin B after oral administration with milk in unweaned dairy calves. JAVMA 219:3 (346-350).

Bullet Points from this article:
- 26 calves 1-5 weeks fed replacer at 12% BW spiked w/ 0.68 mg/kg PPG (same as 0.4 mg/kg Pen G) which is equivalent to a calf consuming milk at 3,333 ng/ml penicillin G at 12% body weight.
- No statistical differences were found between tissue concentrations of calves fed penicillin G for 1 or 14 days. Given the very short terminal tissues T1/2s it is a virtual certainty that SS was attained.
- Liver had highest residues (tissue T ½ 3 hr) kidney next (T ½ 3.5 hr) muscle never had detectable residues. Last sampling time (13 hrs post feeding) liver was only tissue w residue (LOD-22 ppb).
- Using the 95% confidence interval for the 99th percentile of the tissue depletion curve the calculated time for liver residues to decrease below the tolerance at the dose used was 21 hours.
- The authors used their PK model to estimate a 27 hr slaughter withdrawal for a 40-kg calf receiving 120 mg penicillin G. This is equivalent to 3.0 mg/kg or 4.4 times the dose used in this study.
- Importantly urine (LOQ 150, LOD 50 ppb) had higher residues then all tissues (LOQ 5, LOD 1.5 ppb) and serum (LOQ 10, LOD 5 ppb) at all times (when LOD allowed detection of differences. (JAVMA)

24 calves used fed replacer at 12% BW spiked w/ 0.68 mg/kg PPG (same as 0.4 mg/kg Pen G). One group (12 calves, 3-5 weeks old) slaughtered (in groups of 3) at 4, 6.5, 9.5 & 13 hours after the single feeding of spiked replacer. Second group (12 calves 1-3 weeks) slaughtered (in groups of 3) at 4, 6.5, 9.5 & 13 hours after the SID feeding of spiked replacer for 14 days. No statistical differences were found between tissue concentration of calves fed penicillin G for 1 or 14 days. Data from the specific euthanasia times were combined for the 2 groups. “The terminal half-life for penicillin G in kidney was 3.5 hours. The terminal half-life for penicillin G in liver was 3 hours. Using the 95% confidence interval for the 99th percentile of the tissue depletion curve the calculated time for liver residues to decrease below the tolerance at the dose used was 21 hours” “The dose used in this study was selected to simulate the high concentrations of penicillin G that calves may receive when fed milk form cows treated with antimicrobials.....With the knowledge that the actual concentrations of penicillin G in milk fed to calves are quite variable we attempted to maximize the dose while keeping it below the threshold that would cause diarrhea. Extrapolating from prior research, it was calculated that the oral dose of 0.68 mg/kg should induce liver and kidney concentrations of penicillin G above the tolerance value. This dose was equivalent to a calf consuming milk with a concentration of 3,333 ng/ml penicillin G at 12% body weight.” “It has been reported that some practitioners infuse a double dose of an approved antimicrobial product for treatment of mastitis. If the product contained 100,000 units of procaine penicillin G the maximum dose a calf may receive would be approximately 120 mg of penicillin G (3.0 mg/kg or 1.36 mg/lb) in a 40 kg (88 lb) calf.

U.S. tolerance for penicillin is 0.05 ppm in all edible tissues. ** LOD = 0.005 ppm
The upper tolerance limit calculated by simulating liver concentrations to reflect this increased dose compared with the dose used in this study indicates that the withdrawal time would be 27 hours.”

NOTES ON JVPT ARTICLE
Bullet Points from this article:
- 18 calves dosed first at 1 week old then at 5 weeks old.
- Na Pen G given IV and oral and PPG given oral at 4 mg/kg based on Pen G
- For IV Na Pen AUC & clearance indicated faster elimination at 5 wks.
- There were no significant differences between oral Na Pen and oral PPG w/i the same age groups but AUC, terminal T1/2, Tmax and Cmax indicated faster elimination at 5 wks for both Na and Procaine.
- Authors review literature on maturation of calves renal ability to excrete penicillin.
- Bioavailability was 10.2 and 7.4% at 1 and 5 weeks, respectively.

Abstract: Eighteen 1-week-old Holstein calves were randomly assigned to one of three groups: (a) sodium penicillin G administered intravenously, (b) sodium penicillin G administered orally, or (c) procaine penicillin G administered orally. All calves were dosed with penicillin G at 4.0 mg/kg BW. At 5 weeks of age, the calves were dosed again. Blood samples were taken serially for 24 h after both dosings. Plasma was assayed for penicillin G by high performance liquid chromatography (HPLC). For i.v. administration, the area under the concentration-time curve (AUC), 7456 and 5508 ng/mL h, and systemic clearance, 0.54 and 0.73 L/kg h, were significantly different (P < 0.05) at 1 and 5 weeks of age, respectively. There were no significant differences between orally administered sodium and procaine penicillin G within the same age groups. Following oral (p.o.) administration, there were significant differences (P < 0.01) at 1 and 5 weeks of age in the AUC, 760 and 409 ng/mL h, terminal half-life, 2.1 and 1.6 h, time of maximum concentration (TMAX), 3.0 and 2.3 h, and maximum plasma concentration (CMAX), 85 and 58 ng/mL, respectively. Bioavailability was 10.2 and 7.4% at 1 and 5 weeks, respectively.

NOTES ON AJVR ARTICLE
Abstract: OBJECTIVE: To develop a multiple-residue screening method for the detection of beta-lactams in bovine urine. ANIMALS: 6 clinically normal Holstein cows and 6 calves. PROCEDURE: Pooled urine obtained from cows was used as a negative-control sample or spiked with varying concentrations of 6 beta-lactam antibiotics. Urine samples were prepared for liquid chromatography by diluting 1 ml of urine with 9 ml of 0.01M KH2PO4, 0.01 M Na2PO4, and filtering. Filtrate (2,000 ml) was eluted with a mobile phase in a gradient program. A fraction corresponding to each beta-lactam of interest was collected and evaporated to < 1 ml, and water then was added to achieve a 1 ml volume. The collected fraction was tested, using a microbial inhibition test. Then, calves were fed milk spiked with a mixture of 5 beta-lactam antibiotics at a concentration 40X the FDA tolerance in milk. Three hours following the feeding, urine samples were obtained from the calves and tested, as described for the urine samples for the cows. RESULTS: The lowest concentrations of amoxicillin, ampicillin, cephapirin, cloxacillin, desfuroylceftiofurcysteine, and penicillin G that were consistently detected in urine were 100, 10, 100, 250, 1,000, and 10 ng/ml, respectively. Amoxicillin, ampicillin, cephapirin, cloxacillin, desacetylcephapirin, and penicillin G were detected in urine samples of 6/6, 5/6, 0/6, 6/6, 2/6, and 3/6 calves respectively, fed antibiotic-spiked milk. CONCLUSIONS AND CLINICAL RELEVANCE: The integrated method described can be used to detect or identify beta-lactam antibiotics in bovine urine. This method can be used to test cattle for beta-lactam residues.

Amoxicillin Pivotal study (JDS, 84:126-133. 2001) found no serum/urine residues @ 24 hrs after feeding 2,000 ppb. Also, (from previous evaluation) calves getting the label PO dose of amoxicillin (8.9 mg/kg having a 20-day withdrawal) would be consuming equivalent of 50,000-200,000 ppb. A separate study (FARAD citation 9001726) which used 7 mg/kg PO reported tissue residues near tolerance at only 24 hr post
treatment. Several papers were located with serum data following exposure via a variety of routes (see below) but these data are not as directly applicable as those above.

NOTES ON JDS ARTICLE (From penicillin summary above)
Amoxicillin added to reconstituted milk replacer to achieve concentrations 0.25, 1.0 and 2.0 µg/ml (ppm) pen G (250, 1000 and 2000 ppb). Calves fed at 6% body weight every 12 hours for 3 feedings. Serum tested by Charm II and urine by LAST and Charm II. A Charm II LOD for serum and urine was 0.006 and 0.013 ppm respectively (6 and 13 ppb). All serum and urine samples were negative at 0 hours. All but one serum and urine samples were positive at first sampling time of 3 hours post feeding. All samples were negative by 24 hours following the final feeding.

FROM PREVIOUS EVALUATION (CA-012201-8736)
“AMOXICILLIN: The most important information in this section relates to the approved use of amoxicillin oral boluses in calves. The following is a bullet point summary of the salient information:

- Unlike the other compounds in this evaluation, amoxicillin is approved for PO treatment of calves.
- Amoxicillin tolerance is 0.01 ppm for both edible tissues and milk/eggs
- Label dose is one 400 mg bolus/100 lb (approximately 8.9 mg/kg) PO Q 12 hours for 5 days. Label withdrawal is 20 days.
- CDFA’s proposed protocol allows at most 1.6 mg/calf/day with a 45 day withdrawal.
- Once again a remarkable margin of safety is offered by the Texas protocol calling for a 45-day WDT.
- Because this product is approved for use in non-ruminating calves, including veal calves, there are no complicating issues relative to applying adult withdrawal times to calves.”

If the oral bolus label dose (400 mg/100 lb, or 8.9 mg/kg, PO Q 12 hours for 5 days = 800 mg/day) were diluted into an extraordinary daily 16 liters of milk replacer, the concentration in milk would be 50,000 ppb (800,000 µg / 16,000 ml = 50 µg/ml = 50 ppm = 50,000 ppb). For more typical milk replacer rations of 8 or 4 liters per day the concentration jumps to 100,000 and 200,000 ppb respectively.

SEARCH FARAD DATABASES
The following was returned when the FARAD Biblio, Kinetic and Kinentry databases was queried using amoxycillin (with and without “:”) and amoxicillin.

Only paper located that had tissue residue data following oral administration of amoxycillin (7 mg/kg) in calves (n=16, mean BW 43.1 ± 5.7 kg) given as a single oral drench of amoxycillin (sic) trihydrate (Clamoxyl dispersible powder; Beecham) in 200 ml calf milk replacer. Thus calves averaged 43.1 kg X 7mg/kg = 301.7 mg/calf. This is equivalent to 300,000 µg which if diluted into between 16 and 4 liters/day would be equivalent to a single feeding of 18,750 to 75,000 ppb respectively. Samples were taken at 0.5, 1.2, 3, 4, 6, 8, and 24 hrs but time-concentration graphs only report out to 8 hours post treatment. At 8 hours residues were approximately kidney-3 ppm, liver-0.8 ppm, urine-75 ppm, bile-30 ppm and serum/fat/lung/skin all about 0.2-0.4 ppm. Other values available of many GI tract tissues. “At 24 hours less then 0.01 µg/g or ml of amoxycillin was present in the majority of tissues, gut contents and body fluids.” Authors make treatment recommendations.

8004008: Absorption in calves of amoxicillin, ampicillin and oxytetracycline given in milk replacer, water or an oral rehydration formulation
Calves (5-10 days old) fed 7 mg/kg amoxicillin in two liters milk replacer (n=28), water (n=16) or GGES (electrolyte, n=22). Dose was always consumed w/i 5 mins. Blood samples taken at intervals up to 6 hr and once at 24 hr. Curves were similar but absorption was highest for GGES and lowest for milk. In an experiment to measure binding of amoxicillin to milk replacer, binding was 16% and readily reversible.
“Absorption of aminopenicillins appears to be mainly a passive phenomenon (9) therefore it may be that the present results indicating improved bioavailability of ampicillin and amoxicillin when given in GGES rather than in water or milk replacer could be explained on the basis of passive movement of the antibiotics
in solution with the water. Increased bioavailability of orally administered antibiotics is clearly desirable when treating systemic infections and the present results indicate that dosing in water or even better GGES is preferable to dosing in milk. This is particularly true for OTC and, to a lesser extent, amoxicillin and ampicillin. Amoxicillin suspended in GGES was more effective than was treatment alone. Idiot authors did not report 24 hr serum values even through in Materials & Methods they reported taking them. Graphic data is available and could be used to determine terminal serum T½ for both amoxicillin and ampicillin.

Single administration 0.25 g amoxycillin in milk serum concentration peaked at 1.74 ug/ml with Cmax of 2.2, 3.16, 3.79, & 1.40 when probenecid was added at 0.5, 1, 1.5, 2 grams/calf respectively. When 3 consecutive oral doses of 1 gram amoxycillin given the Cmax was 4.45, and 3.4 ppm whereas with probenecid the peaks were 4.8, 5.2 and 4.0 respectively. Serum samples only taken out to 8-10 hours post treatment so data not particularly useful in this consultation.

**9001467**: Oral absorption and bioavailability of ampicillin derivatives in calves. (Ziv)

Abstract: The intrinsic absorption rate of ampicillin trihydrate, amoxycillin trihydrate, pivampicillin HCL, hetacillin potassium and BL-P1761 (an experimental ampicillin ester) the absolute bioavailability and the bioavailability relative to ampicillin trihydrate were investigated in dairy calves after oral drug administration with and without milk. Pivamicillin was absorbed at the fastest rate, and BL-P1761 at the slowest. Doses (1 gram) of amoxycillin and pivampicillin administered with the milk resulted in mean peak antibiotic concentrations of 6.7 and 8.5 ug/ml respectively. After these drugs were administered as a drench to fasting calves the respective peaks were 13.4 and 10.2 ug/ml. Considerably lower peak antibiotic concentrations were found after treatment with other drugs. The absolute oral bioavailability of amoxycillin and pivampicillin was approximately 30% as compared with <10% for the other drugs. The relative bioavailability of amoxycillin and pivampicillin was >6.4 times greater than that of ampicillin trihydrate. Fasting of the calves improved the oral bioavailability of ampicillin by a factor of 2.67 depressed that the BL-P1761 by approximately 30% and did not change the bioavailability of the other 3 derivatives. Results are discussed in relation to the possible therapeutic implications of the observed differences in the bioavailability and serum concentrations to the treatment of diseases in newborn calves.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Elimination T½ in milk replacer</th>
<th>Elimination T½ without milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>163 ± 25.5</td>
<td>99.1 ± 15.0</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>116 ± 28.5</td>
<td>93.3 ± 8.6</td>
</tr>
<tr>
<td>Pivampicillin</td>
<td>133.0 ± 80.6</td>
<td>94.5 ± 31.9</td>
</tr>
<tr>
<td>Hetacillin</td>
<td>121.2 ± 17.7</td>
<td>82.0 ± 1.2</td>
</tr>
<tr>
<td>BL-P1761</td>
<td>162.7 ± 61.1</td>
<td>65.7 ± 4.9</td>
</tr>
</tbody>
</table>

**9001597**: Effects of experimental Escherichia coli endotoxaemia on ampicillin: amoxycillin blood levels after oral and parental administration in calves

Amoxycillin sodium and trihydrate PO in milk replacer with and without IV endotoxin. Ampicillin trihydrate was also given IM with and without IV endotoxin. Graphic data available.

**9004015**: Clavulanate-potentiated amoxycillin: in vitro antibacterial activity & oral bioavailability in calves

**9000900**: Freedom of Information; NADA number: 055-100, Amoxicillin intramammary infusion

Not examined. Unlikely to contain useful information.

**9000272**: The effect of storage at 4 C on antibiotic residues in kidney and meat tissues of dairy cows

Not examined. Unlikely to contain useful information.

**900360**: Tissue distribution and residues of beta-lactam antibiotics in normal dairy cows.

Contains only data (n=6 cows) on IM 6.5 hrs post trt w/ 8.9 mg/kg na amoxicillin reporting mean (SD) for renal cortex & medulla, muscle & muscle drip, liver, serum, urine, and bile.

**9001522**: A kinetic study of beta-lactam antibiotic residues in normal dairy cows

Reports serum PK following IM trt. More useful are tissue/serum ratios but the actual concentrations for the beta-lactam antibiotics were detailed in a previous report (see 900360 above)

**900362**: Kinetic disposition and biodistribution of amoxycillin in Bubalus bubalis.

Na amoxycillin IV once 10 mg/kg in male buffalo calves. Serum & tissue data. Elimination T1/2 in serum 2.05 (± 0.15) hr. Highest tissue levels in liver 10.5 ppm at 0.25 hr. 7.5 ppm at 3 hr. 0.75 ppm at 6 hr. Might allow us to calculate a liver T1/2. Authors calculated optimal dose 11 mg/kg Q6 hrs for buffaloes.
Pharmacokinetics of ampicillin and amoxycillin in bubalus bubalis following IM administration
Serum PK data on ampicillin and amoxycillin only.
Persistence of antibiotic residues at the intramuscular injection site in dairy cows. Not in files.
Ampicillin and amoxycillin residue detection in milk, using microbial receptor assay (Charm II) and liquid chromatography methods, after extra-label administration of the drugs to lactating cows
6 cows given ELUD (2X) dose of 22 mg/kg IM once. Milk ampicillin < 10 ppb by 48 hr (label 48 hr milk w/d). Milk amoxicillin < 10 ppb by 72 hr (label 96 hr milk w/s). Mean milk T ½ amoxicillin 23.1 hrs and ampicillin 11.6 hrs. Maximum injection volume per site was 30 ml.
Pharmacokinetic studies and amoxycillin
Serum PK studies only. Author (David Pugh) recommends a dose.
Microbiology and bioavailability of amoxicillin
Huge review of amoxicillin in a wide variety of species with occasional references to other drugs including ampicillin. In calves it compares serum levels of amoxicillin suspension vs sodium after IM treatment (Figure 7), serum and urine concentrations following IM treatment (Figure 8 & 9), serum amoxicillin and ampicillin in pre-ruminant calves following a PO treatment with a 400-mg bolus equivalent to about 11 mg/kg (Figure 11) and compares amoxicillin bolus vs powder (Figure 13). No tissue data.
Amoxicillin: a new veterinary penicillin
Compares serum PK of both amoxycillin and ampicillin in dogs (PO) and calves (IM) only.
Effect of injection site on bioavailability of aminopenicillins in calves
Authors conclude 1) Site and route influence bioavailability, 2) An salts (soluable) are less influenced than suspensions, 3) Different penicillins (or formulations?) may be influenced by different sites/routes, 4) Overall neck IM in the calf shows maximum bioavailability irrespective of drug physical form or formulation.
Observations on the bioavailability of long-acting amoxycillin injectables for use in cattle
Compares PK parameters for 2 different amoxycillin long acting (oil) formulations given IM.
AMOXICILLIN LITERATURE SEARCH
Used Medline and all combinations of amoxicillin or amoxycillin and liver, calves, cattle. All years. Assay methodology papers were excluded.

Palmer, GH; Bywater, RJ; Stanton, A. Absorption in calves of amoxicillin, ampicillin, and oxytetracycline given in milk replacer, water, or an oral rehydration formulation. AJVR 1983 Jan, 44(1):68-71. (Abstract: Oxytetracycline, amoxicillin or ampicillin suspended in milk replacer, water, or a glucose-glycine-electrolyte solution (GGES) was orally given to calves (n = 64). Oxytetracycline suspended in milk replacer gave lower relative bioavailability than when suspended in water (P less than 0.01) or in GGES (P less than 0.001). Seemingly, the 63% binding (only partly reversible) of oxytetracycline suspended in milk replacer was responsible for low serum concentrations, whereas the greater water absorption from the GGES was responsible for improved uptake of antibiotic. Amoxicillin suspended in milk replacer had a delayed absorption, compared with that suspended in water, but the relative bioavailabilities from milk replacer and water were similar. In contrast, peak serum concentration and bioavailability were higher (P less than 0.05) when amoxicillin was suspended in GGES than when suspended in water. Binding of amoxicillin with milk replacer was comparatively low (16%) and was reversible. Ampicillin suspended in GGES gave a higher peak serum concentration (P less than 0.05) than when suspended in milk replacer. It, therefore, appeared that these antibiotics were more bioavailable when suspended in GGES than when suspended in water or especially in milk replacer. Oxytetracycline was bound particularly strongly to milk replacer.

Nouws, JF; Guelen, P; Mevius, D; Driessens, F. Age difference in pharmacokinetics of an amoxycillin trihydrate-15% formulation administered intramuscularly to ruminants. Vet. Quart. 1986 Oct, 8(4):339-42. (Abstract: Intramuscular administration of an amoxycillin trihydrate-15% formulation to three groups of animals revealed in preruminant calves (age 3-4 weeks) significant higher plasma peak drug concentrations and shorter biological half-lives than in 5-month-old ruminant calves and dairy cows. Differences in pharmacokinetics were related to age-difference in drug absorption capability at the injection site regarding the formulation.

Lashev, L. [Comparative pharmacokinetic research on amoxicillin in agricultural animals]
Abstract: Described is the pharmacokinetic of amoxycillin following i/v and oral administration to calves, pigs, and rabbits at the rate of 10 mg per kg of body mass and to turkeys at 30 mg/kg body mass. The pharmacokinetic was found to be of first order, whereas a bispatial model was applicable after i/v introduction of the sodium salt and a monospatial model--following the oral administration of amoxycillin trihydrate. Discussed are the differences in the absorption, distribution, and excretion of the antibiotic as affected by the mode of application and the species peculiarities of the animals.

Abstract: The kinetics of elimination into milk of sodium penicillin G, procaine penicillin G, benzathine penicillin G, ampicillin and amoxycillin residues have been determined after intramuscular administration of eleven drugs chosen among those commercially available in France. These investigations will be used as a basis to estimate and harmonize the withdrawal times demanded for veterinary drugs. The quantitative analysis of residues was carried out by a cylinder plate microbiological method with Bacillus stearothermophilus as test organism. The threshold of detection is 0.001 unit (or micrograms)/ml of milk. The mean durations of elimination are four milkings for the association sodium penicillin G and procaine penicillin G alone, from 19 to 33 milkings for benzathine penicillin G, and from three to five milkings for ampicillin and amoxycillin.

Abstract: The sodium salts of ampicillin and amoxicillin at the single i/m application in 20 per cent water solutions at the rate of 10 mg per kg of body mass guaranteed close therapeutic concentrations of both antibiotics in the blood serum of birds and rabbits. Amoxicillin-trihydrate in a 20% water suspension applied i/m in a single dose of 10 mg/kg persisted in a therapeutic concentrations in the blood serum of birds for a longer time (10 hours) than ampicillin-trihydrate (8 hours). The oil suspension containing 20% amoxicillin-trihydrate guaranteed bacteriostatic serum concentrations in the course of 24 to 48 hours at single i/m application in doses of 20 and 30 mg/kg, depending on the amount and the species of animal. The optimal doses for calves and sheep were 20 mg/kg and 30 mg/kg injected at intervals of 24, resp., 48 hours, and for pigs and birds it were 30 mg/kg at 24-hour intervals. The choice of one or another dose depended on the sensitivity of the disease agents.

Bywater, RJ; Palmer, GH; Wanstall, SA. Discrepancy between antibiotic (amoxycillin) resistance in vitro and efficacy in calf diarrhoea. Veterinary Record 1978 Feb 18, 102(7):150-1

Abstract: Treatment of experimental Salmonella dublin infection in the calf with amoxycillin is described. In most animals a rapid response occurred when the drug was administered by the parenteral route. Results were moderately good when the oral route was used in calves fed solely on a milk replacer diet. In calves consuming hay and concentrates oral administration was much less efficient.

Abstract: The present investigation was undertaken to improve regimens dosage of amoxycillin, chloramphenicol or trimethoprim-sulphadiazine in Salmonella dublin infected veal calves. The pharmacokinetics of these drugs were studied after i.v., oral, and i.m. administration (bioavailability, local irritation at the injection site, volume of distribution, and elimination half life). The most important conclusion was that amoxycillin, chloramphenicol, and trimethoprim were suitable for oral administration to veal calves, although the bioavailability of chloramphenicol and trimethoprim was significantly less when concurrently administered with a milk replacer. In vitro, the antibacterial activities of these drugs were compared. Addition of trimethoprim to sulphadiazine lowered its MIC for S. dublin, but sulphadiazine reduced the killing rate compared to that of trimethoprim alone. In the efficacy studies the activities of several serum enzymes and the plasma concentrations of Fe, Zn, and Cu were measured, but it appeared, that these biochemical parameters were no better than the clinical parameters body temperature and body weight. Using optimal dosage regimens based on MIC values and blood levels, treatment with either of the three drugs was of equal efficacy.

Abstract: The minimal inhibitory concentrations (MIC) of mecillinam, a novel beta-amidinopenicillanic acid derivative with unusual activity against Gram-negative bacteria, were compared with the MIC of cephalaxin, cephalothin, amoxycillin, oxytetracycline, chloramphenicol, dihydrostreptomycin, neomycin, kanamycin, gentamicin and sulphadiazine in vitro. The MIC values of mecillinam ranged between 0.05 microgram/ml and 12.5 micrograms/ml, and the MIC90 values were 1.56 micrograms/ml and 3.12 micrograms/ml. The activity of mecillinam against salmonella, Escherichia coli and Pasteurella multocida was similar to or slightly greater than the activities of the first-generation cephalosporins, gentamicin and sulfa/TMP. Mecillinam concentrations less than or equal to 3.12 micrograms/ml inhibited the growth of the majority of isolates which were resistant (MIC90 greater than 100 micrograms/ml) to the other antibiotics studied. The minimum bactericidal concentration (MBC) values of mecillinam were two- to three-fold higher than the MIC values. The two-compartment open model was appropriate for the analysis of serum mecillinam concentrations measured after intravenous administration. The distribution half-life (t1/2 alpha) was 11.7 min, the elimination half-life (t1/2 beta) was 53.3 min, and the apparent volume of distribution (Vd (area)) and the distribution volume at steady state (Vd (ss)) were 0.568 and 0.896 l/kg, respectively. The drug was quickly absorbed after intramuscular (i.m.) injection; peak serum drug concentrations were directly related to the dose administered. They were obtained 30 min after treatment and the i.m. t1/2 was approximately 65 min. (ABSTRACT TRUNCATED AT 250 WORDS)


**Ampicillin**

There are a number of rationales which can be applied to the safety of 2000 ppb milk contamination limit/45-day withdrawal for ampicillin. As was reviewed in the previous FARAD consultation (CA-012201-8736) doses which can be given on label via IM route are massive compared to this exposure and this label exposure carries only a 6 day slaughter withdrawal. In addition overwhelming data has demonstrated that following oral administration, ampicillin has considerably smaller bio-availability then does amoxicillin.

New to this evaluation is a summary of a number of papers that investigated serum depletion following oral administration (see table below). In citations which reported serum half-lives (or presented data from which it could be estimated) the half-life following oral administration ranged from about 1 to 3 hours. In citations which only presented graphic data (time concentration figures) the curves were very similar to those which reported both; plasma concentrations peaked 2-3 hours at a low (<1ppm) level and then fell off gradually. This 1-3 hour serum half-life for oral administration is remarkably similar to that reported in 9000110 for IV (1.1 hour) and IM (1.8 hours) administration. This citation also has sufficient tissue data to estimate liver, bile, kidney, spleen and heart tissue half-lives, all approximately 2 hours. In addition urine and bile data 9001723 was modeled to show half-lives in those fluids of between 2-3 hours. Therefore we can extrapolate that ampicillin given IV, IM, or PO will have a short plasma and tissue half-lives on the order of 2-3 hours. Even doubling or tripling that to a 6-hour tissue half-life will mean that after only a 4-day withdrawal the animal will have experienced 16 tissue half-lives. Calves receiving ampicillin at 2000 ppb in 4 liters of milk would be getting only about 8 mg/calf, far less then the doses used below.

Ampicillin serum depletion following oral administration compared with serum depletion after IM & IV trt

<table>
<thead>
<tr>
<th>Citation</th>
<th>Amp. salt</th>
<th>Rt.</th>
<th>n</th>
<th>age or BW</th>
<th>Dose</th>
<th>Vehicle</th>
<th>Term. T ½</th>
</tr>
</thead>
<tbody>
<tr>
<td>9003155</td>
<td>Trihydrate</td>
<td>PO</td>
<td>11</td>
<td>1-5 wks</td>
<td>250 mg/calf</td>
<td>Capsule</td>
<td>Graphic only*</td>
</tr>
<tr>
<td>9001725</td>
<td>Not specified</td>
<td>PO</td>
<td>2</td>
<td>App.10 d</td>
<td>50 mg/calf</td>
<td>Milk</td>
<td>Could not model**</td>
</tr>
<tr>
<td>9001723</td>
<td>Not specified</td>
<td>PO</td>
<td>2</td>
<td>App.10 d</td>
<td>50 mg/calf</td>
<td>Milk</td>
<td>Could not model**</td>
</tr>
<tr>
<td>9001696</td>
<td>Not specified</td>
<td>PO</td>
<td>5</td>
<td>2-6 wks</td>
<td>11 mg/kg</td>
<td>Milk</td>
<td>124 mins***</td>
</tr>
<tr>
<td>9001467</td>
<td>Trihydrate</td>
<td>PO</td>
<td>8</td>
<td>2-3 wks</td>
<td>1 g/calf</td>
<td>Milk Replacer</td>
<td>163 + 25.5 min</td>
</tr>
<tr>
<td>9001467</td>
<td>Trihydrate</td>
<td>PO</td>
<td>7</td>
<td>2-3 wks</td>
<td>1 g/calf</td>
<td>Saline Drench</td>
<td>99.1 ± 15.0 min</td>
</tr>
<tr>
<td>9001424</td>
<td>Not specified</td>
<td>PO</td>
<td>10</td>
<td>Pre-ruminant</td>
<td>400 mg/calf</td>
<td>Bolus</td>
<td>Graphic only*</td>
</tr>
<tr>
<td>8004008</td>
<td>Trihydrate</td>
<td>PO</td>
<td>12</td>
<td>5-10 days</td>
<td>7 mg/kg</td>
<td>Milk Replacer</td>
<td>Graphic only*</td>
</tr>
<tr>
<td>8004008</td>
<td>Trihydrate</td>
<td>PO</td>
<td>12</td>
<td>5-10 days</td>
<td>7 mg/kg</td>
<td>Electrolyte</td>
<td>Graphic only*</td>
</tr>
<tr>
<td>9000110</td>
<td>Sodium</td>
<td>IV</td>
<td>4</td>
<td>105-154 kg</td>
<td>10mg/kg</td>
<td>Solution</td>
<td>1.09 hr</td>
</tr>
<tr>
<td>9000110</td>
<td>Sodium</td>
<td>IM</td>
<td>4</td>
<td>105-154 kg</td>
<td>10mg/kg</td>
<td>Solution</td>
<td>1.81 hr</td>
</tr>
</tbody>
</table>

*Data presented only as time-concentration figures.
**Could not model - Insufficient data. Serum collected at slaughter (like a tissue study) not continuously.
***FARAD calculated from tabulated data

Ampicillin tissue concentrations (in ppm) following 10mg/kg ampicillin IV (from 9000110)

<table>
<thead>
<tr>
<th>Tissue/Route</th>
<th>Con. @ ¼ hr</th>
<th>Con. @ 2 hrs</th>
<th>Con. @ 6 hrs</th>
<th>Tissue T ½ in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver - IV</td>
<td>6.71</td>
<td>4.49</td>
<td>1.18</td>
<td>2.26</td>
</tr>
<tr>
<td>Bile - IV</td>
<td>7.95</td>
<td>6.01</td>
<td>0.98</td>
<td>1.85</td>
</tr>
<tr>
<td>Spleen - IV</td>
<td>5.31</td>
<td>4.76</td>
<td>0.72</td>
<td>1.93</td>
</tr>
<tr>
<td>Heart - IV</td>
<td>5.77</td>
<td>3.49</td>
<td>0.61</td>
<td>1.75</td>
</tr>
<tr>
<td>Serum - IV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.09*</td>
</tr>
<tr>
<td>Serum - IM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.81*</td>
</tr>
</tbody>
</table>

* As reported by the authors derived from 15 (IV route) and 13 (IM route) serum sampling times.
Urine and bile concentrations (ug/ml) following 400 mg ampicillin PO. (9001723)

<table>
<thead>
<tr>
<th>Calf # /fluid</th>
<th>6 hours</th>
<th>10 hours</th>
<th>24 hours</th>
<th>Estimated T 1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 Urine</td>
<td>383.9</td>
<td>100.9</td>
<td>1.32</td>
<td>2.1 hours</td>
</tr>
<tr>
<td>#2 Urine</td>
<td>201.4</td>
<td>87.9</td>
<td>2.01</td>
<td>2.7 hours (weighted)</td>
</tr>
<tr>
<td>#1 Bile</td>
<td>44.0</td>
<td>19.7</td>
<td>0.2</td>
<td>2.3 hours</td>
</tr>
<tr>
<td>#2 Bile</td>
<td>95.8</td>
<td>21.0</td>
<td>0.3</td>
<td>2.2 hours</td>
</tr>
</tbody>
</table>

FROM PREVIOUS EVALUATION (CA-012201-8736)

“AMPICILLIN The following is a bullet point summary of the salient information:
- Ampicillin tolerance is 0.01 ppm for both edible tissues and milk/eggs (the same as amoxicillin).
- The only approved food animal ampicillin product is Polyflex, dosed at 11 mg/kg, IM, SID ≤ 7 days.
- Polyflex’s label withdrawal is 2 days for milk and 6 days for slaughter.
- CDFA’s proposed protocol would allow at most 1.6 mg/calf/day with a 45-day withdrawal.
- Once again a remarkable safety margin of is offered by the 45-day withdrawal Texas protocol.
- To further support the lack of risk, there is a plethora of data proving that ampicillin is not as well absorbed as amoxicillin and actually has lower tissue levels.
- Thus given the identical doses and tolerances between the two drugs, amoxicillin is more likely then ampicillin to result in calf residues. Since the amoxicillin section above shows that (with a 45-day withdrawal) residues are virtually impossible, there need be no concern about ampicillin residues.

Comparison of Ampicillin and Amoxicillin:
A number of sources and lines of evidence indicate that ampicillin is not nearly as well absorbed from the intestine as amoxicillin. As a result, given equivalent oral does, tissue levels will be lower for ampicillin. From Prescott & Baggot 2ed edition, page 90: “Ampicillin amoxicillin, and the related compounds hetacillin and pivampicillin have similar antimicrobial activity, but amoxicillin and possibly pivampicillin have the advantage of achieving higher tissue concentrations because of better absorption form the intestine.” From Plumb, 3ed edition, page 35: “Amoxicillin serum levels will generally be 1.5-3 times greater than those of ampicillin after equivalent oral does.” A number of papers are summarized in Appendix D which demonstrate that ampicillin is not as well absorbed as amoxicillin and actually has lower tissue levels. One last observation: while the maximum label dose and regulatory tolerance for Polyflex (ampicillin trihydrate) and Amoxi-Inject (amoxicillin trihydrate) are identical, ampicillin has a 6 day slaughter withdrawal and amoxicillin has a 20 day slaughter withdrawal.”

CITATIONS FROM FARAD DATABASE
Searches were made of Biblio, Kinetic and Kinetic databases.

Citations dealing with oral administration or having tissue depletion data in calves.
9003155: Elevation and prolongation of serum ampicillin and amoxycillin concentrations in calves by the concomitant administration of probenecid
“Ampicillin trihydrate was administered orally at 250 mg/calf after an overnight fast, alone and with 0.5, 1 or 1.5 gram probenecid. Peak serum ampicillin concentration were elevated form 0.60 to 1.22 ug/ml by the co-administration of probenecid”. At the last sampling time for all combinations (8 hours) had serum ampicillin concentration between 0 and 0.4 ug/ml. When 1 gram Na ampicillin given SQ with 0, 1 or 2 grams probenecid, peak concentrations went from 12 to more than 20 ug/ml, but at final sample time all were similar at about 2 ug/ml. “The co-administration of 2 grams probenecid and 1 gram Na ampicillin or 0.5 grams Na amoxycillin parentally resulted in peak antibiotic concentrations considered to be effective against some of the more resistant pathogenic Gram-negative bacteria associated with diseases in calves. Serum antibiotics concentrations ≤ 5 ug/ml were maintained during 4-6 hours as opposed to 2-3 hours after the antibiotics were injected alone.”. “When given orally to pre-ruminant calves amoxicillin was well absorbed and the serum concentrations obtained by this route were considerably higher than those of ampicillin (Yeoman, 1977a, Ziv, nouws, Groothuis & Ven Miert 1977).” “The present finding confirm the relatively inefficient oral absorption of ampicillin in calves are in agreement with oral reports (Chaleva, 1977, Ziv et al 1977, Thompson & Black 1978)”. “We suggest based on the present findings that oral ampicillin therapy with or without probenecid is not likely to produce effective serum antibiotic concentrations for the majority of these pathogens.”
9001725: The distribution of orally administered ampicillin in calves
50 mg/ each of 12 calves in 2 liters of milk. Two calves killed at 1, 2, 4, 6, 10 and 24 hours post treatment. Only various GI tissues, bile, urine and serum were sampled. Urine and bile almost always had highest values and ranged 0 to 2.1 ppm at 24 hours. Did not include liver, kidney or muscle.

9001723: The distribution of a 400 mg. dose of ampicillin administered orally to calves
400 mg/ each of 12 calves approximately 10 days old in 1/2 liters of milk. Two calves killed at 1, 2, 4, 6, 10 and 24 hours post treatment. Only various GI tissues, bile, urine and serum were sampled. Did not include liver, kidney or muscle. Ampicillin levels climbed until 4-6 hours post treatment and then fell:

Urine and bile concentrations (ug/ml) follow 400 mg ampicillin PO. (9001723)

<table>
<thead>
<tr>
<th>Calf/fluid</th>
<th>6 hours</th>
<th>10 hours</th>
<th>24 hours</th>
<th>Estimated T 1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 Urine</td>
<td>383.9</td>
<td>100.9</td>
<td>1.32</td>
<td>2.1 hours</td>
</tr>
<tr>
<td>#2 Urine</td>
<td>201.4</td>
<td>87.9</td>
<td>2.01</td>
<td>2.7 hours (weighted)</td>
</tr>
<tr>
<td>#1 Bile</td>
<td>44.0</td>
<td>19.7</td>
<td>0.2</td>
<td>2.3 hours</td>
</tr>
<tr>
<td>#2 Bile</td>
<td>95.8</td>
<td>21.0</td>
<td>0.3</td>
<td>2.2 hours</td>
</tr>
</tbody>
</table>

9001696: A study of the influence of the method of oral administration of ampicillin upon plasma drug levels in calves
Ampicillin was given orally to 5 calves (2-6 weeks old) using 1) in milk via stomach tube, 100 mg 2) calf starter, 100 mg 3) milk in pale, 100 mg 4) as 400 mg tablets. All doses were at 11 mg/kg SID for 5 days. Ampicillin was only detected in serum in the group fed by pail. By 24 hours even method 3 did not have detectable ampicillin. Separate experiment involved radiographing abdomen.

9001597: Effects of experimental Escherichia coli endotoxaemia on ampicillin: amoxycillin blood levels after oral and parental administration in calves
Amoxycillin sodium and trihydrate PO in milk replacer with and without IV endotoxin. Ampicillin trihydrate was also given IM with and without IV endotoxin. Graphic data available.

9001467: Oral absorption and bioavailability of ampicillin derivatives in calves. (Ziv)
Abstract: The intrinsic absorption rate of ampicillin trihydrate, amoxycillin trihydrate, pivampicillin HCL, hetacillin potassium and BL-P1761 (an experimental ampicillin ester) the absolute bioavailability and the bioavailability relative to ampicillin trihydrate were investigated in dairy calves after oral drug administration with and without milk. Pivamycin was absorbed at the fastest rate, and BL-P1761 at the slowest. Doses (1 gram) of amoxycillin and pivamycin administered with the milk resulted in mean peak antibiotic concentrations of 6.7 and 8.5 ug/ml respectively. After these drugs were administered as a drench to fasting calves the respective peaks were 13.4 and 10.2 ug/ml. Considerably lower peak antibiotic concentrations were found after treatment with other drugs. The absolute oral bioavailability of amoxycillin and pivampicillin was approximately 30% as compared with < 10% for the other drugs. The relative bioavailability of amoxycillin and pivampicillin was > 6.4 times greater than that of ampicillin trihydrate. Fasting of the calves improved the oral bioavailability of ampicillin by a factor of 2.67 depressed that the BL-P1761 by approximately 30% and did not changes the bioavailability of the other 3 derivatives. Results are discussed in relation to the possible therapeutic implications of the observed differences in the bioavailability and serum concentrations to the treatment of diseases in newborn calves.

Serum Elimination T ½ (minutes) from 9001467

<table>
<thead>
<tr>
<th>Drug</th>
<th>Elimination T ½ in milk replacer Experiment #3 (in minutes)</th>
<th>Elimination T ½ without milk Experiment #4 (in minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>163 ± 25.5</td>
<td>99.1 ± 15.0</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>116 ± 28.5</td>
<td>93.3 ± 8.6</td>
</tr>
<tr>
<td>Pivampicillin</td>
<td>133.0 ± 80.6</td>
<td>94.5 ± 31.9</td>
</tr>
<tr>
<td>Hetacillin</td>
<td>121.2 ± 17.7</td>
<td>82.0 ± 1.2</td>
</tr>
<tr>
<td>BL-P1761</td>
<td>162.7 ± 61.1</td>
<td>65.7 ± 4.9</td>
</tr>
</tbody>
</table>

9001424: Microbiology and bioavailability of amoxicillin
Huge review of amoxicillin in a wide variety of species with occasional references to other drugs including ampicillin. In calves it compares serum levels of amoxicillin suspension vs sodium after IM treatment (Figure 7), serum and urine concentrations following IM treatment (Figure 8 & 9), serum amoxicillin and
ampicillin in pre-ruminant calves following a PO treatment with a 400-mg bolus equivalent to about 11 mg/kg (Figure 11) and compares amoxicillin bolus vs powder (Figure 13). No tissue data.

Pharmacology and chemotherapy of ampicillin - a new broad-spectrum penicillin

Huge review of amoxicillin in a wide variety of species. Mostly rat data. Only bovine data related to serum protein binding.

Absorption in calves of amoxicillin, ampicillin and oxytetracycline given in milk replacer, water or an oral rehydration formulation

Calves (5-10 days old) fed 7 mg/kg ampicillin in two liters milk replacer (n=12) and GGES (electrolyte, n=12). Dose was always consumed w/i 5 mins. “Serum concentrations of ampicillin given in milk replacer were significantly (p< 0.05) lower than that when ampicillin was given in GGES at PDH 1 and 2 (Figure 3.” Peak ampicillin levels were approximately 0.35 ug/ml at about 3 hours and at the last sample time (6 hours) was approximately 0.15 ug/ml. “Absorption of aminopenicillins appears to be mainly a passive phenomenon (9) therefore it may be that the present results indicating improved bioavailability of ampicillin and amoxicillin when given in GGES rather than in water or milk replacer could be explained on the basis of passive movement of the antibiotics in solution with the water. Increased bioavailability of orally administered antibiotics is clearly desirable when treating systemic infections and the present results indicate that dosing in water or even better GGES is preferable to dosing in milk. This is particularly true for OTC and to a lesser extent of amoxicillin and ampicillin.” Idiot authors did not report 24 hr serum values even through in Materials & Methods they reported taking them.

Other ampicillin citations

Pharmacokinetic study of ampicillin in cow calves (Abstract below provided by BIOSIS)


Abstract: Serum concentrations of ampicillin administered in the dose of 10mg/kg intravenously and intramuscularly in cow calves were estimated at different time intervals by microbiological assay technique. Peak serum concentration of 21.03 .plus-minus. 0.97 .mu.g/ml at 2.5 min after iv and 10.28 .plus-minus. 0.65 .mu.g/ml at 30 min after im administration were attained and MIC (.greater than or equal to. 1.5 .mu.g/ml) was maintained up to 2 and 4 hrs, respectively. Based on serum concentrations, various pharmacokinetic parameters were estimated. The elimination half life (t1/2 .beta.) was 1.09 .plus-minus. 0.01 hr after iv and 1.8 .plus-minus. 0.11 hr following im administration. In the repeat studies viz., iv followed by im and im followed by im, there was not much difference in various kinetic parameters. Distribution of ampicillin in body tissues and fluids was estimated at 15, 120 and 360 min after iv administration of 10 .mu.g/kg of the drug. Therapeutic levels of the drug persisted for longer periods in various organs and body fluids in comparison to that in serum.

The tissue and serum half lives below were calculated from data extracted from the paper. Concentrations are in ug/g or ppm. See Kinentry files for models.

<table>
<thead>
<tr>
<th>Tissue/Route</th>
<th>Con. @ ¼ hr</th>
<th>Con. @ 2 hrs</th>
<th>Con. @ 6 hrs</th>
<th>Tissue T½ (in hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver - IV</td>
<td>6.71</td>
<td>4.49</td>
<td>1.18</td>
<td>2.26</td>
</tr>
<tr>
<td>Bile - IV</td>
<td>7.95</td>
<td>6.01</td>
<td>0.98</td>
<td>1.85</td>
</tr>
<tr>
<td>Spleen - IV</td>
<td>5.31</td>
<td>4.76</td>
<td>0.72</td>
<td>1.93</td>
</tr>
<tr>
<td>Heart - IV</td>
<td>5.77</td>
<td>3.49</td>
<td>0.61</td>
<td>1.75</td>
</tr>
<tr>
<td>Kidney - IV</td>
<td>6.77</td>
<td>9.3</td>
<td>1.9</td>
<td>Could not be modeled.**</td>
</tr>
<tr>
<td>Urine - IV</td>
<td>7.69</td>
<td>8.46</td>
<td>1.41</td>
<td>Could not be modeled.**</td>
</tr>
<tr>
<td>Lung - IV</td>
<td>1.62</td>
<td>3.24</td>
<td>0.57</td>
<td>Could not be modeled.**</td>
</tr>
<tr>
<td>Brain - IV</td>
<td>1.16</td>
<td>1.76</td>
<td>0.60</td>
<td>Could not be modeled.**</td>
</tr>
<tr>
<td>Thigh mm - IV</td>
<td>1.21</td>
<td>1.69</td>
<td>0.56</td>
<td>Could not be modeled.**</td>
</tr>
<tr>
<td>Pectoral mm - IV</td>
<td>0.84</td>
<td>1.52</td>
<td>0.56</td>
<td>Could not be modeled.**</td>
</tr>
<tr>
<td>Serum - IV</td>
<td>12.75</td>
<td>5.79</td>
<td>1.09</td>
<td>(Serum at same times)</td>
</tr>
<tr>
<td>Serum - IM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.05*</td>
</tr>
<tr>
<td>Serum - IM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.81*</td>
</tr>
</tbody>
</table>

* As reported by the authors derived from 15 (IV route) and 13 (IM route) serum sampling times.
** Insufficient elimination phase data points to allow for accurate modeling.
9001606: Serum and milk concentrations of ampicillin and amoxycillin in ruminants Not examined.

9003061: Pharmacokinetics of ampicillin and amoxycillin in bubalus bubalis following intramuscular administration. Not examined.

9000360: Tissue distribution and residues of beta-lactam antibiotics in normal dairy cows. Contains only data (n=4 cows) on IM 6 hrs post trt w/ 8.3 mg/kg na ampicillin reporting mean (SD) for renal cortex & medulla, muscle & muscle drip, liver, serum, urine, and bile. Insufficient to model.

9001522: A kinetic study of beta-lactam antibiotic residues in normal dairy cows. Reports serum PK following IM trt. More useful are tissue/serum ratios but the actual concentrations for the beta-lactam antibiotics were detailed in a previous report (see 900360 above).

9001012: Antibiotic residues and their recovery from animal tissues. Not examined.

9000734: Facteurs influencant l'excretion des antibiotiques par le lait. Not examined.

9001315: Treatment of various forms of bovine mastitis with consideration of udder pathology and the pharmacokinetics of appropriate drugs: A review. Not examined.

900159: Persistence of antibiotic residues at the intramuscular injection site in dairy cows. Not examined.

9001420: Absorption of antibiotics by the bovine udder. Not examined.

9001714: Amoxycillin: a new veterinary penicillin. Compares serum PK of both amoxycillin and ampicillin in dogs (PO) and calves (IM) only.

9002044: Intra-abdominal versus intramuscular application of two ampicillin preparations in cows Not examined.

9003163: A comparison of serum antibiotic concentrations achieved in calves with intratracheal administration of procaine penicillin G, ampicillin trihydrate, tylosin, oxytetracycline hydrochloride, chloramphenicol, chloramphenicol sodium succinate, dihydrostreptomycin sulfate and neomycin sulfate with those achieved with intravenous, intramuscular and subcutaneous administration. Not Examined.

9004068: Pharmacokinetics and distribution of ampicillin in plasma, milk and uterine fluid of female buffaloes. Not examined.

9004205: Pharmacokinetic and tissue distribution studies of ampicillin in Bubalus bubalis. Male Buffalo calves(95-120 kg) were used. Sodium ampicillin given IV at 10 mg/kg. Serum PK data (T 1/2 beta 1.69 ± 0.82 hours). Tissue, urine and bile data at 15, 120 and 360 mins. Highest concentrations in kidney (approximately 11ppm @ 2hours and 1.5 ppm @ 6 hours thus rough T 1/2 about 1 hour). Excerpt from the paper: “The levels of ampicillin detected in tissues and fluids at 15, 120 and 360 minutes after an intravenous dose of 10 mg/kg are shown in Fig. 2. At 15 min after dosing the concentrations of ampicillin in kidney, bile liver, urine, heart and spleen were in the range of 5.3-7.4 ug/g or ml. Brain lung and muscles had lower concentrations of the drug (<0.8 ug/g). At 120 min the kidney contained the highest level of ampicillin (10.8 ug/g) followed by urine (7.8 ug/ml) and bile (5.5 ug/ml) Six hours after drug administration < 1 ug/g or ml of ampicillin was present in various tissues and fluids other than kidney (1.9 ug/g) and urine (1.2 ug/ul)” Ziv. Uses PK calculations to suggest an optimal IV dose: 11mg/kg q 4 hours.

9005017: Excretion of penicillins in bovine milk following intramuscular administration. Not examined.


9005420: Ampicillin and amoxicillin residue detection in milk, using microbial receptor assay (Charm II) and in liquid chromatography methods, after extra-label administration of the drugs to lactating cows. Not examined.

9000653: Effect of injection site on bioavailability of aminopenicillins in calves. Authors conclude 1) Site and route influence bioavailability, 2) An salts (soluable) are less influenced than suspensions, 3) Different penicillins (or formulations?) may be influenced by different sites/routes, 4) Overall neck IM in the calf shows maximum bioavailability irrespective of drug physical form or formulation.

**Cloxacillin**

A exhaustive search for new data was conducted using the FARAD and MEDLINE, AGRICOLA, CABI and FSTA databases (see results below). Little relevant data was uncovered that had not been reviewed in
the previous evaluation. Some serum half-lives in various species were located. Following IV treatment in calves with cloxacillin of $T_{1/2}$ was 19.5 +/- 12.8 minutes (Daigneault 1990) and approximately 1.2 hours for cattle and ewes (9000207). Terminal serum $T_{1/2}$ in swine following IV treatment was 0.97 (+ 0.12) hours and following IM treatment absorption from the injection site was more rapid then its elimination (Dimitrova 1997). In humans following oral treatment with cloxacillin bioavailability was 32.9% and $T_{1/2}$ was 32 min (Nauta 1975). As before it was noted that no tissue depletion data exists in the published literature. The references make reference to a sodium cloxacillin product “Orbenin” that may have once been available but several internet searches failed to locate information on any cloxacillin veterinary products other then mastitis tubes and topical (ocular) treatment.

In the earlier evaluation we noted that elimination half life’s of various tissues (including kidney) in hens, rabbits and rats ranged from 0.2 to 21 hours (these estimations extrapolated from raw data in 9001222). Remodeling of much of this data confirms that the 21-hour $T_{1/2}$ was overly conservative. See 9001222 notes below. We substituted the longest tissue half-life reported in any species, assumed 100% bioavailability and accumulation of all residues in the kidney. Assuming complete concentration in the kidney of cloxacillin contained in 4 gallons milk containing 4000 ppb and a half-life of 24 hours, residues should fall below tolerance in that target organ by 16 half lives. By extrapolation increasing the concentration to 6000 or 8000 ppb would increase withdrawal only by one half-life still only approximately 1/3 of the proposed 45 day withdrawal. The conservative assumption in this case includes the impossibility of complete concentration into the calf’s kidneys of the entire mass of ingested cloxacillin (ie no distribution to other organs and no fecal excretion). In addition the tissue half-life assumed is 24 hours compared with a published serum half-life of less then 1/2 hour in calves (Daigneault 1990).

The assumptions made in the previous evaluation allow use to easily conclude that residues from milk containing 6000 ppb cloxacillin will be gone from the calves tissues long before the 45-day withdrawal.

FROM PREVIOUS EVALUATION (CA-012201-8736)

“CLOXACILLIN: Most of the following information was generated during the preparation of FARAD Digest on veal calves, (see Appendix IV). A standard computer assisted literature search could be performed in the future if more data was desired. The following is a bullet point summary of the salient points:

- Tolerance for cloxacillin in the U.S. is 0.01 PPM for both milk and tissue.
- The only cloxacillin veterinary products labeled in the US are mastitis and dry-cow tubes.
- Dry tubes have a 28-30 day slaughter withdrawal, lactating tubes have a 10 day slaughter withdrawal.
- Dry-treated cows will be exposed to 500 mg x 4 = 2000 mg benzathine cloxicillin in an oil vehicle.
- Both the salt and the vehicle make these products particularly persistent in the dry-udder secretions.
- No tissue depletion data exists in the published literature. We substituted the longest tissue half-life reported in any species, assumed 100% bioavailability and accumulation of all residues in the kidney.
- FARAD recommended a conservative 20-day withdrawal for veal calves exposed to one gallon colostrum/milk per day containing 4000 ppb cloxacillin for four days, roughly 61 mg (see below).
- The maximum estimated exposure in CDFA’s protocol is 3.2 mg/calf/day. 20 fold less then above.
- Additionally the CDFA protocol proposes a 45-day slaughter withdrawal rather then 20 days.

References and Calculations for Cloxacillin Examined for JAVMA article

Cloxacillin benzathine (CXB) - A semi-synthetic penicillin resistant to penicillinase, this compound finds it’s primary veterinary application in the treatment and prevention of bovine staphylococcal mastitis (Prescott and Baggot 2ed edition). Both CXB-containing dry cow products marketed in the United States contain 500 mg of the insoluble benzathine salt in an oil vehicle (CVP 4th edition, 9001447). Tolerance for BC in the U.S. is 0.01 PPM for both milk and tissue (FARAD Compendium 10th edition). CXB appears to be particularly persistent in the dry udder secretions (9001447, 9000412, 9000695, 80005115, 9000543, 9001407, 9000410, 9001452) with residues in two studies (9000410 on page 418 figure 3, 9001452) present at between 3 and 4 PPM following a 30 day dry period. A calf consuming 4 gallons of colostrum and milk containing 4 PPM CXB will have a total oral exposure of 60,800 ug (4 days x 4 PPM x 3800 ml/gallon = 60,800ug). Bioavailability and tissue residue depletion data in ruminants does not exist. Elimination half life’s of various tissues (including kidney) in hens, rabbits and rats range from 0.2 to 21
hours (these estimations extrapolated from raw data in 9001222). Assuming complete concentration in the kidney and a half-life of 24 hours, residues should fall below tolerance in that target organ by 16 half lives (60,800 ug/137 grams kidney tissue = 443 ug/gram. 468 x ½^16 = 0.007 ug/gram). The colostrum-exposed veal withdrawal time of 20 days represents a conservative estimate based on the extremely limited data available.

Below from BIBIOL and KINENTRY databases having reference to cloxacillin
9006940 Route IMM with muscle, serum and kidney data. The normal, experimentally treated cows were at the end of their lactation period. Data is also included for emergency-slaughtered cows, for which diagnosis and type of treatment are listed. Muscle was measured by drip. 9006488: Cloxacillin and nafcillin: serum binding and its relationship to antibacterial effect in mice. 9006337 Route IM. Matrix: perilymph and thoracic lymph and plasma. K-9 data. 9006052 - (Waraw Agricultural University, Poland) “The detection of the antibiotic residues in milk of cows after intrauterine infusion of antibiotics for the therapeutic purpose and the evaluation of the effects of the drug on the health state of the mammary gland.” Examined milk residues resulting from intrauterine administration of 7 different drugs. Not useful because IU administration of sodium cloxacillin. 9006022 - (Zomer from Charm Sciences) “Incurred studies with veterinary drugs, pharmacokinetics nd active metabolites using Charm II and HPLC receptorgram”. Only reference to cloxacillin is study using receptorgram which showed after IMM infusion of cloxacillin (no vehicle or dose noted) 48 hour withdrawal time is sufficient. Some potentially good data for Payne thesis write up. Not useful due to no dose or vehicle given.
9005612: “Pharmacokinetics and tissue irritation of sodium dicloxacillin in lactating cows after intravenous and intramuscular administration.”. T ½ beta following dicloxacillin after IM treatment with 10 mg/kg was 10.1 (± SE 1.2) minutes. “The drug was not detected after IM administration In milk no drug was detected after IV or IM administration.” The authors suggested that a more sensitive assay or a higher dose might have been more appropriate. “In adult cattle typical half-lives of B-lactam antibiotics administered as aqueous solution are short”. The authors give several examples. This study is not useful because it is dicloxacillin which appears to have a even shorter T ½ then cloxacillin. 9005575: Excretion of penicillins and cephalixin in bovine milk following intramammary administration. 9005617: Pharmacokinetics and tissue irritation of sodium dicloxacillin in lactating cows after intravenous and intramuscular administration.
9005575 Milk residue only. Cows were in various stages of lactation. Four quarters were infused with cloxacillin, alone or with ampicillin trihydrate or gentamicin sulfate. Several ointments preparations of sodium cloxacillin, alone or in combination with other antibiotics were administered IMAM to lactating dairy, and milk concentration of residues pre- and post-TX is reported. Three doses of 200 mg/quarter, were administered (12-48 h apart) to milking dairy, milked twice per day. Preparations were given in peanut oil, liquid paraffin, propylene glycol, with or without several other vehicle components. The sensitivity of the method was 0.01um/ml (agar diffusion bacteriological method). Maximum concentration occurred the first milking after Tx (42-136 ug/mL), and max milk levels fell below detection between 6-10 milkings after Tx. Percent recoveries in milk were 21-59%. Max WDT’s were between 4.9 - 8.7 days. Sodium and lactating cows.
9005531: “Antibiotic residues in goats milk following intramammary treatment”. (New Zealand) Eight normal lactating goats were treated either with cloxacillin (200 mg, three times at 48 hour intervals) or oxytetracycline (426 mg, three time at 24 hour intervals). No mention was made of the vehicle used however the paper referred to them as “lactational antibiotic therapies (that) are designed for milking cows” so it was most likely sodium cloxacillin. Cloxacillin was still detectable at low levels (mean 0.029 ug/ml) at the 13th milking 156 hours after the final treatment. Not useful because unknown vehicle, probably sodium cloxacillin, in lactating goats.
9004466: Persistence of antibiotic residues in bovine mammary secretions throughout nonlactating period after intramammary infusion at drying off. Matrix: mammary secretion. Breed: Holstein and Jersey. Dose: 500 mg. Health: Cloxacillin was infused in all quarters after the last milking of lactation. SOM: 0.05 - 0.08 ug/ml. # of animals: 11 – 13 Samples collected at 7 days and at 14 days tested positive for residues. 9003147: Antibiotic residues in milk from individual quarters after intramammary treatment. Lactating dairy cows were treated with a cloxacillin preparation (salt not specified). Not useful, lactating cow. This reference makes reference to another paper we did not have in BIB which the above says examined residues in both slow and fast release bases. We looked up this reference in Medline and found Wilson

9003009: Antibiotic levels in bovine lacrimal fluid after a single application of ointments containing procaine benzylpenicillin plus dihydrostreptomycin and benzathine cloxacillin” Not useful.

9002083: (Daigneault 1990) Ocular and serum disposition kinetics of cloxacillin after topical administration of benzathine cloxacillin and intravenous administration of sodium cloxacillin to calves. Sodium cloxacillin, at 10 mg/kg in calves IV, serum levels measured for 10 h- Serum half life was 19.5+12.8 minutes. Topical ocular benzathine cloxacillin, 50-375 mg dose given to calves, and blood and tears were collected for 72h. Cloxacillin concentrations in lacrimal fluid are provided. Not useful.

9001993: Penetration of ampicillin and dicloxacillin into tissue cage fluid in rabbits: relation to serum and tissue protein binding

9001707: Tissue distribution of penicillins; Rodent data. Blood and peritoneal fluid after IM dose given immediately after an i.p. injection of 0.1 ml turpentine. Distribution of cloxacillin (salt not stated) in granuloma tissue, blood and lymph rats, after IM administration of 100 mg/kg. Not useful.

9001522: (Ziv et al, Zbl. Vet Med. 25(312-326), 1978) “A kinetic Study of Beat lactam antibiotic residues in normal dairy cows. Serum data after IM dose. In this study sodium cloxacillin (Orbenin, by Beecham), in the form marketed by the manufacturer, was given 2.15 (SD = 0.1) mg/kg IM to 8 cull dairy cows. Terminal T serum ½ was 0.79 (SD = 0.23) hours. This is the study where serum data and serum to tissue ratios (Table 2), are used to provide with tissue WDT and some t1/2’s. The actual concentrations for the beta-lactam antibiotics were detailed in a previous report by the same authors (FARAD citation 9000360). For cloxacillin kidney cortex/syringe ratios and kidney cortex/muscle-drip ratios could not be determined because of unmeasurable kidney drug levels. Did not give estimated withdrawal time for cloxacillin in tissues as for other tissues. The muscle-drip/syringe ratio was reported as 0.7 (+ 0.24) meaning that less cloxacillin was in the muscle fluid then the serum.

9001452: (Smith) “The persistence of cloxacillin in the mammary gland when infused immediately after the last milking of lactation” Only 3 quarters treated. Early (1967) paper that was probably the first to examine cloxacillin in various bases. Sodium and benzathine salts and vehicles containing aqueous, silicone, aluminum monostearate, and mineral oil were tried variously with 0.2, 0.5 and one gram of cloxacillin. All preparations were experimental but the one with the closest formulation (0.5 grams benzathine salt in 3% aluminum monostearate) had residues ranging from 0.5-6.2 ug/ml at 21 days and up to 3ug/ml at 28 days. Limit of detection 0.1-0.5 ug/ml.

9001448: (Nauta 1975) Pharmacokinetics of flucloxacillin and cloxacillin in healthy subjects and patients on chronic intermittent haemodialysis. PK of cloxacillin in healthy subjects and patients on chronic intermittent hemodialysis after oral administration of the sodium salt. In the healthy subjects, oral absorption of cloxacillin was 32.9%, and elimination t1/2 was 32 min. Absorption was slower in the hemodialysis patients.

9001447: This FOI has also a page with figures where Orbenin is compared to Cefa-dri. It shows levels (ug/mL) weeks after Tx, probably in dry cow secretions. These figures show residues of less than 1 ug/mL by the third week post-Tx. There is also a report in “Modern Veterinary Practice” attached in this pack, by Thomas J. Keefe “Benzathine cloxacillin as a dry-cow mastitis product”. In it “cloxacillin activity higher than 0.5 ug/mL after 4-5 weeks post-Tx is reported, as well as a half life of 8.07 days in the dry udder for cloxacillin benzathine (half lives of 5.68, 1.47, and 1 d for DHS, PenG and erythromycin are also reported in that table (from refs 9 and 10: J Dairy Res 34:47, 1967, and Zbl Vet Med B 20:415, 1973) - these data look familiar (Ziv’s - 9000412). FOI provided by Pfizer on Dariclox (sodium cloxacillin, for lactating cows). The treatment is three doses of 200 mg/quarter, 12h apart. Milk residues decreased to below 0.01 mg/mL in less than 48h for both normal and mastitic cows, and urine levels fell below detection by 48h too. Tissue residues persisted in kidney and liver at “trace levels” for seven days. At ten days all tissues of all cows were negative. There are two tables with u/mL levels in 24 samples (probably from 12 cows) each - maybe one is for mastitic and the other for non-mastitic cows. All samples were below 0.10 g/mL by the fifth time period.

9001434: This record contains milk data from the producer of Dry-Clox (cloxacilline benzathine). Dose: 500 mg cloxacillin per syringe given IMM. Number: Not reported.

9001420: Absorption of antibiotics by the bovine udder. Cloxacillin and dicloxacillin (both sodium salts) were in aqueous solutions or suspensions with [C14]-labeled urea (2uCi/quarter). Dose was administered
after last milking and before dying off. Absorption rate and half-life given as ratio to that of [C14]-labeled urea.

9001409 - (Ziv) “Binding of antibiotics to dry udder secretion and to udder tissue homogenates.” Done because Ziv believes that retention time of antibiotics in the udder is primarily determined by rate of drug absorption from the udder, and so he wanted to examine the effect of protein binding. Each udder was treated with 1 gram of one of several antibiotics in 15 mls of saline in two cows. Fourteen days following drying off the cows were slaughtered and udder secretions and tissue were assayed. For cloxacillin dry udder secretion concentrations ranged from 5.0 to 12.5 ug/ml (86 % ± 6.5 binding) and 25% udder tissue concentration was 1.5-3.0 ug/ml (< 50% binding). Authors conclude that neomycin, DHS, spiramycin, polymyxin B, cloxacillin, novobiocin, and phenoxymethyl penicillin most suitable for dry cow therapy because of higher binding and retention times. Not useful because experimental preparation.

9001407: Dose: Following the last milking of lactation, cows were infused i.m.m. in all four quarters with 500 mg benzathine cloxacillin. Matrix: Colostrum.186 cows were dry-treated with Quartermaster, NB, BC, or CB. Of the 186 colostrum composite samples only 4 were positive with Delvo but only one was confirmed with BSDA. This was a cow treated with CB and having a dry period of 50 days.

9001359: “Persistence of Antibiotic residues at the intra-muscular injection site in dairy cows”. I did not find any reference to cloxacillin in this paper.

9001315: “Treatment of various forms of bovine mastitis with consideration of udder pathology and the pharmacokinetics of appropriate drugs: a review”. The paper touches on (and gives references for) a number of issues which might be useful in so other papers including: anaerobic mastitis pathogens, International Dairy Federation definition of normal SCC (500,000), reasons for lack of drug dispersal, drug binding to serum and milk proteins, blood circulation through udder, overview of parenteral mastitis therapy and the ideal antibiotic for it, one way to kill a quarter (ether), treatment for various types of mastitis (including toxic) brief reviews of major classes of antibiotics including erythromycin. Only information about cloxacillin is that it is a narrow-spectrum semi-synthetic penicillin highly lipid soluble and resistant to staphylococcal penicillinase, that it is has good as pen G against streptococcal mastitis pathogens. The paper also references Ziv in saying that it is highly bound to serum protein (about 75%) and has limited distribution when given both parenterally and in intramammary infusion. Only withholding time relates to label directions in a lactating cow product. Not useful.

9001291: (Italian) This study was more on developing a method to detect several antibiotics in lactating cows, cloxacillin among them. It does not state dose and salt type. Not useful.

9001222: (Further pharmacology and chemotherapy of cloxacillin. Acred and Brown, Brit. J Pharm. 1963.) Summary of 19 different studies in hens, rabbits, dogs, and rats using either IM or PO routes is located in 9001222 hard copy file in as Kinetic Database entries. Longest T1/2 calculated was 25.36 hours (rat urine following IM treatment with 100 mg/kg sodium cloxacillin). However when I remodeled this same urine data on WinNonlin even severely weighting the terminal data points (1/Yhat*Yhat) I only get 5.88 hours for the urine T1/2. I suspect that earlier calculations used data during the absorption phase of the curve as well. This would mean that the previous evaluations were even more conservative. Serum data and tissue residue (in rats) might be more applicable. In male albino white rats after oral administration of 100 mg/kg the kidney residues were higher then liver > 0.5 hours. The kidney residues in PPM are found in chart below. T1/2 (no weighting) using IV bolus model using IM data was only 0.95 hours. T1/2 (no weighting) using IV bolus model using PO data was only 7.76 hours but that figures is highly biased by the single high data point. Still the PO values are much shorter then the 24 hours used in the original evaluation. Kidney residue data in mice following IM and PO treatment with 100 mg/kg Na cloxacillin.

<table>
<thead>
<tr>
<th></th>
<th>0.5 hrs</th>
<th>1 hrs</th>
<th>2 hrs</th>
<th>4 hrs</th>
<th>6 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO 100 mg/kg</td>
<td>2.15</td>
<td>28.0</td>
<td>12.6</td>
<td>7.5</td>
<td>22.1</td>
</tr>
<tr>
<td>IM 100 mg/kg</td>
<td>242.5</td>
<td>76.0</td>
<td>15.2</td>
<td>3.9</td>
<td>1.8</td>
</tr>
</tbody>
</table>

9001159: “Persistence of detectable residues of penicillin and cloxacillin in normal and mastitic quarters following intramammary infusion.” Cows were treated with either 500,000 IU of penicillin (14 normal and 5 mastitic quarters) or 500 mg of sodium cloxacillin (12 normal and 5 mastitic quarters) twice and milk measured with BSDA with minimum sensitivity of 0.003 IU and 0.02 ug/ml respectively. Cloxacillin residues were detected for 64 hours following the last treatment in normal and 48 hours in mastitic quarters. No significant difference in the excretion of penicillin or cloxacillin in normal or mastitic quarters was detected. Not useful because lactating cows and sodium cloxacillin vehicle. May be useful in sick vs. Healthy residue paper.
9001155: (Ziv) Practical pharmacokinetic aspects of mastitis therapy - 3: Intramammary Treatment. Extremely valuable review on intramammary treatment related to both dry and lactating therapy. May be useful in future papers because the many different areas addressed. The only data which relates to cloxacillin is as follows: Figure 17 gives concentration of cloxacillin (500 mg in slow release base, no product named) in dry cow secretions. At day 29 of the dry cow period 7 cows (of approximately 79 cows) had residues ranging from 0.1 to 3.5 ug/ml. Similar levels on day 32. Residues of greater than 1 PPM were found as late 36 days post treatment and detectable residues of less then 0.1 PPM were found as late 44 days post dry treating. Remember the label dry period in the US is 30 days. The only other data relevant to this drug was Table 9 which reported that benzathine cloxacillin had moderate relative absorption from the udder, was 80% bound to dry udder secretions and < 25% bound to a udder tissue homogenate, had a T ½ in the udder of 8 days (the longest one reported) and had an estimated duration of effective concentration of 15-25 days. Paper has useful antibiotic depletion data in dry cow secretions.

9001063: Residus de cloxacilline et de neomycine dans le lait apres leur administration, en association, par voie galactophore. Cloxacillin sodium (with neomycin sulfate) in an oil vehicle was administered to lactating dairy and milk residue concentrations were qualitatively measured. In French. Not useful, lactating.

9001060: SOM for detection in MILK: 0.03-0.05 ug/ml Health: end of lactation Breed: Holstein and Jersey Matrix: mammary secretions, Dose: 500 mg infusion per quarter, all quarters Number of animals: 10-13. At 49 days post infusion 14.6% of samples were still positive.

9001059 - Milk Concentration of cloxacillin in ug/ml *Zone size > 16 mm + fail.

<table>
<thead>
<tr>
<th>Method</th>
<th>Disk*</th>
<th>.041</th>
<th>.081</th>
<th>.122</th>
<th>.270</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone mm</td>
<td>0.0</td>
<td>13.9 sd 0.34</td>
<td>16.4 sd 0.26</td>
<td>18.2 sd 0.31</td>
<td>28.8 sd 0.64</td>
</tr>
<tr>
<td>% Pass</td>
<td>100</td>
<td>100</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% Reject</td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Angenics</td>
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<td></td>
</tr>
<tr>
<td>% Pass</td>
<td>100</td>
<td>13</td>
<td>0</td>
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</tr>
<tr>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>Charm II</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>46</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>54</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Delvo</td>
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</tr>
<tr>
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<td>0</td>
</tr>
<tr>
<td>% Caution</td>
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<td>0</td>
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</tr>
<tr>
<td>% Reject</td>
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<td>100</td>
<td>100</td>
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</tr>
<tr>
<td>Penenzyme Farm</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Pass</td>
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</tr>
<tr>
<td>% Caution</td>
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<td>17</td>
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<td>0</td>
</tr>
<tr>
<td>% Reject</td>
<td>0</td>
<td>8</td>
<td>83</td>
<td>100</td>
<td>100</td>
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<td>Penzyme Lab III</td>
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</tr>
<tr>
<td>% Pass</td>
<td>100</td>
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<td>0</td>
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<tr>
<td>% Caution</td>
<td>0</td>
<td>83</td>
<td>8</td>
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</tr>
<tr>
<td>% Reject</td>
<td>0</td>
<td>17</td>
<td>92</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Interpretation is that BSDA test using (16 mm as pass/fail) will start to detect cloxacillin at 0.081 PPM, (failing approx. 1/5 of samples) and fail 100% at 0.122 PPM (tolerance 0.01 PPM in milk and same in tissue) Bottom Line: Somewhere between 0.04 and 0.1 BSDA starts picking up cloxacillin in all samples.

9000959: Practical pharmacokinetic aspects of mastitis therapy-1: parenteral treatment.

9000739: “Actualités bibliographiques a propos du traitement des mammites et de la detection des residus d’anti-infectieux dans le lait” In French so not useful.

9000734: “Facteurs influençant L’excretion des antibiotiques par le lait”. The English summary talks of specific behavior of macrolide antibiotics that may be helpful in another paper. All data on cloxacillin in the tables seems to come from Ziv. In French so not useful.

9000712: Same Data set as 9001407.
Drug: 500 mg of benzathine cloxacillin in 7.5 g of suitable base per infusible preparation. Dose: two treated quarters; each quarter receiving one infusion. Other treatment: sterile water placebos were given to non-treated quarters. Health: treatment at 7-18 days prior to parturition. Tabular data available in citation. No detectable residues were present in nontreated quarters. At 0 hr post-calving, only one quarter of one animal tested positive with 0.165 µg/ml concentration. At 12 hrs post-calving, two quarters of another animal tested positive with 0.83 and 1.65 µg/ml concentration. At and after 24 hrs post-calving, no residues were detected in any samples tested. (Johnson) Milk from eight dry treated cows was analyzed for benzathine cloxacillin residues, dry treatment periods were shorter from label specifications. Cows were treated in two quarters only with 500 mg of benzathine cloxacillin each, 7-18 days before parturition. Residues were present for one milking in two of the cows (interval between treatment and calving was 8 d), and were absent thereafter - maximum residue levels were 1.65 µg/mL. The authors warn that this value could be higher since colostrum does not diffuse as well as milk in the agar assay. LOD was 0.25 µg/mL.

Compliance with recommended drug withdrawal requirements for dairy cows sent to market in Michigan

Pharmacokinetics of dicloxacillin sodium following intravenous and intramuscular administration to domestic cats. Cat serum data and PK parameters after Dicloxacillin sodium given IV (T ½ beta = 0.85 + 0.09 hours) and IM (T ½ d = 0.92 + 0.04 hours). SOM=0.16 µg/ml. Weight: 2.2-4.5 kg. Graphical data. This study is not useful because it is dicloxacillin which appears to have a even shorter T ½ then cloxacillin.

Mammary secretion & colostrum data following IMM treatment in cattle. (Oliver) “Persistence of antibiotics in bovine mammary secretion following intra-mammary infusion at cessation of milking”. Drug used was benzathine cloxacillin (500 mg, Beecham Laboratories). All quarters were treated. All quarters were sampled at either 7, 14, 21, 28, 35, 42, or 49 days after infusion. Quarters were sampled only once presumably to avoid inappropriate depletion of the drug in the udder. Samples were also taken at or within 3 days of calving. The author does not report sensitivity of the assay in their hands but reports others as saying the BSDA detected cloxacin down to 0.03-0.05 PPM. Samples for cloxacillin were frozen at -20 degrees C and assayed within 4-6 weeks of collection. For benzathine cloxacillin the authors report the following results: “All samples of mammary secretion collected at 7 days and 91.5% of samples collected at 14 days after intra-mammary infusion of 500 mg of benzathine cloxacillin were positive for antibiotic residues by the disc assay (Table 1). At 28 and 35 days after infusion of cloxacillin 69.8 and 61.5% of samples respectively were positive for residues. Results of this study are not consistent with a report by Smith et al (Smith et al, The persistence of cloxacillin in the mammary gland when infused immediately after the last milking of lactation. Journal of Dairy Research 34: 47-57 1967) who showed that 0.5 or 1.0 gram of benzathine cloxacillin in a 3 % aluminum monostearate in mineral oil base usually persisted for only 3 weeks after intra-mammary infusion. In the present study benzathine cloxacillin persisted for up to 5 weeks in 32 or 52 quarters infused at cessation of milking. Smith (1967) indicated that cloxacillin could be detected at 0.5 µg/ml using Bacillus subtilis and 0.2 µg/ml using Sarcina lutea. The Bacillus stearothermophilus disc assay that was used in this study has been reported to detect cloxacin in milk at concentrations as low as 0.03-0.05 µg/ml (Ouderkirk LA. Bacillus stearothermophilus disc assay for detection of residual penicillins in milk: Collaborative study Journal of the Association of Official Analytical Chemists 62:985-988, 1979). Consequently differences in results could be due to different methods of antibiotic detection method used. On the other hand the results of the present study are consistent with those of Ziv et al (Ziv G. et al Retention of antibiotics in dry-udder secretions after infusion of several “dry cow” antibiotic products. Zentralbl. Veterinaermed. 20:415-424, 1973) who showed that intra-mammary infusion of 500 mg of benzathine cloxacillin after the last milking of lactation resulted in detectable cloxacillin concentrations 32-36 days after infusion. Mean cloxacillin concentrations at 14 and 21 days after infusion were 3.6 and 3.0 mg/ml respectively. ” Also under Colostral Samples paper reports only one of 1174 samples of colostrum or milk obtained within 3 days of parturition was positive for antibiotic residues and that sample was from a quarter of a cow infused with benzathine cloxacillin with a dry period of 61 days. The author makes the rash statement that “These data demonstrate quite clearly that antibiotic residues in colostrum and milk during early lactation should not be a problem if manufacturer’s recommendations are followed.” The reason this is rash is because many cloxacillin residues were detected past 30 days (the label dry period) and the only positive 3 day post calving milk sample was in a dry cow with 61-day dry period. Note that there is NO milk discard for CB in US products. Summary at 28 days 69.8 % (30/43 in 11 cows) of sampled quarters were still positive with an average zone size of 26.8 mm
porin derivatives in serum and milk of
usidic acid and lincomycin on the mechanical properties of
R to ewes (n=4) and cows (n=3). In this
preps were used. Drug A (cloxacillin 500 mg) Drug B (PPG 300mg + DHS 500 mg) and Drug C nafcillin
therapy” This was done in response to
ultra concentrations for cloxacillin serum was 58.00 mg/kg) with IM maintenance doses or 6 mg/kg at 2 and 4 hour
single and the combined values were reported. Serum and milk PK in lactating ewes (n=4) and cows (n=2) a given
lactating cows and ewes”. In this study plasma cloxacillin concentration was measured in cattle and sheep
900377: (Valman 1970) Serum unbound levels of cloxacillin and erythromycin in cystic fibrosis. Serum
levels of cloxacillin in children after oral administration, comparison of patients with and without cystic
fibrosis, @ 90 minutes and 4 h after Tx.
9000360: Data on tissue residues after a single IM administration of sodium cloxacillin to culled dairy
cows. Dose of sodium cloxacillin (Orbenin8 ) was 2.1 - 2.2 mg/kg (S.D. ± 0.1) IM. When 4 cattle were
slaughtered 3 ¾ hours after treatment, residues levels in PPM were: renal cortex-trace, renal medulla - 0.91 (+ 0.74 ),
muscle homogenate- < 0.15, muscle drip-0.065 (+ 0.012 ), liver- 0.66 (+ 0.09 ), serum- 0.089 (+ 0.044 ), urine-287 (+ 160 ), bile- 18.3 (+ 17.1). When 4 cattle were slaughtered 20 ¾ hours after
treatment, residues levels in PPM (standard deviations) were: renal cortex-0.62 (+ 0.21 ), renal medulla -
0.83 (+ 0.27 ), muscle homogenate- < 0.15, muscle drip-0.059 (+ 0.001 ), liver- 0.57 (+ 0.06 ), serum
trace, urine-0.92 (+ 0.8 ), bile- 1.0 (+ 1.1). Given the fact that there are only two time points and very large
standard deviations the data in not particularly useful.
900282: Effects of cloxacillin, doxycyline, fusidic acid and lincomycin on the mechanical properties of
bone and skin in young rats. Safety studies fore several antibiotics, cloxacillin included, in rats. Not useful.
9000272: The effect of storage at 4 C on antibiotic residues in kidney and meat tiss
9000360: Data on tissue residues after a single IM administration of sodium cloxacillin to culled dairy
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standard deviations the data in not particularly useful.
Not all teats were dry treated. Milk was tested at calving with large plate modification of the IDF’s BSDA assay (standards of 0.0025-0.1 IU of penicillin “equivalents”). Testing was continued until antibiotic concentration fell below 0.003 IU/ml of penicillin or equivalent. Residues were detected and examined to find out if residues were found beyond the mandatory 4 day (8 milking) colostrum/milk withhold time in New Zealand. When cow dry period ranged from 8-19 weeks 7 of 30 teats had residues but only one cow (Drug A/cloxacillin) had residues detectable beyond 4 day withhold. When cow dry period ranged from 1-11 weeks, residues at calving and past the 4 day withhold were much more common. When dry period was 6 weeks or less residues were detected up to 27 milking post calving. With dry periods of less then 4 weeks residues from both Drug B (PPG/DHS) and Drug C (nafcillin) were detected for long periods. Study determining that New Zealand dry cow labels were insufficient and actually resulted in changing the label recommendations. This study in 1983 lead to New Zealand changes in label requirements for dry cow products. Recommendations: “Unless scientific data about residue release for a particular formulation indicate otherwise the implementation of the following guidelines for all dry-cow formulations should minimize the risk of antibiotic residues gaining access to the milk at the beginning of lactation. 1) Use dry-cow formulation only at drying off. 2) Avoid dry-cow products where the dry period is expected to be less than six weeks. 3) Use short acting product where treatment is required during the dry period. 4) Withhold milk for the full four day colostrum withholding time. (this will exclude both Colostrum and residual antibiotics from the milk supply. Not generally useful because: no commercial products listed, not all teats treated, no concentration data just number of milkings where residues were detected after calving, not clear sensitivity of the assay for anything other then penicillin, did not differentiate DHS and PPG with product B. Only useful aspect of this paper is that a six week dry period and a four day withhold will prevent most residues with these products. What is most interesting is that with a withdrawal period of only 30 days (as is required for US cloxacillin products) a number of the treated quarters would be positive for up to 30 milkings. While we don’t know the vehicle, it was prob. benzathine and if not the residue picture would have been even worse. Furthermore if all the quarters were treated there would have most likely been more persistent residues due to larger drug mass.

8004661: Liquid chromatographic determination of benzylpenicillin and cloxacillin in animal tissues and its application to a study of the stability at -20 degree C of spiked and incurred residues of benzylpenicillin in ovine liver. This article is a methodology one, spiked tissues for cloxacillin and incurred samples with PPG in tissues were analyzed. Not useful for cloxacillin, but useful for penicillin.

Other Resources
Neither JECFA nor EMEA has monographs on cloxacillin.

LITERATURE SEARCH
MEDLINE was searched using cloxacillin combined with calf, calves, cow, cattle, pharmacokinetics. AGRICOLA, CABI and FSTA were searched using the keyword cloxacillin combined with calf, calves, pharmacokinetics, liver and kidney.

Potentially Applicable Citations

Daigneault, J; George, LW; Baggot, JD. Ocular and serum disposition kinetics of cloxacillin after topical administration of benzathine cloxacillin and intravenous administration of sodium cloxacillin to calves. American Journal of Veterinary Research 1990 Mar, 51(3):381-5

Abstract: Disposition kinetics of cloxacillin were examined in calves after topical administration of benzathine cloxacillin and single IV administration of sodium cloxacillin, and the susceptibility of 17 field isolates of Moraxella bovis was measured. For the IV pharmacokinetic phase, sodium cloxacillin was administered at dosage of 10 mg/kg of body weight to male Holstein calves (n = 6, weighing 146 to 170 kg), and serum concentration of cloxacillin was measured thereafter for 10 hours. For the ocular pharmacokinetic phase, 6 calves were given either of 4 benzathine cloxacillin topical formulations consisting of 50-, 125-, 250-, or 375-mg doses. Treatment was repeated every 10 days until all 4 benzathine cloxacillin dosages were tested in the same 6 calves. Blood and tears were collected for 72 hours after each benzathine cloxacillin formulation was administered, and the concentration of cloxacillin in each specimen was measured, using a bioassay. The minimal inhibitory concentration of cloxacillin for 17 field isolates of M bovis was determined by use of an agar pour-plate dilution assay. After single IV administration of
sodium cloxacillin, its half-life, body clearance, and volume of distribution were 19.5 +/- 12.8 minutes, 
18.3 +/- 2.2 ml/min.kg, and 496 +/- 290 ml/kg, respectively. After topical administration of benzathine 
cloxacillin, cloxacillin concentration in lacrimal fluid peaked between 30 and 45 minutes and ranged 
between 963 micrograms/ml and 3,256 micrograms/ml for the 125- and 375-mg doses, respectively. There 
was no detectable cloxacillin activity in the lacrimal fluid of any calf by 36 hours after topical 
administration of benzathine cloxacillin, and cloxacillin was not detected in the serum at any 
time. (ABSTRACT TRUNCATED AT 250 WORDS)

Nouws, JF; Ziv, G. Tissue distribution and residues of beta-lactam antibiotics in normal dairy cows. 
Tijdschrift voor Diergeneeskunde 1977 Oct 15, 102(20):1173-86. FARAD citation 9000360 see above 
Abstract: Tissue residues and concentrations of benzylpenicillin, cloxacillin, ampicillin, amoxycillin, 
cephapirin, and cephacetrile were determined in normal dairy cows after parenteral administration of 
several forms of these drugs. Assay methods included the Sarcina lutea Kidney Test of Van Schothorst, the 
Bacillus subtilis BGA Tests at pH 6.0 and 8.0, the Escherichia coli Test and a Sarcina lutea Test performed 
at pH 8.0 of the agar, and specific quantitative assay methods. The E. coli test method demonstrated an 
insensitivity for the beta-lactam antibiotic residues. Identical results in residue testing of meat and kidney 
were obtained with the B. subtilis BGA tests and S. lutea test at pH 8.0, and these test methods replicated 
each other. The S. lutea Kidney Test was very often positive at times after treatment when the antibiotics 
were no longer detected in the meat. The qualitative and quantitative residue data from the renal cortex 
were higher than the data obtained from the muscle meat. The concentration relationship between renal 
cortex and muscle meat dependent on the formulation and type of drug used, and on the time of sampling 
after treatment. After treatment with products containing ampicillin trihydrate and procaine penicillin an 
unexpectedly long persistence of these drugs in the renal cortex was observed. It is suggested that, in 
the case of beta-lactam antibiotics, meat tests are more accurate indicators for the residue status of the carcass.

Musser, JM; Anderson, KL; Moats, WA. Screening method for identification of beta-lactams in bovine 
Abstract: OBJECTIVE: To develop a multiple-residue screening method for the detection of beta-lactams 
in bovine urine. ANIMALS: 6 clinically normal Holstein cows and 6 calves. PROCEDURE: Pooled urine 
obtained from cows was used as a negative-control sample or spiked with varying concentrations of 6 beta-
lactam antibiotics. Urine samples were prepared for liquid chromatography by diluting 1 ml of urine with 9 
ml of 0.01M KH2PO4, 0.01 M Na2PO4, and filtering. Filtrate (2,000 ml) was eluted with a mobile phase in 
a gradient program. A fraction corresponding to each beta-lactam of interest was collected and evaporated 
to <1 ml, and water then was added to achieve a 1 ml volume. The collected fraction was tested, using a 
microbial inhibition test. Then, calves were fed milk spiked with a mixture of 5 beta-lactam antibiotics at a 
concentration 40X the FDA tolerance in milk. Three hours following the feeding, urine samples were 
obtained from the calves and tested, as described for the urine samples for the cows. RESULTS: The lowest 
concentrations of amoxicillin, ampicillin, cepahirpin, cloxacin, desfurolcyctiofurcysteine, and penicillin 
G that were consistently detected in urine were 100, 10, 100, 250, 1,000, and 10 ng/ml, respectively. 
Amoxicillin, ampicillin, cepahirpin, cloxacin, desacetylcephahirpin, and penicillin G were detected in 
urine samples of 6/6, 5/6, 0/6, 6/6, 2/6, and 3/6 calves respectively, fed antibiotic-spiked milk. 
CONCLUSIONS AND CLINICAL RELEVANCE: The integrated method described can be used to 
detect or identify beta-lactam antibiotics in bovine urine. This method can be used to test cattle for beta-
lactam residues.

Moats, WA. Determination of penicillin G and cloxacinil residues in beef and pork tissue by high-
Abstract: Tissues were homogenized and then deproteinized with acetonitrile. The acetoniitrile extract was 
partitioned between dichloromethane and pH 2.2 buffer and then extracted with pH 7 buffer. After addition 
of ammonium sulfate to the aqueous solution, it was mixed with acetonitrile. The acetoniitrile extract was 
separated and evaporated, and the residue was taken up in water. The aqueous solution was analyzed by 
high-performance liquid chromatography (HPLC) on a C18, 10-microns particle size, reversed-phase 
column using gradient elution with 0.01 M orthophosphoric acid-acetonitrile (from 80:20 to 40:60 in 20 
min) at a flow-rate of 1 ml/min and UV absorbance detection at 220 nm. Recoveries were generally greater 
than 90% with all tissues. Data on incurred residues from a treated cow showed recoveries of penicillin 
which were frequently several times higher by HPLC than by bioassay. Sensitivity limits in muscle were
about 0.05 ppm for both penicillin G and cloxacillin, but higher in liver and kidney because of interferences. The method is suitable for other monobasic penicillins but not for dibasic or amphoteric penicillins.

Ziv, G; Shani, J; Sulman, FG. Pharmacokinetic evaluation of penicillin and cephalosporin derivatives in serum and milk of lactating cows and ewes. American Journal of Veterinary Research 1973 Dec, 34(12):1561-5. FARAD citation 9000207 see above


Nauta, EH; Mattie, H. Pharmacokinetics of flucloxacillin and cloxacillin in healthy subjects and patients on chronic intermittent haemodialysis. British Journal of Clinical Pharmacology 1975 Apr, 2(2):111-21. Abstract: 1 A pharmacokinetic study on flucloxacillin and cloxacillin was performed to investigate the factors contributing to the higher serum concentrations reported for the former after oral administration. 2 The results obtained in a study performed in a group of volunteers with flucloxacillin administered orally and by continuous infusion, were compared with the results of a similar investigation with cloxacillin. Patients on chronic intermittent haemodialysis received flucloxacillin orally and as a single i.v. injection. The results of this part of the study were compared with those of an earlier study on cloxacillin in haemodialysis patients. Serum and urine concentrations of flucloxacillin and cloxacillin were determined by bio-assay, and a one-compartment model was used for the calculations. 3 Higher serum concentrations reached after oral administration of flucloxacillin as compared with cloxacillin were based not only on better oral absorption (53.7% and 32.9%, respectively) but also on slower (renal and extra-renal) elimination (T1/2 : 46 and 32 min, respectively). A significant difference between the apparent volumes of distribution of flucloxacillin and cloxacillin, which could contribute to higher serum concentrations, could not be demonstrated. Considerable individual variation occurs in the rate and amount of oral absorption, especially in patients. The elimination rate of flucloxacillin in haemodialysis patients (T1/2 : 2h 53 min) corresponds with the extra-renal elimination rate in healthy subjects. No influence of haemodialysis on the elimination rate constant of flucloxacillin was found; total plasma clearance was, however, slightly but significantly higher during dialysis.

Visser, LG; Arnouts, P; van Furth, R; Mattie, H; van den Broek, PJ. Clinical pharmacokinetics of continuous intravenous administration of penicillins. Clinical Infectious Diseases 1993 Sep, 17(3):491-5. Abstract: Theoretically, continuous intravenous administration of beta-lactam antibiotics has advantages over intermittent administration because of the close relationship between efficacy and the time the plasma concentration remains above the minimal inhibitory concentration that has been found in vitro. The aim of the present study was to establish the pharmacokinetic parameters of benzylpenicillin and cloxacillin in patients receiving high-dose benzylpenicillin or cloxacillin therapy by continuous infusion. A major part of the interindividual variation in the plasma concentrations at steady-state was attributable to variation in renal function, as estimated by the creatinine clearance. On the basis of these results, a nomogram was constructed that can be used to determine on an individualized basis the total daily dose of benzylpenicillin or cloxacillin necessary for each patient to obtain therapeutic plasma concentrations.


AB: This paper describes the distribution of antibiotics in blood, tissues, gut and milk after administration to different animal species. These studies enable suitable withholding times to be established so that no detectable antibiotics are present in edible tissues or fluids. Problems associated with the detection of combined antibiotics in animal tissues are discussed and a method used for overcoming this difficulty in the
analysis of milk from cows treated with a combined ampicillin-cloxacillin intramammary preparation is described.

AB: After solvent extraction and clean-up an aqueous solution was analyzed by high-performance liquid chromatography (HPLC) on a C18, 10-µm particle size, reversed-phase column using gradient elution with 0.01 M orthophosphoric acid-acetonitrile (from 80:20 to 40:60 in 20 min) at a flow-rate of 1 ml/min and UV absorbance detection at 220 nm. Recoveries were generally greater than 90% with all tissues. Measured residues from a cow killed 2 h after injection of penicillin G (6 X 106 units i/m) were frequently several times higher by HPLC than by bioassay. Sensitivity limits in muscle were about 0.05 mg/kg for both penicillin G and cloxacillin, but higher in liver and kidney because of interference. The method is suitable for monobasic penicillins but not for dibasic or amphoteric penicillins.

Protein Binding Citations


Phillips, GO; Power, DM; Robinson, C; Davies, JV. Interactions of bovine serum albumin with penicillins and cephalosporins studied by pulse radiolysis. Biochimica et Biophysica Acta 1973 Jan 25, 295(1):8-17.

Abstract: Interaction of oxacillin, cloxacillin, dicloxacillin, phenoxymethylpenicillin, methicillin, nafcillin and benzylpenicillin with human serum albumin (HSA) was studied with flow microcalorimetry and differential scanning calorimetry. The measured thermodynamic parameters of complex formation between the penicillins and HSA were compared with similar characteristics of their binding to bovine serum albumin. It was shown that there were species differences between these two globular proteins in their interaction with the above antibiotics in relation to both the number of the biopolymer active sites and the nature of the molecular forces in the complex formation. The effect of the first bound molecule of oxacillin, cloxacillin, dicloxacillin, nafcillin, phenoxymethylpenicillin and benzylpenicillin on HSA conformation was observed. It was demonstrated that there was thermostabilization of HSA on its interaction with the above drugs with preserving cooperative nature of thermal denaturation of the complexes in relation to HSA melting.

Abstract: Using dynamic and equilibrium dialysis methods, it has been demonstrated that ascorbic acid (CAS 50-81-7) inhibits the binding of cloxacillin sodium (CAS 7081-44-9) to bovine serum albumin (BSA) in vitro. Normally, ascorbic acid has a lower number of binding sites and a much lower binding constant for BSA than cloxacillin sodium. There is an indication that ascorbic acid inhibits the binding of cloxacillin to BSA through a noncompetitive mechanism. The probable interactions leading to the non-competitive inhibition were suggested.

Abstract: The ability of bilirubin to displace antimicrobial agents from their binding sites on albumin was studied in vitro by equilibrium dialysis. Sulfonamides, tetracyclines, penicillins and cephalosporins of different degrees of protein binding were tested. It was found that bilirubin reduced the protein binding of all four classes of antimicrobial agents, although by varying degrees. This effect was most pronounced with the compounds which had the highest degree of protein binding, such as cloxacillin, cephalolin, methacycline and sulfisoxazole, all of which are bound greater than 80% by albumin. On the other hand,
the drugs with less than 25% binding, such as ampicillin, cephalexine and tetracycline were not ostensibly displaced by bilirubin. Scatchard plots of the binding of sulfamethoxazole to albumin in the presence of 400 mumol/1 of bilirubin, showed that bilirubin almost completely displaced the sulfonamide molecule from the high affinity site on the albumin molecule. Reduced protein binding of drugs in hyperbilirubinemic infants may have pharmacokinetic significance.

Abstract: Unpredictable inactivation of antimicrobial agents may cause erratic results in pharmacokinetic studies. In this study we followed the inactivation of the high protein bound beta-lactams flucloxacillin, dicloxacillin and ceftriaxone in vitro. The antibiotics were added to pools of human and rabbit sera, ultrafiltrates of these pools, rabbit interstitial fluid, phosphate buffered saline (PBS), rabbit albumin in PBS and sodium dodecyl sulphate (SDS) treated preparations of human sera. Ceftriaxone was relatively stable but different serum pools varied significantly in their flucloxacillin and dicloxacillin inactivating capacity. The dominating inactivation took place within five minutes after the addition of antibiotics to serum. The inactivating factor was heat stable at 56 degrees C, 0.5 h, of relatively high molecular weight, and not related to albumin. The inactivating capacity could be diminished by SDS-treatment of serum suggesting a lipoprotein nature.

Treatment of Pinkeye
Daigneault, J; George, LW; Baggot, JD. Ocular and serum disposition kinetics of cloxacillin after topical administration of benzathine cloxacillin and intravenous administration of sodium cloxacillin to calves. American Journal of Veterinary Research 1990 Mar, 51(3):381-5 See first result in this search for abstract.

Abstract: A field study was performed to determine the effectiveness of benzathine cloxacillin for the treatment of infectious bovine keratoconjunctivitis in cattle from 2 farms located in northern California. The study was performed between June and September of 1987. Affected calves ranging from 2 to 9 months of age were selected from the main herd when signs of corneal ulceration were observed. The study was conducted in 2 phases. For phase I, the affected calves of herd 1 (n = 21; Holsteins) and herd 2 (n = 43 Angus crossbred), were randomly assigned to 1 of 3 groups, and were either treated with 250 (n = 23) or 375 mg (n = 21) of benzathine cloxacillin, or mineral oil (n = 20) on days 1 and 4. For phase II, affected calves (n = 16; Angus crossbred, 3 to 9 months of age) from herd 2 were treated with benzathine cloxacillin (250 mg). Eight of these calves were retreated on day 4. After treatment, all calves were examined every 72 hours for 16 days. For examinations, a clinical score was assigned to each eye, and the surface areas of photographed corneal ulcers were measured. The ocular secretions were collected and examined culturally for Moraxella bovis. On days 7, 10, and 13, the calves treated with benzathine cloxacillin had significantly (P less than 0.05) lower lesion scores, compared with the controls.(ABSTRACT TRUNCATED AT 250 WORDS)

Daigneault, J; George, LW. Topically applied benzathine cloxacillin for treatment of experimentally induced infectious bovine keratoconjunctivitis. AJVR 1990 Mar, 51(3):376-80.
Abstract: The efficacy of an ophthalmic ointment containing benzathine cloxacillin for treatment of infectious bovine keratoconjunctivitis was determined in 2 experiments. In the first experiment, Holstein calves (n = 6/group) were inoculated with Moraxella bovis and treated on postinoculation days 3 and 6 with either topically applied benzathine cloxacillin (250 mg/eye) or long-acting oxytetracycline formulation (20 mg/kg of body weight, IM). A third group of inoculated calves remained untreated as controls. For the second experiment, 4 groups of calves (n = 6/group) were inoculated and treated on postinoculation days 3 and 6 with 50, 125, 250, or 375 mg of benzathine cloxacillin; a fifth untreated group served as controls. Ocular specimens were obtained for microbiologic culture, and eyes were observed and assigned a clinical score daily. Eyes were photographed on alternate days. Ulcer surface area was measured, using a planimeter. In experiment 1, the week-2 ulcer surface area measurements for both groups of treated calves were smaller than those for controls. There was a greater frequency of M bovis isolation from the ocular secretions of controls than from those of benzathine cloxacillin-treated calves during postinoculation weeks.
2 and 3. The number of M bovis isolations from the benzathine cloxacillin- and oxytetracycline-treated calves was not significantly different at any sample collection interval. On week 3, the scores of the benzathine cloxacillin-treated calves were smaller than those of controls. In experiment 2, calves of the 250- and 375-mg groups had smaller ulcer surface area measurements than did controls on week 2. By week 3, calves of the 375-mg group had smaller scores than did controls. (ABSTRACT TRUNCATED AT 250 WORDS)


Binkhorst, GJ. Antibiotic levels in bovine lacrimal fluid after single application of ointments containing procaine benzyl penicillin plus dihydrostreptomycin; and benzathine cloxacillin. Veterinary Record 1987 Aug 8, 121(6):124-5.

Abstract: The efficacy of an experimental slow-release formulation, containing procaine benzyl penicillin and dihydrostreptomycin, was investigated in a cross-over study with a cloxacillin eye ointment in 12 cows with clinically normal eyes. After a single topical application the therapeutic concentrations of penicillin were sustained for 48 to 92 hours and of cloxacillin for 32 to 48 hours. These long-acting ointments will simplify the successful treatment of painful eye disorders such as keratoconjunctivitis. A practical and non-irritant method for sampling tears is described.

Cephapirin
The highest kidney tissue residue reported following any route of administration was 67.3 ppm of the major metabolite deacetylcephapirin following IM treatment at 30 mg/kg, 3 times the normal dose (9007319, see below). The longest reported plasma half-life was 13.3 hours following IM treatment with the long acting benzathine salt (9001522). Extending the half-life to 24 hours and applying it to tissue, kidney residues will fall to below tolerance by 10 days. The are a number of conservative assumptions in this. The prolonged cephapirin serum half-life used was based on IM injection of the benzathine salt where a “flip flop” of absorption and excretion occurred and absorption dictated excretion due to the presence of a slow release vehicle. In fact, serum half-lives of the sodium salt all species are uniformly less than several hours and almost always less then 1 hour. Also conservative in the use of the major metabolite rather then the parents compound (and target residue) which will be nearly 5000 folds smaller in the kidney (9007319). Even Lastly the oral bioavailability of cephapirin will be far less then 100%.

An 84 kg calf receiving 30 mg/kg is dosed with 2520 mg. Diluted in 16, 8 and 4 liters of milk replacer this would be equivalent to 157.5, 315, and 360 mg/kg (ppm) cephapirin concentration in milk respectively. This is equivalent to between 157,500 and 360,000 ppb.

Other tissue data:
- Kidney 1.5 – 6.8 ppm @ 4.5 hours following 7.5 mg/kg IV (Na salt) in dairy cows (EMEA monograph)
- Kidney 1 – 5 ppm @ 4.5 hours following 8.5 mg/kg IM (benzathine salt) in dairy cows (EMEA monograph)
- After IU trt to dairy cows w/ 500 mg of benzathine cephapirin, levels in kidney, liver, meat, fat and udder tissue were less then LOD (0.015 ppm) by day 2-4. (EMEA monograph)
- After IMM trt of 300 mg benzathine cephapirin levels in fat, muscle, kidney and liver less then LOD (0.02 – 0.05 ppm) on day 14-21. (EMEA monograph)
- After IMM trt of 381 mg benzathine cephapirin/quarter, levels in fat, muscle, udder kidney and liver less then LOD (0.02 ppm) on day 21-42. (EMEA monograph)
- Five cows given 8.5 mg/kg IM of an aqueous form of CB and slaughtered 41/2 hours later. Renal cortex 0.97 ± S.D. 0.75 and renal medulla = 4.76 ± S.D. 2.16 PPM. (9000360)
- Nouws/Ziv for benzathine salt suggested a WDT 4.4 -6.5 days after 8.3 mg/kg IM (9001522)
- Nouws/Ziv for sodium salt suggested a WDT was 14 (SD 18) hrs after 8.5 mg/kg IM (9001522)
When a 84 kg calf was treated with cephapirin (30 mg/kg IM) and slaughtered 4 hours post-treatment. The compound was almost completely converted deacetylcephapirin in tissues. Additional milk assay data.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CEP (ppm)</th>
<th>DACEP (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected Muscle</td>
<td>2.74</td>
<td>11.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0032</td>
<td>0.67</td>
</tr>
<tr>
<td>Liver</td>
<td>None Detected</td>
<td>2.23</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.015</td>
<td>67.3</td>
</tr>
<tr>
<td>Blood Serum</td>
<td>0.56</td>
<td>0.75</td>
</tr>
</tbody>
</table>

FROM PREVIOUS EVALUATION (CA-012201-8736)

“Most of the following comes from FARAD consultation CA-032800-3979, the EMEA-CVMP monograph and the FARAD Digest on veal calves, (see Appendix IV). A standard computer assisted literature search could be performed in the future if more data was desired. The following is a bullet point summary of the salient points:

- The only veterinary cephapirin products approved in the US are lactating and dry-cow preparations.
- Cephapirin (marker residue) has a milk tolerance of 0.02 ppm in milk and 0.1 ppm in bovine tissue.
- Very little tissue depletion data are available in the published literature (see EMEA report) but the serum elimination half life of calves following IM treatment with 8.5 mg/kg is approximately 1 hour. We conservatively used 13 hours (see Calculations for Veal Digest below).
- FARAD recommended a highly conservative 7-day withdrawal for veal calves exposed to one gallon milk per day containing 320 ppb for three days. In that assessment we assumed 100% per os absorption and complete accumulation of all residue in the kidney.
- The estimated exposure in CDFA’s protocol totals 3.2 mg/calf/day, 100 fold less then above.
- In cattle treated IU with 500 mg benzathine residues were cleared in 2-4 days.
- In cattle treated IMM with 381 mg residues were cleared in 21-42 days.
- U.S. label withdrawal following IMM use of 300 mg benzathine (sustained release) is 42 days.
- U.S. label withdrawal following IMM use of 300 mg sodium salt is 4 days.
- In pigs (mongastics like calves) treated IM with 20 mg/kg residues, were cleared in 1-5 days.
- As with penicillin a remarkable margin of safety is offered by the Texas protocol calling for a 45-day withdrawal.

References and Calculations Examined for Veal Digest

Cephapirin Benzathine (CB): A first generation cephalosporin, this compound has an established United States tolerance level of 0.02 parts per million (PPM) in milk and 0.1 PPM in uncooked tissue of cattle. Both CB-containing products marketed in the U.S. contain 300 milligrams (mg) of CB per syringe. If label requirements of a 30-day interval between dry-treatment and calving are observed, the vast majority of cows will contain milk CB residues of < 0.02 PPM (9001458, 9000543, 9001407). If we use the highest colostrum/milk residue reported (0.32 PPM, see above) following label treatment and assume calf fluid consumption of one gallon/day for three days (the label milk discard time) we calculate an exposure of (0.32 ug/ml x 3800 x 3 = 3648 ug) 3.65 mg. Assuming total per os absorption and complete concentration in the kidney this would result in approximately (3.65 mg ÷(60 lb / 2.2 kg/lb = 27.3 kg calf) x 0.5% BW (% of BW that is kidney) = 137 grams of kidney tissue) 26.7 PPM in kidney. (This is extremely conservative compared to renal medulla = 4.76 ± S.D. 2.16 PPM in five cows given 8.5 mg/kg IM of an aqueous form of CB 9000360) Using the longest reported plasma half-life (13.3 hours 9001522 for CB, but reported sodium cephapirin serum T1/2 in humans, dogs, cattle, foals, calves are approximately 0.5-2 hours 9000287, 9004097, 9000902) it would require (13.3 x 8 = (106.4 hours) approximately 4.43 days which is within the calculated withdrawal time by Ziv of 4.4 -6.5 days following 8.3 mg CB IM to reach 0.01 PPM in kidney 9001522) approximately 7 days to reach tolerance. This calculation assumes a similar plasma and tissue half-life. Information supplied by manufacturer (9001458): Milk is usually 0.02 PPM 12 hours following calving but occasionally spikes to 0.25 to 0.32 PPM.”
Check JECFA
The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has not yet produced a monograph on cephapirin. The summary chart in their latest publication (41/13) has no cephapirin entry.

Re-evaluation of information from the European Agency for the Evaluation of Medicinal Products

EMEA/MRL/0128/96-FINAL July 1996
Committee For Veterinary Medical Products
Cephapirin Summary Report
Summary points:
1. Due to acid susceptibility, bioavailability of cephapirin following PO trt is claimed to be very low. Several studies point to low oral bioavailability of cefapirin. Authors consider this circumstantial.
2. In species examined the plasma elimination half life of cefapirin and desacetylcefapirin the major metabolite was 0.4-0.9 hours. In IV dog studies 98% of the drug was recovered in urine within 8 hours.
3. The recommended dose for parenteral (IV,IM, SQ) sodium cefapirin in Europe in cattle, sheep, goats and pigs is 10/mg/kg.
4. Cephapirin has very low toxicity and no evidence of reproductive tox, immunotoxicity, mutagenicity or carcinogenicity were evident. An oral NOEL of 20 mg/kg BW/day was established based on 3-month dog and rats studies. An ADI of 0.1 mg/kg BW/day (6 mg/day for 60 kg adult) was established. A provisional microbiological ADI of 0.001 mg/kg BW/day was also established.
5. In humans allergic cross reactivity between penicillins and cephalosporins is low and occurs only in 5% of patients hypersensitive to penicillins.
6. After 8.5 mg/kg IM (sodium salt) serum elimination t ½ approx. = 1 hour for dairy cows and calves.
7. After 8.5 mg/kg IV (sodium salt) serum elimination t ½ = 1.1 hour in dairy cows.
8. The tissue distribution of total residues of cephapirin in cattle is unknown.
9. After 7.5 mg/kg IV (sodium salt) in dairy cows, @ 4.5 hours kidney = 1.5 – 6.8 ppm, muscle = < 0.008 ppm and liver = 0.37 ppm.
10. After 8.5 mg/kg IM (benzathine salt) in dairy cows @ 4.5 hours kidney = 1 – 5 ppm, muscle = < 0.008 ppm and liver = < 0.045 ppm.
11. After IU trt to dairy cows w/ 500 mg of benzathine cephapirin, levels in kidney, liver, meat, fat and udder tissue were less then Limit of Detection (0.015 ppm) by day 2-4.
12. After IMM trt of 381 mg benzathine cephapirin/quarter, levels in fat, muscle, udder kidney and liver less then Limit of Detection (0.02 ppm) on day 21-42.
13. After IMM trt of 300 mg benzathine cephapirin levels in fat, muscle, kidney and liver less then Limit of Detection (0.02 – 0.05 ppm) on day 14-21.
14. In piglets after intramuscular trt with 20 mg/kg sodium cephapirin, on residues could be detected in liver, kidney, spleen, lung and injection site at 24-120 hours post treatment.
15. No studies were reported on metabolites in tissues with other routes of treatment and other species.
16. The recommended dose for parenteral (IV, IM, SQ) Na cephapirin treatment of infections in cattle, sheep, goats, and pigs is 10 mg/kg.
17. After IM trt with 10 mg/kg sodium salt 0.03-0.11 ppm in milk @ 1-4 hours and 0.01 ppm @ 4-8 hours.
18. After SQ trt with 10 mg/kg sodium salt no cefapirin could be detected in milk.
19. After IU trt 500 mg benzathine (3 doses) @ ≥ 2 milkings milk cefapirin was < LOD (0.01 ppm).
20. After IU trt 500 mg benzathine (1 doses) cefapirin was < LOD (0.01 ppm).
21. After IMM trt 500 mg benzathine/quarter in dry cows cefapirin levels in milk (after 5 to 86 days of dry period) were less then 20 to 1500 ppb at 1st to 3ed milking, less than 20 to 130 ppb at 4th and 5th milking and less than 20 ppb from the sixth milking.
22. Other milking studies (primarily IMM in lactating cows) were reported.
23. Provisional MRL of 50 ppb were established for muscle, liver, and fat, 100 for kidney and 10 for milk.

EMEA/MRL/745/00-FINAL February 2001
Committee for Veterinary Medical Products
Cephapirin Summary Report (2)
Summary points:
1. An appeal to the “incomplete” data assessment (see first summary above) was made to the CVMP. New information was provided.

2. In an in vitro gut model the effect of simulated gastrointestinal conditions on cefapirin resides was studied in the presence of meat, low fat milk and artificial saliva. It was estimated that 89% of the oral intake will pass in active form the stomach and duodenum of humans under average gastrointestinal conditions.

3. A new microbiological ADI of 2.54 ug/kg (153 ug/person) was established.

4. The sum of the parent compound and its metabolite desacetylcefapirin was identified as a suitable marker residue for milk and tissues (the estimated ration marker to total antimicrobially active residues is 1).

5. In kidney and muscle only desacetylcefapirin will be found.

6. Residues in liver are not stable and therefor it was not considered necessary to establish an MRL for liver.

7. The Committee for Veterinary Medical Products reversed their earlier opinion and recommended that cefapirin be included in Annex I of Council Regulation (EEC) No 2377/90 such that MRLs were established for muscle and fat at 50 ppb, 60 ppb for milk and 100 ppb for kidney. Based on these MRL levels the daily intake from bovine tissues and milk will represent approximatley 73% of the ADI.

From the FARAD Database
(See Appendix B for detailed information on these references.)

CEPHAPIRIN REFERENCES
The following FARAD citations were obtained by checking all available citations in the BIBILO, KINETIC, and KINENTRY databases.

<table>
<thead>
<tr>
<th>FARAD Citation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>8005105</td>
<td>Comparative pharmacokinetics of cefazolin, cephalothin, cephacetrile, and cephapirine after intravenous administration. Human data. Not examined.</td>
</tr>
<tr>
<td>8005115</td>
<td>Pharmacokinetics of cephapirin sodium in neonatal foals. 20 mg/kg IM t1/2 of 0.7 hours. Includes raw data.</td>
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<tr>
<td>9000020</td>
<td>Not incurred data. Determined cephapirin detection of BSDA and Delvo BSDA Not examined.</td>
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<tr>
<td></td>
<td>Cephalosporin (ug/ml)</td>
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<tr>
<td>1.00</td>
<td>31.78</td>
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<td>29.46</td>
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<tr>
<td>Control</td>
<td>13.00 (diameter of disk)</td>
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<td></td>
<td>Results were similar for Delvotest-P assay</td>
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<tr>
<td></td>
<td>Log 10 (cephapirin concentration) = (zone-31.823)/7.674</td>
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<td>Validated by making 0.006 PPM and running 40 assays all measured 15 mm.</td>
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<td></td>
<td>Interpretation is test will detect cephapirin to approx. 0.006</td>
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<tr>
<td>9000287</td>
<td>Cephapirin Sodium comparative PK in lab animals and humans. No tissue residue data was included. Dog T1/2 was &lt; 1 hours.</td>
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<tr>
<td>9000300</td>
<td>Antimicorobial agents in rats. IM dose 150 mg/kg T1/2 beta 0.3 hours.</td>
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<tr>
<td>9000312</td>
<td>Comparative pharmacokinetic studies of sodium cephapirin and cephalothin following intravenous and intra-muscular administration. Human plasma T1/2 for terminal beta values was approximately 0.55 hours for cephapirin and cephalothin. The pharmacokinetic profile of the drugs is essentially similar and little therapeutic difference should be expected in clinical practice.</td>
</tr>
<tr>
<td>9000360</td>
<td>Five cows given 8.5 mg/kg IM of an aqueous form of CB and slaughtered 41/2 hours later. Renal cortex 0.97 ± S.D. 0.75 and renal medulla = 4.76 ± S.D. 2.16 PPM. Four-tissue data point but all taken 4.5 hours after treatment. Has sodium cephapirin data as well.</td>
</tr>
<tr>
<td>9000440</td>
<td>Pharmacokinetics of cephalosporins in patients with normal or reduced function. Human data not examined.</td>
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<td>9000438</td>
<td>Detection of residual penicillins in milk by using a bacillus stearothermophilus disk assay.</td>
</tr>
<tr>
<td>9000413</td>
<td>Goat effectiveness data of dry treatment with CB. Also some residue testing. Only one of 29 goats had residues but average dry period 107.7 days ± S.D. 33.2 days. Not examined.</td>
</tr>
</tbody>
</table>
Laboratory studies with a new cephalosporinic acid derivative Mouse serum levels of BL-P 1322 (which is the same compound as cephapirin) compared to cephalothin. Mouse serum T ½ = 0.134- 0.3 hrs.

Retention data for antibiotic commonly used for bovine infections. Only IMM cephapirin data.

Comparison of the pharmacokinetics of cefamandole and other cephalosporin compounds. Human serum t ½ 0.71 hours after IV (unknown if Sodium or Benzathine).

Persistence of antibiotic in mammary secretions (LOD 0.005-0.008). At least 10 cows were positive (of 116) at 28 days post treatment. (8.6%). All 116 cows were negative by 49 days post treatment.

Compliance with recommended drug withdrawal requirements for dairy cows sent to market…

Same data as 543. (Oliver got a lot of mileage out of this one data set)

Calf PK data following IM IV & PO administration of a number of cephalosporins. Cephapirin (no mention of sodium or benzathine, probably sodium) at 10 mg/kg IM : Cmax 8 ug/ml serum t1/2 65 min. Other 1” generation cephalosporins at 10 mg/kg PO Cmax 3 PPM. Maximum serum T1/2 of a number of different cephapirins was 180 min.

See Cloxacillin section for complete chart. Interpretation is that BSDA test will detect cephapirin at 0.007 PPM, and using 16 mm as pass/fail will fail approx. 2/3 samples at 0.01 PPM. Fail 100% at 0.18 PPM (tolerance 0.2 PPM) Bottom Line: somewhere between 0.005-0.02 BSDA starts picking up cephapirin 9001060: Paper by Oliver appears to be the same data as 543.

Cephapirin benzathine IMM with milk data.

Paper compared effectiveness of systemic dry cow therapy with norfloxacin (SQ) and oxytetracycline (IM) with CB (IMM). No meat or milk residue data.

Review of absorption of antibiotics from udder. Cephapirin percentage that was non-ionized at pH 6.8 0.08, degree of lipid solubility : low, # infused quarters: 7. Ratio of t1/2 antibiotic to t1/2 carbon 14 urea Mean: 0.08 and SD was 0.18.

Pharmacokinetics and body fluid and endometrial concentrations of cefapirin in mares.

Depletion of cephapirin in goat’s milk after IMM treatment was longer then the recommended milk WDT in cattle.

Interspecific allometric analysis of the comparative pharmacokinetics of 44 drugs using the FARAD database. Results: A total of 11 drugs (ampicillin, apramycin carbencillin, cephapirin, chlortetracycline, diazepam, erythromycin, gentamicin oxytetracycline, prednisolone, and tetracycline. Show statistically significant (P<0.05; r² from 0.7- 0.99) correlation and consequently may be excellent candidates for inter-species extrapolations of half-life in species of relevance to veterinary medicine. These drugs has a mean beta of 0.236 ± 0.09. The average number of species/drug in this group was 6.2 with an average log body weight of 8.1. The remaining 33 drugs were classified into two groups which showed various degrees of lack of correlation. Bottom Line cephapirin was a “well behaved” drug.

Of 186 1st milking colostrum sample from cows treated with 4 dry cow products only 1 cow treated 50 days earlier with benzathine cephapirin was positive with BSDA (0.008-0.001)

Dose: 200 mg cephapirin sodium infused into one or two cow quarter with milk data.

Freedom of Information Summary; NADA 108-114; CEFA-DRY (Cephapirin benzathine) “Separate studies were submitted for the cephapirin and benzathine moieties. Separate studies were also carried out demonstrating the complete depletion of benzathine before the depletion of cephapirin in milk, and the complete depletion of major metabolites in milk before the parent compound, thus establishing that cephapirin is the marker residue.” Much milk data virtually no tissue data.

8 mg/kg Cep. Benzathine IM = t1/2 beta 13 (SD 2.62) hours. (longest serum t1/2 we have seen.) Estimated sodium cephapirin T ½ beta = 1.04 (SD 0.2) hours. Probably “flip flop” of absorption occurred with slow release benzathine containing vehicle. Nouws and Ziv used a equation to estimate withdrawal times based on ratios of serum to kidney ratio. They suggested a kidney cortex withdrawal time of 4.4 to 6.5 days following a 8.3 mg/kg IM dose... Kidney cortex to serum ration 4.8 ± SD 3.5. For sodium cephapirin the calculated withdrawal time was 14 (SD 18) hours following a 8.5 mg/kg IM dose.

“Determination of milk and mammary tissue concentrations of cefiofur after intramammary and intramuscular therapy” Only IMM cephapirin data.

Seymour: “Persistence of resides in milk following antibiotic treatment of dairy cattle”. Only milk data following IMM administration.

Seymour: “Comparisons of on-farm screening tests for detection of antibiotic residues”. Compared Penzyne, Delvo and BSDA with a number of antibiotics. Used Sodium cephapirin IMM with 4 day WDT. No pertinent data for this study.
“Pharmacokinetics of sodium cephapirin in lactating dairy cows”. PK of Sodium cephapirin in lactating dairy cows. Six cow at 10 mg/kg IM with 4 doses every eight hours. Plasma T1/2 1.16 hours. Not enough urine data to create depletion curve.

Development of a HPLC Mass Spec method to detect Sodium Cephapirin in bovine milk and serum. Used incurred residue but used lactating cows and preparation. Limits of detection approximately 10-50 ug/kg. PB. The principal metabolite was tentatively identified as desacetylcephapirin.

Pharmacokinetics and serum concentration of cephapirin in neonatal foals. 20 mg/kg IM = Cmax = 21.2; tmax =0.17; t1/2=0.7; kelim = 1.06; Cl = 18.4 kelim = 1.06.

Effect of probenecid administration on cephradine pharmacokinetic and concentration in mares...

Same data as 543. (Oliver got a lot of mileage out of this one data set)

Folder not in files. Medline abstract describes a cylinder plate method for determination of Pen G, ampicillin and cephapirin in animal tissues. LOD reported as 0.02-0.32 PPM. Unknown if incurred residue samples used.

Determination of penicillin G, ampicillin, and cephradine residues in tissues. No incurred data.

incurred residues using Charm II. No pertinent/useful data.

“Compliance with recommended drug withdrawal requirements for dairy cows sent to market in Michigan”. Documents Cephapirin mastitis treatments as a cause of cull cow tissue residues. Also references Kaneene Journal of Dairy Science 1987, vol 70 2176-2180 which stated that treatment for mastitis and metritis was a leading cause of milk drug residues.

Absence of cephradine residues after treatment with Metricure in milk from healthy cows…

When a 84 kg calf was treated with cephradine (30 mg/kg IM) and slaughtered 4 hours post-treatment. The compound was almost completely converted deacetylcephapirin in tissues. Additional milk assay data.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CEP (ppm)</th>
<th>DACEP (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected Muscle</td>
<td>2.74</td>
<td>11.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0032</td>
<td>0.67</td>
</tr>
<tr>
<td>Liver</td>
<td>None Detected</td>
<td>2.23</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.015</td>
<td>67.3</td>
</tr>
<tr>
<td>Blood Serum</td>
<td>0.56</td>
<td>0.75</td>
</tr>
</tbody>
</table>

**Ceftiofur**

If the label IM dose for a 100 lb calf was diluted into an extraordinary daily 16 liters of milk replacer, the concentration in milk would be more then 6,000 ppb (2.2 mg/kg X 45 kg = 99mg / 16 kg = 6.1875 mg/kg = 6.1875 ppm = 6,1875 ppb). For more typical milk replacer rations of 8 or 4 liters per day the concentration jumps to approximately 12,000 and 24,000 ppb respectively. Ceftiofur is poorly absorbed after oral administration (See EMEA below) and probably less then 30% would be absorbed. We can conclude that total exposure would be less then the label dose, which has a withdrawal (depending on the salt of the ceftiofur product) of 0-2 days.

**FROM PREVIOUS EVALUATION (CA-012201-8736)**

Most of the following information was generated during the preparation CA-110900-8708

- In 1998 Upjohn obtained a tolerance for ceftiofur in milk of 0.1 ppm (or 100 ppb.)
- My most recent communication with FDA on this subject suggests that they would not object to the marketing of milk that contained parent ceftiofur at or below the “old” safe level of 50 ppb.
- Tissue depletion data following oral exposure to cattle is not available in the published literature.
- Currently there exist two ceftiofur veterinary products in the US:
  - Ceftiofur Sodium (Naxcel®): 1.1-2.2 mg/kg SID, IM, up to 5 days duration.
  - Ceftiofur Hydrochloride (Excenel®): 1.1-2.2 mg/kg SID, IM or SQ, up to 5 days duration.
- Slaughter withdrawal for Excenel® is 2 days and for Naxcel® zero days although it is prudent to allow at least 12 hours post Naxcel® treatment prior to slaughter.
- CDFA’s protocol would allow oral exposure of up to 8 mg ceftiofur/calf/day.
- Additionally the CDFA protocol proposes a 45-day slaughter withdrawal rather then 2 days.
Following weaning and a 45-day withdrawal the calf will be “metabolically mature” alleviating any concerns relative to applying adult withdrawal times to calves.

**Background on Ceftiofur Tolerance**
In 1998 Pharmacia-Upjohn has obtained its first-ever tolerance for ceftiofur in milk of 0.1 ppm, or 100 ppb (the Federal Register, Oct 6, 1998 page 53579). The tolerance is based on total desfuroylceftiofur (marker residue) not the parent compound, which appears in milk only as the result of intra-mammary infusion or vandalism. There was some confusion with the various regulators I talked to about whether an assay which converted parent compound to the desfuroylceftiofur marker was a problem. A representative from CVM indicated that if the amount of parent ceftiofur was known to be less then the historical unofficial “Safe Level” of 50 ppb (previously used by CVM/FDA for PARENT compound) FDA would not object to releasing the milk for sale. The caveats were that they wanted to be approached and feel comfortable with the assay methodology. In cattle the tissue tolerances are kidney (target tissue) 8 ppm, liver 2 ppm, muscle 1 ppm, and milk 0.1 ppm (or 100 ppb.)

**Background on Availability of Ceftiofur Products**
Ceftiofur has previously been available in the US as ceftiofur sodium (Naxcel®). As part of the 1998 Excene® RTU (ceftiofur hydrochloride, “Ready-To-Use” because no reconstitution is required) approval, Upjohn obtained its first-ever tolerance for ceftiofur. This tolerance was published in the Federal Register, Vol. 63 No. 193, Oct 6, 1998 page 53579. Tolerances are based on total desfuroylecftiofur (marker residue) in edible cattle tissues. In cattle tissue tolerances are kidney (target tissue) 8 ppm, liver 2 ppm, muscle 1 ppm, and milk 0.1 ppm (or 100 ppb.)

**Background on Regulatory Issues**
In spite of the new milk tolerance, unresolved are a host of questions, the answers to which must be agreed upon by CVM/FDA and the State of California:

1) Is the State even allowed to perform confirmatory testing under Appendix N?
2) Is the State allowed to release for sale or dispose of milk based on the results of such testing?
3) If the State uses testing which converts parent compound to marker desfuroylecftiofur and therefor does not know if parent compound was present, does that change the course of required action?

In order for the State milk testing program to run smoothly it will need to be granted or confirm its authority to perform its own confirmatory testing at least on tanker milk and possibly on silo milk. This will allow tanker milk that been misidentified as violative (so called “false-violative”) to be co-mingled with other milk and released for processing and sale. This would seem to be reasonable and prudent based on extensive literature that indicates that screening assays can and do falsely identify fluid milk as containing violative concentrations of antibiotics when in fact the product contains legal levels of those compounds. A CVM representative indicated that strictly speaking (under the PMO) the State did not have the authority to release milk for sale based on its own testing. In spite of this however, he indicated that was unlikely that they would object to the State clearing milk for sale if they felt that that the State’s confirmatory methodology was adequate. He said that CVM/FDA would support the State (or other agencies or organizations) in their effort to set up laboratories which were formally recognized as performing official validated confirmatory assays. Because the presence of parent compound indicates that an extra-label drug use had occurred and a residue had resulted, the CVM representative also indicated that he would expect the State and/or the FDA to take regulatory action against the producer.

**Background On Ceftiofur Excretion Into Milk**
Within minutes of IM, IV or SQ injection, all ceftiofur as the parent compound is converted to desfuroylecftiofur metabolites. Even administering 16 times the label dose IV may only result in milk metabolites up to 250 ppb, but parent ceftiofur will not be present. The only way to have parent drug present in milk is by intra-mammary treatment or direct adulteration of already-harvested milk (sabotage from disgruntled employee etc). About 12 hours after label IM use, peak ceftiofur metabolites will be present at about 120 ppb of metabolites. Just prior to the next daily treatment 24 hours following the first, a treated cows metabolite levels will fall trough level of approximately 20 ppb. Therefore in pooled milk from a number of animals treated and milked at different times, the level could be expected to be about 70 ppb.
Background On Assay Sensitivity

The BSDA picks up ceftiofur metabolites at about 60 ppb, Delvo-P and Penzyme at about 30-45 ppb and SNAP at about 20 ppb. You can see that as long as you have 3 to 5 cows worth of milk going into the tank with every treated cow you will still not get a positive test, even using the most sensitive assay. Thus if ceftiofur is being used on-label (IM or SQ but not infused into the quarter), unless you are treating more than 1/5 to 1/3 of your milking herd, it is virtually impossible to get a positive bulk tank or tanker using any assay. Treating 20 to 30% of your herd is unlikely given both the disease incidence and expense that would be involved. The problem with bulk tank, tanker-truck and silo milk arises because of parent compound. Most of the screening assays extremely sensitive to the parent compound, far more so than the metabolite. SNAP for instance may show positive when exposed to 1-5 ppb of parent ceftiofur. This has resulted in intense education by P&U to veterinarians, producers and processors. One P&U representative says that if you use Naxcel® in the quarter you might as well get your checkbook out, since your going to buy a tanker. I contacted Dr. Biget Prushner (530-752-6322) at CVDLS. The LC-MS-MS screening assay used looks at seven beta-lactams. They are ceftiofur, cephapirin, penicillin G, penicillin V, amoxicillin, ampicillin, and cloxacinil. The limits of detection for ceftiofur is approximately 1ppb with all the others being approximately 5 ppb. Dr. Prushner makes it clear that the LC-MS-MS assay will only detect ceftiofur parent compound and not ceftiofur present as metabolites. Additional information concerning assay sensitivity is available in the JECFA monographs.

Background Concerning Ability of Calves to Excrete Ceftiofur (see also JECFA section below)

The most pertinent data comes from the proceedings from the 1994 AABP meeting: “Plasma disposition of ceftiofur and metabolites after intravenous and intramuscular administration of ceftiofur sodium to calves of various ages” (Brown SA & Robb EJ FARAD citation 8005178). The salient point that we draw from this data is that at some point between 1 and 3 months of age a calf’s ability to excrete ceftiofur fully matures. Up to that point a neonatal calf’s plasma half life is approximately 2 to 3 times longer then that of older cattle. It should also be noted however that peak plasma concentrations are no higher in neonatal calves than in mature cattle suggesting that the tissue concentrations (and similarly tissue residues) are no higher in neonatal calves than in mature cattle. This assumption is strongly supported by the studies reviewed by JECFA (see below). Even if tissue residues were higher, by the time the calves are weaned from the contaminated milk (approximately 2 months) and the Texas withdrawal time completed (an additional 45 days) the calf will be fully metabolically matured.

<table>
<thead>
<tr>
<th>Value *</th>
<th>7 days</th>
<th>1 month</th>
<th>3 month</th>
<th>6 month</th>
<th>9 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ug/ml)</td>
<td>9.36</td>
<td>9.31</td>
<td>8.35</td>
<td>8.68</td>
<td>9.25</td>
</tr>
<tr>
<td>AUC (ug x h/ml)</td>
<td>162</td>
<td>150</td>
<td>66.2</td>
<td>64.6</td>
<td>73.8</td>
</tr>
<tr>
<td>t ½ (h)</td>
<td>12.3</td>
<td>11.4</td>
<td>5.2</td>
<td>4.15</td>
<td>4.42</td>
</tr>
<tr>
<td>Clb (ml/h/kg)</td>
<td>14.9</td>
<td>14.9</td>
<td>33.4</td>
<td>34.2</td>
<td>30.4</td>
</tr>
<tr>
<td>Vdss (ml/kg)</td>
<td>263</td>
<td>251</td>
<td>260</td>
<td>214</td>
<td>203</td>
</tr>
</tbody>
</table>

* Standard Deviations have been excluded from the original table.

From the Joint WHO/FAO Expert Committee on Food Additives (JECFA)

Two reports had ceftiofur monographs 41/8 (1995) and 41/10 (1997). Useful data is combined below.

1. In calves (94-136 kg) given 2.2 mg/kg ceftiofur Na IM tissue levels at eight hours were: kidney 3.5 ppm (tolerance 8 ppm), liver 1.29 (tolerance 2 ppm), muscle 0.21 (tolerance 1ppm) and fat 0.32 (no tolerance). Thus even at 8 hours in calves residues are less then US tolerance. The above study and others suggest a bio-phasic tissue depletion. Authors suggest kidney should be target tissue until 20 days after which the liver (which may then have higher residues) be used as the target tissue.
2. In a second study 25 veal calves (39-68 kg) received 1 mg/kg ceftiofur Na IM SID for 5 days. All tissue samples were at the first two slaughter times at 5 and 10 days post treatment.

3. In a third study 6 calves (54-73 kg) received 1 mg/kg ceftiofur Na and killed at 2.5 hours (n=3) or 7.5 hours (n=3). Kidney residues were all less than US tolerance.

4. Oral studies in bovines were not presented.

5. Data on other species and ages of bovines was present and may be of use in future FARAD consultations.

From the European Agency for the Evaluation of Medicinal Products (EMEA)
EMEA/MRL/498/98-FINAL July 1999
Committee For Veterinary Medical Products
Ceftiofur Summary Report (1) & (2)

Summary points:
1. Ceftiofur is poorly absorbed after oral administration.
2. In all species ceftiofur is rapidly (initially) metabolized to desfuroylceftiofur (DFC) within 2-4 hours.
3. More than 95% of the administered dose is excreted within 24 hours (60-80% in urine, rest in feces).
4. Very low acute oral & parenteral toxicity The oral LD50 > 7760 mg/kg in rats.
5. Due to extremely low toxicity, EMEA established a microbiological-based ADI (1.2 mg/person/day).
6. Residues deplete slowest from the kidney although occasionally liver residues are significant.
7. Oral studies in bovines were not presented.
8. Data on other species and ages of bovines was present and may be of use in future FARAD consultations.