California Department of Food and Agriculture

Protocol for Diversion of Confirmed Positive Bulk Raw Milk Tankers

To Calf Ranches – Tetracyclines and Sulfonamides

08 March 2013

Protocol

All bulk raw milk tankers confirmed positive for tetracycline or sulfonamides intended for diversion to calf feed shall be cleared for such use by a negative test obtained from a dilution of 1:100 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 tetracycline and sulfonamide.

Loads testing negative after dilution may be diverted to animal feed provided the shipping invoice includes: the negative test result, dilution used, test used, the recorded test reading, certified laboratory identification, signature of certified analyst, and load identification verification plus a clear statement “MEDICATED ANIMAL FEED –WITHDRAW 20 DAYS BEFORE SLAUGHTER”.

Loads testing positive following dilution must be disposed of by an approved manner other than animal feed use.

The table below represents the maximum expected tetracycline or sulfonamide drug concentration that could result in a negative test after a dilution of 1:100 with confirmed negative milk and the estimated time required for all tested calf tissues to fall below tolerance levels as defined in 21 Code of Federal Regulations Part 556.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tolerance</th>
<th>Charm II Performance Standard*</th>
<th>Maximum concentration resulting in a negative Charm II test @ 1:100 dilution</th>
<th>Estimated Time to Calf Tissue Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>300 ppb</td>
<td>257 ppb</td>
<td>25,600 ppb</td>
<td>20 days</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>300 ppb</td>
<td>119 ppb</td>
<td>11,900 ppb</td>
<td>10 days</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>300 ppb</td>
<td>67 ppb</td>
<td>6,700 ppb</td>
<td>10 days</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>100 ppb</td>
<td>4 ppb</td>
<td>400 ppb</td>
<td>20 days</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>0 ppb</td>
<td>4.9 ppb</td>
<td>490 ppb</td>
<td>-</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>0 ppb</td>
<td>9.4 ppb</td>
<td>940 ppb</td>
<td>-</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>0 ppb</td>
<td>7.3 ppb</td>
<td>730 ppb</td>
<td>-</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 Response

CDFA requests the maximum amount of antibiotics that could still be present in a sample test-positive when undiluted and then test-negative after a 1:100 dilution with confirmed test-negative (for tetracycline and sulfonamide) milk. Additionally, CDFA requests the approximate time required for calf tissues to fall below tolerance for each drug if fed at that specified level. It is FARAD’s stance that the most conservative recommendations are made allowing for a large safety factor. The two primary factors in the selection of these levels are 1) demonstrating to state and federal regulatory authorities that a large safety factor exists inherent in the recommendation and 2) the recommendations make sense, logistically, with the most common milk assays used in California. In fact, the concentration of antibiotics which could be fed to calves in all cases is likely many folds higher then these recommendations. Briefly, withdrawal intervals were calculated by the following system independently for each drug: First, tissue half-life (or serum half-life if data were limited) was rounded up to either 12 or 24 hours for one small safety factor. Next, that number was taken times 10 (10 half-lives equal 99.9% elimination) for a preliminary withdrawal interval. The preliminary WDI was finally taken times 2, for an additional safety factor, to reach a final recommended WDI.

FARAD recognizes that Charm II Tetracycline Drug Test and Charm II Sulfa Drug Test are limited in the ability to differentiate between compounds when presented with a test-positive sample. In light of this, the recommendation for a withdrawal interval is made from the compound within each drug class with the longest withdrawal interval (WDI). More specifically, chlortetracycline’s WDI is 20 days and therefore any test-positive milk for tetracycline will have a 20 day WDI. The recommended WDI for any test-positive milk for sulfonamides is also 20 days, based on sulfadimethoxine. Sulfadimethoxine has the longest tissue elimination half-life, and the most consistent plasma elimination half-life, and the most wealth of applicable data of all sulfonamides in this document. It must be reiterated, however, that there are no licensed sulfadiazine, sulfamethazine, or sulfathiazole products approved for use in lactating dairy cattle in the United States and extra-label use of sulfonamide-class antibiotics in lactating dairy cattle is strictly prohibited. Any milk samples that are later found to be positive for sulfadiazine, sulfamethazine, or sulfathiazole by more specific analytical procedures would constitute evidence of illegal drug use by the producer and should be dealt with by the appropriate agency. This is beyond FARAD’s charge.

The following table compares the plasma, liver, kidney, and muscle elimination half-lives (hours) found in the literature review of each sulfonamide drug. Inclusion criteria for the data in the table are that the publication used dairy breed calves, with a reported body weight less than 330 kg, or a reported age less than 6 months of age. Published data not meeting these requirements are not summarized in this table but can be found in the complete summarized review at the end of this document.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sulfadimethoxine (hr)</th>
<th>Sulfadiazine (hr)</th>
<th>Sulfamethazine (hr)</th>
<th>Sulfathiazole (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>13.8-16.4</td>
<td>4.3-22.0</td>
<td>4.7-26.0</td>
<td>3.93</td>
</tr>
<tr>
<td>Liver</td>
<td>19</td>
<td>15.6</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Kidney</td>
<td>16.8</td>
<td>14.7</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Muscle</td>
<td>n/a</td>
<td>16.1</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>
The chart below compares the current tolerance, the Charm II detection levels, and FARAD’s very conservative estimation of the time for concentrations in calf tissue to fall below tolerance which can be equated to a recommended WDI. A brief explanation for the basis of the recommendation and references for each drug is also included in the footnotes below the table.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tolerance</th>
<th>Charm II LOD</th>
<th>FARAD Recommended WDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline*</td>
<td>300 ppb</td>
<td>257 ppb</td>
<td>20 days</td>
</tr>
<tr>
<td>Oxytetracycline*</td>
<td>300 ppb</td>
<td>119 ppb</td>
<td>10 days</td>
</tr>
<tr>
<td>Tetracycline*</td>
<td>300 ppb</td>
<td>67 ppb</td>
<td>10 days</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>100 ppb</td>
<td>4 ppb</td>
<td>20 days</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>0 ppb</td>
<td>4.9 ppb</td>
<td>-</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>0 ppb</td>
<td>9.4 ppb</td>
<td>-</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>0 ppb</td>
<td>7.3 ppb</td>
<td>-</td>
</tr>
</tbody>
</table>


B Charm II Sulfa Drug Test and Charm II Tetracycline Drug Test – Level of detection (LOD) which can be detected 90% of the time with 95% confidence [5].

C Chlortetracycline Assessment: Calculated maximum tissue half-life for calves fed 22 mg CTC/kg by rumen intubation and slaughter in pairs at various time points were approximately 24 hours. Tissues were below tolerance in this study at a 12 hour withdrawal interval [6].

D Oxytetracycline Assessment: Serum half-life is reported at a maximum of approximately 9 hours in the literature [7-10] this half-life was ‘rounded-up’ to 12 hours for a WDI calculation. No residues were detected in 48 calves fed 80, 120 or 500 ppm (500,000 ppb) OTC in milk substitute feeds at slaughter in tissues following 3 day withdrawal interval by three different testing procedures with a reported sensitivity of methods at 0.01 µg/ml (10 ppb) [11].

E Tetracycline Assessment: Only one study was found on the pharmacokinetics of tetracycline administration in cattle and the reported serum elimination half-life for the IV administration group was 5.73 hours and 6.0 hours for IM administration [12]. Several tetracycline oral products are labeled for administration in the drinking water of calves at 10 mg/lb or 220,000 ppb (22 mg/kg fed at 10% body weight) with withdrawal times of either 4 or 5 days [12-14].

F Sulfadimethoxine Assessment: Sulfadimethoxine has the longest elimination half-life reported in the literature of all the sulfonamides in this diversion protocol [1-3, 15-22]. Kidney residues were below the 0.1 ppm (100 ppb) tolerance for all calves slaughtered after a 114 hour withdrawal in 9 Holstein calves (193-330 kg) administered 550,000 ppb (55 mg/kg fed at 10% BW) initially followed by 275,000 ppb (27.5 mg/kg fed at 10% BW) at 24 hours and again at 48 hours. Elimination half-life was calculated to be 16.8 hours in the kidney, 19.0 hours in the liver, and 15.2 hours in the plasma [1]. Nearly identical half-lives are found in the plasma of veal calves as found in the above study and it can therefore be assumed that the tissue elimination half-lives would likely be very similar too [2, 3, 15, 16, 20-22].

G Sulfamethazine Assessment: There are no licensed sulfamethazine products approved for use in lactating dairy cattle in the United States and extra-label use of sulfonamide-class antibiotics in lactating
dairy cattle is strictly prohibited [23]. Due to lack of data, FARAD is unable to provide a recommendation for this drug.

**Sulfadiazine Assessment:** There are no licensed sulfadiazine products approved for use in lactating dairy cattle in the United States and extra-label use of sulfonamide-class antibiotics in lactating dairy cattle is strictly prohibited [23]. Due to lack of data, FARAD is unable to provide a recommendation for this drug.

**Sulfathiazole Assessment:** There are no licensed sulfathiazole products approved for use in lactating dairy cattle in the United States and extra-label use of sulfonamide-class antibiotics in lactating dairy cattle is strictly prohibited [23]. Due to lack of data, FARAD is unable to provide a recommendation for this drug.

* Tolerances are established for the sum of residues of the tetracycline’s including chlortetracycline, oxytetracycline, and tetracycline [4].

**New Analytical Methods and Sampling Procedures**

The Food Safety and Inspection Service (FSIS) recently announced a restructuring of the United States National Residue Program. In addition to a new approach to sampling and scheduling, the Agency has implemented multi-residue methods for analyzing samples of meat, poultry, and egg products for animal drug residues, pesticides, and environmental contaminants in its inspector-generated testing program [24]. The table below presents the new level of quantification of each drug in bovine tissue.

**Analytes and Applicability Level**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Level of Quantification&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bovine Kidney</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>1 ppm</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>0.05 ppm</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>0.05 ppm</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>0.05 ppm</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>0.05 ppm</td>
</tr>
</tbody>
</table>

<sup>+</sup> Federal Register, Vol. 77, No. 130, July 6<sup>th</sup>, 2012 [24]
Tolerances for chlortetracycline in calf tissue are as follows (ppm): Fat (12.0), Kidney (12.0), Liver (6.0), and Muscle (2.0). Note: Tolerances are established for the sum of residues of the tetracycline’s including chlortetracycline, oxytetracycline, and tetracycline [4].

A series of IV and orally dosed CTC experiments were performed on 18 pure-bred, Holstein calves utilizing a 2 period cross-over design. The final experiment consisted of 8 milk-fed and 6 conventionally fed Holstein calves, at approximately fourteen weeks of age (76.0-118.0 kg), that were administered a single dose of 22 mg CTC/kg PO (by rumen intubation) and slaughtered in pairs at 12, 24, 48, and 72 hours. [6]
  o Bioavailability was reported to be 24.1 ±6.1% for milk fed and 4.9 ±0.9% for conventionally fed calves in this study.
  o CTC concentrations (ppm) in plasma and tissue, n=2 at each time point
    - Cylinder plate assay with a reported sensitivity of 0.1 µg/g (100 ppb)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.80</td>
<td>5.87</td>
<td>1.06</td>
<td>0.53</td>
</tr>
<tr>
<td>24</td>
<td>1.79</td>
<td>3.87</td>
<td>0.48</td>
<td>1.18</td>
</tr>
<tr>
<td>48</td>
<td>0.58</td>
<td>0.84</td>
<td>0.22</td>
<td>0.24</td>
</tr>
<tr>
<td>72</td>
<td>0.10</td>
<td>0.20</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Conventionally fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1.09</td>
<td>1.98</td>
<td>0.31</td>
<td>0.57</td>
</tr>
<tr>
<td>48</td>
<td>0.46</td>
<td>0.61</td>
<td>0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>72</td>
<td>0.12</td>
<td>0.22</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

10 Holstein and Jersey calves, three days of age, were administered 50 mg CTC daily PO SID (divided and fed in milk and calf starter ration). Three calves were slaughtered at 16 weeks of age, with no mention of withdrawal of medication prior to slaughter. [25]
  o No quantifiable residues in tissue (liver, kidney, muscle, etc) or plasma at slaughter
  o Detected in liver and kidney (<100 ppb)
    - Reported sensitivity of method 0.1 µg/g (100 ppb)

3 Francaise frisonne calves, three weeks of age (35 kg) were orally administered 50 mg CTC/kg (with 50mg chloramphenicol/kg) PO daily for 5 doses [26]
  o No residues detected at 20 days
    - Reported sensitivity of method 0.01 µg/ml (10 ppb)

48 calves weighing approximately 90 kg were treated with 80, 120 or 500 ppm CTC in milk substitute feeds until they were slaughtered at ~150 kg [11]
  o No residues detected in tissue following 3 day WDT by three different testing procedures
    - Reported sensitivity of method 0.001 µg/ml (1 ppb)

6 calves, four to six weeks of age were fed 50 mg CTC/kg with 2 g citric acid in milk replacer [8]
  o Serum half-life was 6.23 hr

18 three-to-four week old calves (43-54 kg) on a milk diet, were administered 50 mg CTC/kg (1,075-1,350 ppm) in water, milk replacer, and cow’s milk (n=6 per treatment) utilizing a cross-over design with 4 days washout between the three treatments. [7]
  o Serum half-life ranged from 2.695-8.685 depending on the vehicle used

4 male Red Holstein/Simmental cross calves, 3-4 weeks of age administered 50 mg CTC/kg PO once [10]
  o Bioavailability 53 ±17%

PennChlor is labeled for calves up to 250 lbs at a dose of 0.1 mg CTC/lb PO administration in feed with 0 day withdrawal [27]
  o Residue studies were conducted in ruminating calves (n=12) at the labeled dosage
    - Concentrations (ppm) 7.167 ±2.906 in kidney when slaughtered with a 0 day withdrawal
Aureomycin (CTC) is labeled for calves up to 250 lbs at a dose of 0.1 mg CTC/lb PO administration in feed with 0 day withdrawal [28]
  o Several residue studies were conducted in ruminating calves (n=27) at the labeled dosage
    ▪ Concentrations of liver, kidney, muscle, and fat were all below tolerance levels when slaughtered with a 0 day withdrawal

### Oxytetracycline (OTC)

Tolerances for oxytetracycline in calf tissue are as follows (ppm): Fat (12.0), Kidney (12.0), Liver (6.0), and Muscle (2.0). Note: Tolerances are established for the sum of residues of the tetracycline’s including chlortetracycline, oxytetracycline, and tetracycline [4].

- 48 calves weighing approximately 90 kg were treated with 80, 120 or 500 ppm OTC in milk substitute feeds until they were slaughtered at ~150 kg [11]
  o No residues detected in tissue following 3 day WDT by three different testing procedures
  ▪ Reported sensitivity of method 0.01 µg/ml (10 ppb)
- 18 three-to-four week old calves (43-54 kg) on a milk diet, were administered 50 mg OTC/kg (1,075-1,350 ppm) in water, milk replacer, and cow’s milk (n=6 per treatment) utilizing a cross-over design with 4 days washout between the three treatments. [7]
  o Serum half-lives varied from 2.695-8.685 hr across the three treatments
- 5 calves, five-to-seven weeks old, were fed 6.6 mg OTC/kg with and without 2 g citric acid in milk [8]
  o Serum half-life varied from 0.14-3.13 hr across treatments
- 48 calves, five-to-ten days old were fed 9 mg OTC/kg in milk replacer (n=24), water (n=12), or electrolyte solution (GGES, n=12) [9]
  o Serum half-life 8.5 hr (milk replacer), 8.5 hr (water), 6.6 hr (GGES)
- 12 calves of Swedish Red and White breed, five-to-six weeks old (~50 kg) were administer 50 mg OTC/kg in a cross-over design (2 week wash out period) with OTC administered PO in either milk, water, or electrolyte solution [29]
  o Compared to water the bioavailability was significantly reduced (53.5%) when OTC was mixed in the milk replacer. The results from this, and earlier studies, show that the bioavailability of OTC is significant reduced when mixed in milk.
- 4 male Red Holstein/Simmental cross calves, four-to-six weeks of age were administered 50 mg OTC/kg PO once in milk replacer. [10]
  o Serum terminal half-life reported is 10.66 ±3.15 hr
  o Bioavailability 46.35 ±12.00%
- Terramycin (OTC) is approved for inclusion in the diet of calves <250 lbs 0.05-0.1 mg/lb of body weight daily, fed continuously with a 0 day withdrawal [30]
  o Residue studies conducted in ruminating calves (n=6) at 11 mg/lb for 14 days
    ▪ LOQ: liver, kidney 100 ppb, muscle and fat 75 ppb
    ▪ Concentrations (ppm) were 1.247 ±0.563 in kidney, 0.743 ±0.196 in liver, 0.189 ±0.073, and undetectable in fat at time of slaughter
- BIVI Oxy (OTC) calf boluses are approved for use in non-ruminating dairy calves at 10/mg lb divided and given twice daily for four consecutive days with 0 day withdrawal [31]
  o Residue studies were conducted in ruminating calves (n=20) at the labeled dosage
    ▪ LOQ (ppm): liver 0.176, kidney 0.255, muscle 0.238, fat 0.129
    ▪ Concentrations (ppm) were 1.13 ±0.18 in liver, 1.51 ±0.38 kidney, and undetectable in muscle and fat when slaughtered at a 12 hour withdrawal
Tetracycline (TTC)

Tolerances for tetracycline in calf tissue are as follows (ppm): Fat (12.0), Kidney (12.0), Liver (6.0), and Muscle (2.0). Note: Tolerances are established for the sum of residues of the tetracycline’s including chlortetracycline, oxytetracycline, and tetracycline [4].

- 18 Israeli-Friesian lactating dairy cows (520-630 kg) were administered TTC either by IV (n=6) or IM (n=12) at a dosage of 20 mg/kg [32].
  - Reported serum elimination half-life for the IV administration group was 5.73 hrs and 6.0 hours for IM administration
- Duramycin-10 (aka—Tet-Sol 324) is licensed for administration of 10 mg/lb (22 mg/kg) to be administered in drinking water for 3-5 days consecutively with a 5 day withdrawal time [12].
- Tetramed 324 HCA is licensed for administration of 10 mg/lb (22 mg/kg) to be administered in drinking water for 3-5 days consecutively with a 5 day withdrawal time [13].
- Polyotic is licensed for administration of 10 mg/lb (22 mg/kg) to be administered in drinking water with a 4 day withdrawal time [14].

Sulfadimethoxine (SDM)

A tolerance of 0.1 ppm is established for negligible residues of sulfadimethoxine in uncooked edible tissues of cattle [4].

Extra-label use of sulfadimethoxine in lactating dairy cattle is strictly prohibited [23].

- 9 Holstein calves aged four-to-six months (193-330 kg) were administered 55 mg SDM/kg initially followed by 27.5 mg SDM/kg at 24 hours and again at 48 hours [1].
  - Kidney residues had fallen below the 0.1 ppm (100 ppb) tolerance for all calves (n=4) slaughtered at 114 hours post-last dose
  - Tissue elimination half-life was calculated to be 16.8 hours in the kidney and 19.0 hours in the liver
    - The lower quantitation limit reported for the parent drug and metabolite was 10 ng/g (ppb) in kidney and liver and 2 ng/mL (ppb) in plasma
  - Elimination half-life was 15.2 hours in the plasma
- 6 calves weighing 106-114 kg were divided into three groups of two and administered 16.7, 25.0, and 33.4 mg SDM/kg PO [3]
  - Group 1 (16.7 mg/kg) had a plasma elimination half-life of 13.8 hours
  - Group 2 (25.0 mg/kg) had a plasma elimination half-life of 15.6 hours
  - Group 3 (33.4 mg/kg) had a plasma elimination half-life of 15.5 hours
- 10 calves of the Polish Red Breed weighing between 54-83 kg were administered SDM PO in a suspension at doses of 100 and 150 mg/kg in single doses and in another experiment with an initial loading dose of 100 mg SDM/kg and followed by 50 mg SDM/kg in sustaining doses for the following two days successively [2]
  - Calves receiving the 100 mg/kg of SDM had a blood elimination half-life of 16.4 hours after a single administration
  - Calves receiving the 150 mg/kg of SDM had a blood elimination half-life of 13.9 hours after a single administration
- 6 Holstein calves, six-to-eight months of age were administered sulfadimethoxine-ormetoprim orally at a dosage of 33 mg/kg. A separate experiment was performed on the same calves with IV administration [22]
- Plasma elimination half-life for the IV dosed calves was 7.91 hours (data not available for PO dosed elimination half-life)
- Oral bioavailability at 24 hours was 56.6 ±15.9%, however the authors speculated that absorption would reach 100%
- 4 cross bred calves ten-to-twelve months of age (161-202 kg) were administered SDM at 107 mg/kg IV and three months later were administered sulfadimethoxine at 107 mg/kg via stomach tube [15]
  - Blood elimination half-life for IV administration as 10.3 hours and for PO administration the half-life was reported to be 11.5 hours.
  - Oral bioavailability was reported to be 59.1 ±5.9%
- 4 seven month old Angus steers (182-191 kg) were administered SDM PO at 55 mg/kg followed by doses of 27.5 mg/kg every 24 hours thereafter for a 3 additional days. The same 4 Angus steers were administered 55 mg/kg of sulfadimethoxine IV approximately 5 months later in a separate experiment [16]
  - Plasma elimination half-life was 8.2 hours for IV administration
  - Plasma elimination half-life was 12.5 hours for PO administration
- 10 adult lactating Holstein-Friesian cows were administered SDM in one of the following three dosage regimens: 214 mg/kg IV (n=6) or PO (n=6). Separately, an initial loading dose of 55 mg/kg, followed by 27.5 mg/kg at 24, 48 and 72 hours (n=2) and finally an initial loading dose of 110 mg/kg, followed by 55 mg/kg at 24, 48 and 72 hours (n=2) [21]
  - For the group dosed at 214 mg/kg IV elimination half-life in blood was 13.4 hours and in plasma 12.6 hours
  - For the group dosed at 214 mg/kg PO once elimination half-life in blood was 13.0 hours and in plasma 12.7 hours
  - The cows receiving an initial loading dose of 55 mg/kg, followed by 27.5 mg/kg for three doses had reported elimination half-life of 10.1 hours in blood and 8.8 hours in plasma
  - The cows receiving an initial loading dose of 110 mg/kg, followed by 55.5 mg/kg for three doses had reported elimination half-life of 11.7 hours, and 13.2 hours in plasma
- 5 twelve-to-eighteen month old cross-bred calves (217-330 kg) were administered SDM at 100 mg/kg PO once or 50mg/kg of SDM IV once [20]
  - Elimination half-life in the plasma for IV administration was 9.1 hours
  - Elimination half-life in the plasma for PO administration was 11.3 hours and the oral bioavailability was calculated to be 81.9%

### Sulfadiazine (SDZ)

There are no tolerances established in calf tissue for sulfadiazine [4].

There are no licensed sulfadiazine products approved for use in lactating dairy cattle in the United States. Extra-label use of sulfonamide-class antibiotics in lactating dairy cattle is strictly prohibited [23].

- Two groups of 6 Holstein calves each, one was fed milk-replacer throughout the experiment and one was weaned from milk at 5 weeks of age and fed a chopped grain-fiber mixture. Each group was dosed with 30 mg/kg Tribrissen (1:5 mixture w/w of TMP/SDZ) at weeks 1 (only milk fed calves dosed), 6, and 12 weeks of age [33].
  - Serum elimination half-life ranged from 4.3-15.5 hours
- 9 Holstein-Friesian of average age of 9 days at dosing (39-46 kg) were medicated with 1 g SDZ and 0.2 g TMP PO for 5 consecutive days. Three calves were sacrificed on days 1, 3, and 7 days after last dosing. Plasma, liver, kidney, and muscle samples were analyzed with quantitative thin-layer
chromatography using fluorescamine derivatization specific for SDA and sensitive to 0.01 mg/kg. [34]
  - Tissue elimination half-lives are as follows: kidney 14.7 hr, liver 15.6, muscle 16.1
- 2 calves one-to-two weeks of age received radioactive $^{14}$C-SDZ SDZ 1 g + 0.2 TMP PO for five consecutive days. Calves were slaughtered at 14 days post last treatment and radioactivity measured [35].
  - Day 14 residues (ppm) in plasma (0.105), muscle (0.045), liver (.315), and kidney (0.35)
- 6 calves age 6-10 days and 5 calves aged 11-15 days and 7 calves aged over 15 days of age were administered 25 mg SDZ + 5 mg TMP PO once [36].
  - Serum elimination half-life for calves 6-10 days of age were 22.0 ±7.1 hours, 11-15 days of age were 9.1 ±1.2 hours, and over 15 days of age were 11.1 ±2.2
- 6 buffalo calves weighing 80-100 kg administered 150 mg/kg of SDZ PO once [37].
  - Serum elimination half-life 13.75 ±1.94 hours

<table>
<thead>
<tr>
<th>Sulfamethazine (SMZ)</th>
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<tbody>
<tr>
<td>A tolerance of 0.1 ppm is established for negligible residues of sulfamethazine in uncooked edible tissues of cattle [4].</td>
</tr>
<tr>
<td>There are no licensed sulfamethazine products approved for use in lactating dairy cattle in the United States. Extra-label use of sulfonamide-class antibiotics in lactating dairy cattle is strictly prohibited [23].</td>
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</table>

- Bob veal 3-5 days old (39-59 kg, n=20), fancy veal 12-13 weeks old (109-173 kg, n=24), and replacement calves 12-13 weeks old (75-114 kg, n=20) were dosed with 220 mg/kg SMZ boluses the first day and 110 mg/kg for 4 additional days and then slaughtered at various withdrawal periods from 0-14 days after the last dose [38].
  - Average concentration (ppm) in the liver of replacement calves were 0.0725, 0.0275, 0.015, and undetectable at days 5, 7, 10, and 14 after withdrawal
  - Average concentration (ppm) in the liver of bob veal calves were 3.388, 0.358, 0.108, and 0.013 at days 5, 7, 10, and 14 after withdrawal
  - Average concentration (ppm) in the liver of fancy veal calves were 1.075, 1.493, 0.098, and 0.098 at days 5, 7, 10, and 14 after withdrawal
    - The authors reported concentration of SMZ in diaphragm graphically falling below the 0.1 tolerance at 3 days in replacement calves, and 9 days in both bob and fancy veal calves.
    - The authors report (data not shown) that tissue concentrations in muscle collected from the thigh, loin, and shoulder on a few animals didn’t differ significantly from diaphragm concentration data
- Fancy veal and bob veal calves dosed with 220 mg/kg SMZ boluses the first day and 110 mg/kg for 4 additional days and then slaughtered at various withdrawal periods from 0-14 days after the last dose [38].
  - Concentrations (ppm) at slaughter on day 7 after the last dose for fancy veal (n=1) for liver 0.39, kidney 0.42, and diaphragm 0.35
Concentrations (ppm) at slaughter on day 7 after the last dose for bob veal (n=1) for liver 0.36, kidney 0.25, and diaphragm 0.17

- 30 Friesian and Czech Red cross-bred calves from 2-22 days old (30-60 kg) received 50 mg SMZ/kg as either a 20% solution SMZ IV (n=6) or PO 4 hours after morning feeding (n=18 healthy, n=6 diarrheic) [39].
  - Healthy calf (PO administration) kinetic parameters were elimination half-life of 26 ±1.0 hours and a bioavailability of 0.76 ±0.06
  - Diarrheic calf (PO administration) kinetic parameters were elimination half-life 17.7 ±0.5 hours and a bioavailability of 0.59 ±0.06
- 4, three-to-five day old Holstein calves were given 396 mg/kg PO of a 8 g SMZ bolus composed of a 3 g outer shell for rapid disintegration and 5 g core for gradual disintegration [40].
  - Plasma half-life 23.26 hr
- 6, eight-month old (200 kg) Herford-Angus cross calves were administered 214.3 mg/kg or 71.4 mg/kg SMZ in drinking water each day for four days. A separate experiment was carried out with 5 head receiving 142.9 mg/kg each day for seven days [41].
  - Blood half-life calculated from graphical data for 214.3 mg/kg was 27.04
  - Blood half-life calculated from graphical data for 142.9 mg/kg was 9.90 hr
  - Plasma half-life calculated from graphical data for 71.4 mg/kg was 13.27 hr
- 12 calves of an unreported age were administered 214.5 mg/kg on day one and 107.2 mg/kg SMZ for the next four days and slaughtered in groups of 3 at 0-6 hr, 5, 8, and 10 days post final treatment. Fat, muscle, kidney and liver were analyzed by assayed by HPLC for residues [42].
  - Mean residues (ppm) at 0-6 hr: fat 197, muscle 47.2, kidney 82.2, and liver 34.6
  - Mean residues (ppm) at 5 days: fat 0.23, muscle 0.08, kidney 0.49, and liver 0.44
  - Mean residues (ppm) at 8 days: fat 0.07, muscle <0.04, kidney <0.04, and liver <0.04
  - Mean residues (ppm) at 10 days: fat 0.05, muscle <0.04, kidney <0.04, and liver <0.04
- Sulk-S Boluses are approved for use in calves at 10 g/100 lbs (220 mg/kg) for the initial day of treatment and at 5 g/100 lbs (110 mg/kg) for up to four additional days of treatment [43].
  - Twenty-four dairy calves weighting 105-255 pounds were administered the labeled dosage over 5 days and then divided into seven groups and slaughtered at intervals to determine tissue depletion
    - An analysis of the data for a tolerance of 0.1 ppm resulted in a 99% statistical tolerance limit (95% confidence) of 0.07 ppm at 11 days withdrawal.
- 20 calves were administered 44 mg/kg SMZ PO on the tenth and twentieth days of their lives to determine effects on pharmacokinetics due to age or time of day administration. They found significant differences in PK values depending on age and on day-night differences [44].
  - Blood elimination half-life ranged from 4.7-5.9 hours for the ten day old calves and 6.6-7.4 hours for the twenty day old calves
- 6 buffalo calves weighing 80-100 kg were administered sulfamethazine at 150 mg/kg body weight PO in water at a concentration of 7.5 g/100ml [45].
  - Serum elimination half-life was 11.94 hours
- Pharmacokinetic studies on buffalo of an unreported age found the half-life of SMZ to be 6.5 hours when dosed at 200 mg/kg PO once [46].
There are no tolerances established in calf tissue for sulfathiazole [4].

There are no licensed sulfathiazole products approved for use in lactating dairy cattle in the United States. Extra-label use of sulfonamide-class antibiotics in lactating dairy cattle is strictly prohibited [23].

3 calves weighing from 34-111.5 kg were dosed with 100 mg/kg STZ PO once [47].
  - Serum elimination half-life 3.93 hours

Pharmacokinetic studies on buffalo of unreported age found the half-life of sulfathiazole to be 2 hours when dosed at 200 mg/kg PO once [46].
5. United States Food and Drug Administration, Beta Lactam and Other Test Methods for Use Under Appendix N and Section 6 of The Grade "A" Pasteurized Milk Ordinance (PMO), 2012: College Park, MD.