# Pharmacokinetics and tissue elimination of flunixin in veal calves

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**OBJECTIVE**
To describe plasma pharmacokinetic parameters and tissue elimination of flunixin in veal calves.

**ANIMALS**
20 unweaned Holstein calves between 3 and 6 weeks old.

**PROCEDURES**
Each calf received flunixin (2.2 mg/kg, IV, q 24 h) for 3 days. Blood samples were collected from all calves before the first dose and at predetermined times after the first and last doses. Beginning 24 hours after injection of the last dose, 4 calves were euthanized each day for 5 days. Plasma and tissue samples were analyzed by ultraperformance liquid chromatography. Pharmacokinetic parameters were calculated by compartmental and noncompartmental methods.

**RESULTS**
Mean ± SD plasma flunixin elimination half-life, residence time, and clearance were 1.32 ± 0.94 hours, 12.54 ± 10.96 hours, and 64.6 ± 40.7 mL/h/kg, respectively. Mean hepatic and muscle flunixin concentrations decreased to below FDA-established tolerance limits (0.125 and 0.025 μg/mL, respectively) for adult cattle by 3 and 2 days, respectively, after injection of the last dose of flunixin. Detectable flunixin concentrations were present in both the liver and muscle for at least 5 days after injection of the last dose.

**CONCLUSIONS AND CLINICAL RELEVANCE**
The labeled slaughter withdrawal interval for flunixin in adult cattle is 4 days. Because administration of flunixin to veal calves represents extralabel drug use, any detectable flunixin concentrations in edible tissues are considered a violation. Results indicated that a slaughter withdrawal interval of several weeks may be necessary to ensure that violative tissue residues of flunixin are not detected in veal calves treated with that drug. (*Am J Vet Res* 2016;77:634–640)

Flunixin is the only NSAID approved for use in cattle in the United States and is labeled for the modulation of inflammation in endotoxemia and for the control of pyrexia associated with bovine respiratory tract disease and acute bovine mastitis.[1] Although flunixin is approved for use in adult cattle, there is not a specific approval for its use in calves that are to be processed for veal. However, the drug is occasionally used in an extralabel manner as a supportive treatment for calf diarrhea or pneumonia.[2–4] In the United States, drug residues in human food products derived from animals cannot persist at concentrations greater than those established as safe by regulatory agencies. The greatest concentration of a drug residue allowed in edible tissue is called the tolerance limit. The tolerance limit for flunixin in adult cattle is 0.025 μg/mL (25 ppb) for muscle and 0.125 μg/mL (125 ppb) for liver.[1] Because flunixin is not specifically labeled for use in veal calves, tolerance limits (and slaughter withdrawal intervals) have not been established, and any flunixin residue detected in veal calves is considered a violation. Therefore, the unofficial tolerance limit becomes less than the lowest concentration of the drug that can be detected by the analytic method used.

Flunixin residue violations are not uncommon in veal calves. In 2010, flunixin residue violations in veal calves accounted for 60 of the 285 (21%) flunixin residue violations in cattle as reported by the USDA-FSIS.[5] From 2006 through 2012, 261 violative flunixin residues were detected in bob veal (meat from calves ≤ 3 weeks old or ≤ 68 kg[6]), which made flunixin the third most frequently identified drug residue in that type of meat behind neomycin and sulfonamides. Nonsteroi-

**ABBREVIATIONS**

| | |
|---|---|
| 5OH | 5-hydroxy flunixin |
| $C_{max}$ | Maximal concentration of a drug |
| FFA | Flunixin-free acid |
| FSIS | Food Safety and Inspection Service |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| MRT | Mean residence time |
| UPLC | Ultraperformance liquid chromatography |

dal anti-inflammatory drugs, particularly flunixin, are widely used in the cattle industry. In a 2007 survey[2] of bovine practitioners, 86% of the cattle treated with NSAIDS were dairy cattle. Similarly, results of an earlier survey[3] of dairy veterinarians indicate that NSAIDs were the second most prescribed drug in dairy cattle behind antimicrobials. Because of the high incidence of violative flunixin residues in cattle, it is regularly included on the FSIS repeat violator list.[7] In adult cattle, the majority of violative flunixin residues have been attributed to noncompliant drug use[8,9] and impaired tissue elimination subsequent to disease processes.[10] Data to predict appropriate slaughter withdrawal intervals for flunixin following administration to veal calves are limited. Furthermore, marked differences in drug pharmacokinetics have been observed between young and adult animals.[11] Differences in drug distribution and elimination in young food-producing animals are of particular concern because they may increase the likelihood of violative drug residues. The primary objective of the study reported here was to examine the pharmacokinetics and tissue elimination of flunixin in veal calves following multiple IV injections of the drug so that a suggested withdrawal interval for flunixin in veal calves could be determined.

## Materials and Methods

### Animals

The study was approved by the North Carolina State University Institutional Animal Care and Use Committee. Twenty unweaned Holstein bull calves between 3 and 6 weeks old, with a mean ± SD weight of 53.3 ± 9.5 kg, were used in the study. Calves were individually housed in calf hutches and fed 2.5 L of milk replacer (protein content, 24%; fat content, 16%) from a bucket twice daily throughout the duration of the study.

### Experimental design

All calves received flunixin meglumine[a] (2.2 mg/kg, IV, q 24 h) for 3 days. All injections were administered into the left jugular vein. Blood samples (approx 6 mL each) were collected into an evacuated blood collection tube that contained sodium heparin as an anticoagulant by repeated venipuncture of the contralateral jugular vein with an 18-gauge, 1.5-inch needle immediately prior to and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after the first dose of flunixin and at 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 36 hours after the last dose of flunixin. Blood samples were centrifuged at 1,500 × *g* for 10 minutes at 4°C. The plasma from each sample was harvested and stored frozen at −20°C until analyzed for FFA and 5OH concentrations.

Calves were randomly assigned to 1 of 5 groups (n = 4 calves/group) by pulling ear tag numbers from a hat. Calves were euthanized by administration of pentobarbital sodium (60 mg/kg, IV) at 24 (group 1), 48 (group 2), 72 (group 3), 96 (group 4), or 120 (group 5) hours after administration of the last dose of flunixin. Immediately after euthanasia, the entire liver, both kidneys, and an approximately 10-g specimen of tissue from both the semimembranosus and semitendinosus muscles were harvested from each calf and stored frozen at −80°C until analysis.

### Plasma extraction and quantification

For extraction, plasma samples were thawed and 0.5 µL of each sample was combined with 250 µL of 0.5% citric acid in acetonitrile. Samples were sonicated for 5 minutes and then centrifuged at 1,500 × *g* for 10 minutes. The supernatant from each sample was transferred into a clean glass culture tube and dried at 55°C with an evaporator[b] under a 20-psi stream of nitrogen. Each sample was then reconstituted in 100 µL of 50:50 acetonitrile:water and filtered through a 0.22-µm nylon syringe filter. The injection volume was 5 µL for samples with low flunixin concentrations and 0.3 µL for samples with high flunixin concentrations. Concentrations were derived by comparison of the peak areas for the samples with those of an external standard curve made from spiked plasma samples put through the sample cleanup process. The flunixin concentration of the standard curve ranged from 0.125 to 125 ng/mL for plasma or 0.5 to 500 ng/g in tissue samples.

Concentrations of FFA and its primary plasma metabolite, 5OH, were quantified by UPLC with mass spectrometric detection.[c] A gradient was used, and the initial mobile phase was a 70:30 (vol/vol) mixture of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, with a flow rate of 0.2 mL/min for the first 2.5 minutes. The mobile phase was adjusted to 10:90 (vol/vol) from 2.5 to 3.5 minutes and then back to 70:30 (vol/vol) for the last 1.5 minutes of the run. The quadrupole mass spectrometer[d] was run in an electrospray ionization positive mode. The quantification trace used was 297 to 279 for flunixin and 313 to 295 for 5OH. The column temperature was 35°C, the sample temperature was ambient, and the run time was 5 minutes. The LOQ was 0.001 µg/mL, and the LOD was 0.0005 µg/mL, which was the lowest concentration on the calibration curve. The linear calibration range was 0.0005 to 20 µg/mL for FFA and 5OH. Both interday and intraday variations were < 5% for flunixin and 5OH, and accuracy was > 96% for spiked concentrations of 0.001, 0.02, and 0.1 µg/mL.

### Tissue extraction and quantification

Concentrations of FFA and 5OH in tissue were quantified by use of UPLC with mass spectrometric detection as described by Boner et al[12] with minor modifications. Samples were fortified with the deuterated forms of flunixin (flunixin-d3) and 5OH (5OH-d3)[e] as internal standards prior to an initial acid hydrolysis, followed by pH adjustment (approx 9.5) and partitioning with ethyl acetate. A portion of the ethyl acetate extract was loaded onto a strong cation exchange cartridge[f] for further cleanup. The eluate was then evaporated to dryness with a nitrogen evapo-

rator[g] and reconstituted for analysis. Concentrations were determined by comparison of the peak area ratios for the samples with those of an external standard curve.

Analysis was performed by use of a UPLC[h] coupled to a mass spectrometer[i] with a heated electrospray ionization source operated in the positive ion mode. The column[c] was maintained at 30°C. The UPLC mobile phase was 0.4% formic acid in water (A) and 0.4% formic acid in 55:45 methanol:acetonitrile (B). The samples were analyzed at 0.4 mL/min of 50:50 A:B, followed by a solvent wash at 0.55 mL/min of 5:95 A:B. Injection volume was 10 µL. Ions were monitored in the selected reaction monitoring mode with transitions of 297 to 279 for FFA, 300 to 282 for flunixin-d3, 313 to 295 for 5OH, and 316 to 298 for 5OH-d3.

The LOD for FFA was 0.8 ng/g for liver, 1.1 ng/g for muscle, and 1.3 ng/g for kidney. The LOQ for FFA was 1.9 ng/g for liver, 2.5 ng/g for muscle, and 3.4 ng/g for kidney. The LOD for 5OH was 2.6 ng/g for liver, 2.0 ng/g for muscle, and 4.2 ng/g for kidney. The LOQ for 5OH was 6.5 ng/g for liver, 4.4 ng/g for muscle, and 8.7 ng/g for kidney. The relative SDs for interday and intraday variation were < 10% for both FFA and 5OH at spiked concentrations of 62.5, 125, and 250 ng/g. The mean recovery ranged from 99.2% to 102.5% for flunixin and from 98.7% to 100.2% for 5OH.

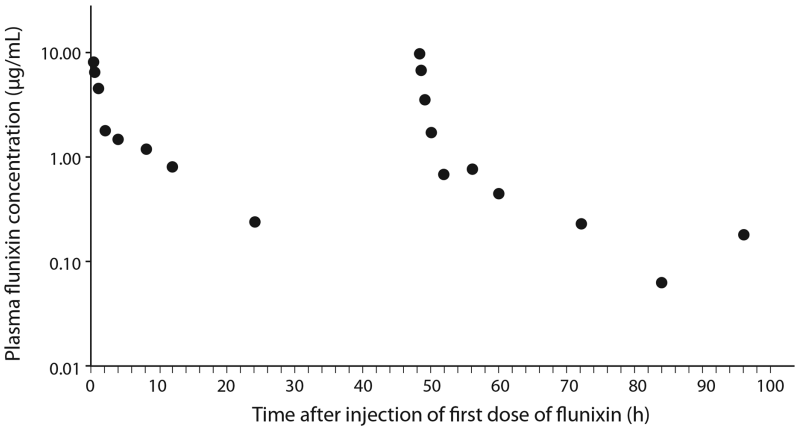


**Figure 1**—Mean plasma flunixin concentration over time for 20 unweaned Holstein calves that were administered flunixin (2.2 mg/kg, IV, q 24 h) for 3 days. The first dose was injected at 0 hours, the second dose was injected at 24 hours, and the third dose was injected at 48 hours. Blood samples were collected from each calf immediately prior to and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after the first dose of flunixin and at 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 36 hours after the third dose of flunixin. The LOD of flunixin for the assay used was 0.0005 µg/mL.

## Pharmacokinetic analysis

The FFA equivalent versus time-concentration data were analyzed with commercial pharmacokinetic modeling software.[j] The best model was selected on the basis of comparison of the Akaike information criterion values among various models (lowest value preferred) and visual inspection of plots of the predicted versus observed data. Standard equations were used to calculate pharmacokinetic parameters.[13]

**Table 1**—Mean ± SD values for pharmacokinetic parameters for flunixin in veal calves as determined by a 2-compartment model derived from data obtained from 20 unweaned Holstein calves following administration of flunixin (2.2 mg/kg, IV, q 24 h) for 3 days.

| Parameter | Mean ± SD |
|---|---|
| $k_{10}$ ($h^{-1}$) | 0.93 ± 1.36 |
| $k_{12}$ ($h^{-1}$) | 1.12 ± 0.53 |
| $k_{21}$ ($h^{-1}$) | 0.20 ± 0.14 |
| $k_{12:21}$ | 8.59 ± 9.33 |
| $k_{10half\text{-}life}$ (h) | 1.32 ± 0.94 |
| $t_{1/2\alpha}$ (h) | 0.43 ± 0.26 |
| $t_{1/2\beta}$ (h) | 12.88 ± 8.71 |
| $AUC_{0-\infty}$ (h•µg/mL) | 48.40 ± 30.91 |
| MRT (h) | 12.54 ± 10.96 |
| $V_{d(ss)}$ (L/kg) | 0.634 ± 0.30 |
| $V_c$ (L/kg) | 0.097 ± 0.057 |
| Cl (mL/h/kg) | 64.60 ± 40.7 |

$AUC_{0-\infty}$ = Area under the concentration-time curve from time 0 to infinity. Cl = Clearance. $k_{10}$ = Elimination rate constant. $k_{10half\text{-}life}$ = Elimination half-life. $k_{12}$ = Distribution rate constant from compartment 1 to compartment 2. $k_{12:21}$ = Ratio of the distribution rates from compartments 1 to 2 to compartments 2 to 1. $k_{21}$ = Distribution rate constant from compartment 2 to compartment 1. $t_{1/2\alpha}$ = Distribution half-life. $t_{1/2\beta}$ = Beta elimination half-life. $V_c$= Volume of central compartment. $V_{d(ss)}$ = Volume of distribution at steady state.

# Results

The calves tolerated flunixin administration well, and no adverse reactions were observed. The mean plasma flunixin concentration over time was plotted **(Figure 1)**. A 2-compartment pharmacokinetic model with first-order elimination provided the best fit for the data. The pharmacokinetic parameters for flunixin in veal calves following IV administration were summarized **(Table 1)**. The time-concentration data for 5OH, the primary plasma metabolite of flunixin, were analyzed with a noncompartmental method. For 5OH, the mean ± SD plasma $C_{max}$, area under the concentration-time curve from time 0 to infinity, elimination half-life, and MRT were 0.079 ± 0.048 µg/mL, 0.416 ± 0.222 hours•µg/mL, 10.24 ± 4.73 hours, and 13.52 ± 6.78 hours, respectively.

The mean ± SD 5OH concentration in the liver and flunixin concentrations in the liver, muscle, and kidney for each group of calves that were euthanized between 24 and 120 hours after injection of the last dose of flunixin were summarized **(Table 2)**. 5-hydroxy flunixin was detected in only 1 of 4 calves in group 4 (calves euthanized 96 hours after injection of the last dose of flunixin) and was not detected in any calves in group 5 (calves euthanized 120 hours after injection of the last dose of flunixin). Flunixin was detected in the liver, muscle, and

**Table 2**—Mean ± SD concentrations of 5OH in the liver and of flunixin in the liver, muscle, and kidney for the calves in Table 1 following random allocation to 1 of 5 groups (n = 4 calves group).

| Group | Hepatic 5OH concentration (μg/mL) | Hepatic flunixin concentration (μg/mL) | Muscle flunixin concentration (μg/mL) | Renal flunixin concentration (μg/mL) |
|---|---|---|---|---|
| 1 | 0.167 ± 0.085 | 3.19 ± 1.35 | 0.096 ± 0.032 | 0.926 ± 0.421 |
| 2 | 0.013 ± 0.005 | 0.126 ± 0.050 | 0.005 ± 0.002 | 0.028 ± 0.011 |
| 3 | 0.003 ± 0.002 | 0.09 ± 0.013 | 0.010 ± 0.002 | 0.028 ± 0.006 |
| 4 | 0.0042* | 0.052 ± 0.013 | 0.002 ± 0.001 | 0.017 ± 0.004 |
| 5 | — | 0.101 ± 0.024 | 0.007 ± 0.002 | 0.023 ± 0.008 |

Calves were euthanized by administration of pentobarbital sodium (60 mg/kg, IV) at 24 (group 1), 48 (group 2), 72 (group 3), 96 (group 4), or 120 (group 5) hours after administration of the last dose of flunixin.
*SD not reported because 5OH was detectable in the liver of only 1 calf.
— = Not determined because 5OH was not detected in the liver of any calf.
***See*** Table 1 for remainder of key.

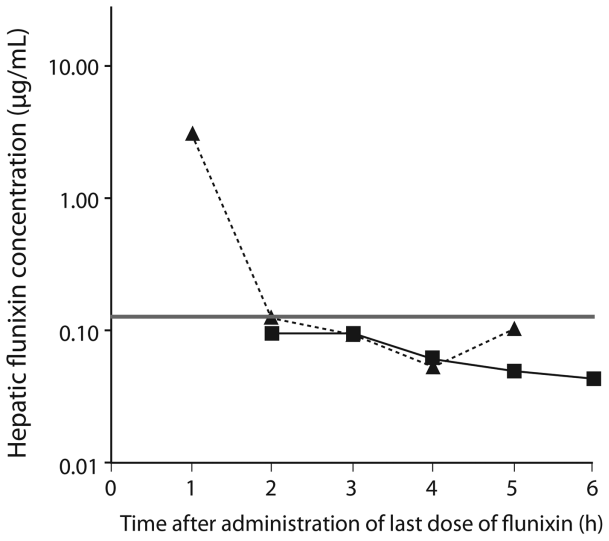


**Figure 2**—Mean hepatic flunixin concentration for the calves of Figure 1 that were euthanized 1 (group 1; n = 4), 2 (group 2; 4), 3 (group 3; 4), 4 (group 4; 4), and 5 (group 5; 4) days after injection of the last dose of flunixin (dotted line with triangles) overlaid on a plot of the mean hepatic flunixin concentration for healthy adult cows over time following administration of the same dosage regimen of flunixin (2.2 mg/kg, IV, q 24 h for 3 days; solid line with squares).[28] The solid horizontal line represents the tolerance limit (0.125 μg/mL) for flunixin in the liver tissue of adult cattle established by the FDA.

kidney of all calves regardless of group assignment. Interestingly, the mean ± SD hepatic flunixin concentration for group 5 (0.101 ± 0.024 μg/mL) was greater than that for group 4 (0.052 ± 0.013 μg/mL).

## Discussion

Results of the present study indicated that flunixin was rapidly transferred from the central compartment to the peripheral compartment in veal calves as evidenced by the short mean distribution half-life (0.43 hours). However, that distribution half-life was longer than that previously reported in calves[14] (0.10 hours) and adult cattle[15–17] (0.19 to 0.29 hours). The mean ratio of distribution rates from compartments 1 to 2 to compartments 2 to 1 in the present study was high, which suggested that there was excellent distribution of flunixin from the central compartment to the peripheral compartment. The mean apparent volume of the central compartment for the calves in the present study (0.097 ± 0.057 L/kg) was substantially greater than that (0.03 L/kg) previously reported in calves.[14] The calves of the present study were < 6 weeks old and had a mean weight of 53.3 kg, whereas the calves of the other study[14] had a mean weight of 118.9 kg. Age is inversely associated with total body water; therefore, the younger the animal, the greater its volume of distribution for drugs.[11,18] The mean volume of distribution at steady state for flunixin was 0.634 ± 0.30 L/kg for the calves in the present study, which was similar to that reported for adult cattle[14–16,19–23] (0.254 to 0.782 L/kg) and indicated good distribution of flunixin throughout the body. The clearance rate of flunixin from the plasma of the calves of the present study (64.60 ± 40.7 mL/kg/h) was similar to that for diseased cows[10] (67.02 ± 47.24 mL/kg/h) but was approximately half that reported for healthy adult cattle[14–16,19–24] (90 to 263 mL/kg/h). Young animals have low phase I and II enzyme activity; thus, the clearance rate of a drug changes with age.[11] The slower clearance of flunixin from the calves of the present study, compared with that of healthy adult cattle, was likely attributable to the slower rate of hepatic drug metabolism and elimination in calves relative to adult cattle. The mean elimination half-life for flunixin in the calves of the present study (12.8 hours) was longer than the elimination half-life for flunixin reported in healthy weaned calves[14,25,26] (6 to 7 hours) and adult cattle[15,16,19–24,27] (3.14 to 8.12 hours). This indicated that, compared with adult cattle, flunixin had prolonged elimination from veal (unweaned) calves, which was consistent with the slow clearance of flunixin observed in those calves.

For the calves of the present study, the mean $C_{max}$ of 5OH (0.079 ± 0.048 μg/mL), the primary plasma metabolite of flunixin, was substantially lower than that reported for healthy adult cattle,[17,22] whereas the mean elimination half-life and MRT for 5OH were both longer than those for adult cattle.[22] The low $C_{max}$ for flunixin in calves was likely the result of the low enzymatic activity and decreased flunixin metabolism inherent in young animals, and the prolonged elimina-

tion half-life and MRT were likely caused by immature excretory pathways.[11,18]

The mean hepatic flunixin concentrations for calves euthanized 48, 72, and 96 hours after injection of the last dose of flunixin (groups 2, 3, and 4, respectively) were similar to those for adults cows[28] **(Figure 2)**. Interestingly, the mean ± SD hepatic flunixin concentration (0.101 ± 0.024 µg/mL) for the 4 calves in group 5 (calves euthanized 120 hours after injection of the last dose of flunixin) was greater than that for group 4 (0.052 ± 0.013 µg/mL) and adult cattle 5 days after administration of the last dose of flunixin, although it was still below the tolerance limit for flunixin in the liver of adult cattle (0.125 µg/mL) established by the FDA.[1] However, because flunixin is not approved for use in veal calves, that established tolerance limit does not apply, and any flunixin residue detected in the tissues of veal calves is considered a violation. The apparent increase in the mean hepatic flunixin concentration for group 5 relative to that for group 4 was unexpected. However, it should be remembered that the mean hepatic flunixin concentrations reported in the present study were derived from different groups of calves that were euthanized at predetermined times after administration of the last dose of flunixin and did not represent results from serial biopsy specimens obtained from the same calves over time. It is possible that the disposition, metabolism, or elimination of flunixin varied among calves sufficiently to affect the means for each group, especially since each group contained only 4 calves. We observed that calves in groups 4 and 5 that were heavier and older (ie, closer to 6 weeks old than 3 weeks old) tended to have lower hepatic flunixin concentrations than did the calves that were lighter and younger. Regardless, it was not possible to draw any mechanistic conclusions regarding the increase in mean hepatic flunixin concentration between groups 4 and 5 given that those concentrations were derived from different calves.

Similar to hepatic flunixin concentrations, the mean muscle flunixin concentrations for groups 2, 3, 4, and 5 were all below the tolerance limit for flunixin in the muscle of adult cows (0.025 µg/mL) established by the FDA.[1] However, that tolerance limit does not apply for veal calves because flunixin is not approved for use in calves, and detection of any concentration of flunixin in the muscle of veal calves is considered a violation. The mean muscle flunixin concentration decreased between groups 1 and 2 and increased between groups 2 and 3 and between groups 4 and 5. As with the mean hepatic flunixin concentrations, the apparent up-and-down fluctuation among the mean muscle flunixin concentrations was likely the result of variation in the disposition and elimination of flunixin among calves.

The labeled slaughter withdrawal interval for flunixin in adult cattle is 4 days,[29] but the tissue flunixin concentration data for the calves of the present study suggested that a 4-day interval would not be sufficient to avoid tissue residue violations in veal. Consequently, the hepatic flunixin concentrations for the calves of the present study were used to estimate a slaughter withdrawal interval for unweaned calves following IV administration of flunixin at 2.2 mg/kg every 24 hours for 3 days in accordance with the FDA's *Guidance for Industry: General Principles for Evaluating the Safety of Compounds Used in Food Animals*.[30] This method considers the rate of depletion and variability among individual animals to determine the 95% confidence interval for when 99% of the population will have a drug concentration below a given target concentration. The estimated time for the hepatic flunixin concentration to decrease to < 0.125 µg/mL (the tolerance limit established for adult cattle) in veal calves was 10 days. However, the hepatic flunixin concentration in veal calves at slaughter must be undetectable because flunixin is not approved for use in calves. Therefore, the time required for the hepatic flunixin concentration to decrease to < 0.313 µg/mL (the LOD of the currently used FSIS analytic method for flunixin[31]) was calculated, and the estimated slaughter withdrawal interval was 13 days. Unfortunately, we only had actual hepatic flunixin concentrations for calves up to 5 days after administration of the last dose of flunixin, and extrapolation of data beyond that point was subject to substantial variability. Thus, on the basis of the data obtained in the present study, it was not possible to reliably predict when the hepatic flunixin concentration in all veal calves would become undetectable. For adult cattle that are slaughtered for human consumption, the marker tissue analyzed for flunixin residues is the liver, although muscle tissue may occasionally be tested for flunixin residues in addition to the liver in some cattle. For the calves of the present study, the calculated time for muscle flunixin concentrations to become undetectable was estimated as 6 days, which was less than the time required for hepatic flunixin concentrations to become undetectable.

Most calves raised for veal are not consumed in the United States. Over the past few decades, veal consumption in the United States has been declining, with the average American consuming only 0.14 kg of veal/y.[6] Most US veal is exported to other countries such as Japan, Mexico, and Canada.[6] Like the United States, those countries consider detectable concentrations of any drug used in an extralabel manner in edible tissue to be a violation, and the tolerance limit for flunixin in those tissues becomes less than the lowest concentration detectable by the currently used analytic method. Consequently, the estimated 13-day slaughter withdrawal interval for flunixin may not be sufficient for veal calves exported to foreign countries for slaughter because the analytic methods used to detect flunixin in those countries might be more sensitive than the method currently being used by the FSIS.

On the basis of the results of the present study, flunixin appears to have a slow terminal elimination phase from the tissues of veal calves following IV administration of 2.2 mg/kg, every 24 hours, for 3 days,

and detectable concentrations of the drug may persist in the tissues for several weeks after administration of the last dose. In the United States, the tolerance limit for flunixin in tissues of adult cattle cannot be used for veal calves because flunixin is not approved for use in unweaned calves; thus, the tolerance limit of flunixin in the tissues of veal calves becomes less than the lowest concentration of the drug detectable by the analytic methods currently being used by the FSIS. Given the pharmacokinetic parameters calculated for flunixin in the present study, we estimated that a slaughter withdrawal interval of 13 days would be sufficient for hepatic flunixin concentrations to become undetectable in veal calves. Unfortunately, calculation of that withdrawal interval required extrapolation of data beyond that actually observed. Thus, we were unable to accurately predict when hepatic flunixin concentrations would reliably become undetectable in all calves, and we recommend caution and the observation of an extended (ie, several week) slaughter withdrawal interval when flunixin is used in veal calves.

## Acknowledgments

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## Footnotes

a. Banamine, Merck Animal Health, Madison, NJ.
b. TurboVap LV evaporator, Zymark Corp, Hopkinton, Mass.
c. Acquity I class UPLC with an HSS T3 column (1.8 μm, 2.1 X 100 mm) and guard column, Waters Corp, Milford, Mass.
d. Xevo TQD tandem quadrupole mass spectrometer, Waters Corp, Milford, Mass.
e. Internal standards from Santa Cruz Biotechnology, Santa Cruz, Calif.
f. Bond Elut cartridge, Agilent Technologies, Lake Forest, Calif.
g. N-Evap, Organomation, Berlin, Mass.
h. Acquity UPLC-MS/MS, Waters Corp, Milford, Mass
i. Thermo TSQ Quantum Discovery Max tandem quadrupole mass spectrometer, Thermo Electron, West Palm Beach, Fla.
j. Phoenix, Pharsight Corp, St Louis, Mo.

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