Development of a physiologic-based pharmacokinetic model for estimating sulfamethazine concentrations in swine and application to prediction of violative residues in edible tissues

Jennifer L. Buur, DVM; Ronald E. Baynes, DVM, PhD; Arthur L. Craigmill, PhD; Jim E. Riviere, DVM, PhD

Objective—To develop a flow-limited, physiologic-based pharmacokinetic model for use in estimating concentrations of sulfamethazine after IV administration to swine.

Sample Population—4 published studies provided physiologic values for organ weights, blood flows, clearance, and tissue-to-blood partition coefficients, and 3 published studies provided data on plasma and other tissue compartments for model validation.

Procedure—For the parent compound, the model included compartments for blood, adipose, muscle, liver, and kidney tissue with an extra compartment representing the remaining carcass. Compartments for the N-acetyl metabolite included the liver and the remaining body. The model was created and optimized by use of computer software. Sensitivity analysis was completed to evaluate the importance of each constant on the whole model. The model was validated and used to estimate a withhold interval after an IV injection at a dose of 50 mg/kg. The withhold interval was compared to the interval estimated by the Food Animal Residue Avoidance Databank (FARAD).

Results—Specific tissue correlations for plasma, adipose, muscle, kidney, and liver tissue compartments were 0.93, 0.86, 0.99, 0.94, and 0.98, respectively. The model typically overpredicted concentrations at early time points but had excellent accuracy at later time points. The withhold interval estimated by use of the model was 120 hours, compared with 100 hours estimated by FARAD.

Conclusions and Clinical Relevance—Use of this model enabled accurate prediction of sulfamethazine pharmacokinetics in swine and has applications for food safety and prediction of drug residues in edible tissues. (*Am J Vet Res* 2005;66:1686–1693)

Sulfamethazine is an antimicrobial commonly used as a feed additive in swine production. It is currently labeled for use in the prevention and treatment of cervical abscesses, colibacillosis, swine dysentery, and bacterial pneumonia as well as for increased feed efficiency in swine with other diseases, such as atrophic rhinitis.[1] The FDA of the United States and other international regulatory agencies have set a residue tolerance limit for sulfamethazine at 0.1 µg/g in all edible swine tissues.[2] However in 2000, sulfamethazine represented the drug found most often as a cause of violative residues in swine.[3]

Under the provisions of AMDUCA, veterinarians in the United States are allowed to use drugs in an extralabel manner only when there is a valid veterinarian-client-patient relationship, the drug is used for therapeutic use, no other product is approved for use in that species, the drug is an FDA-approved human or animal drug, no violative residues in food will result, and the drug is not specifically prohibited. In addition, drugs used in this manner cannot be feed additives unless they are used in a minor species. To ensure that no violative residues are found in food, an appropriate extended withdrawal period must be specified by the attending veterinarian. The **Food Animal Residue Avoidance Databank (FARAD)** helps veterinarians provide these extended withdrawal times by applying principles of pharmacokinetics to scenarios for alternate routes and doses. Currently, this is accomplished through intensive literature searches, use of classical pharmacokinetic models, and use of techniques for modeling population pharmacokinetics.[4] Often there is insufficient information regarding absorption, distribution, metabolism, and excretion of a drug to allow for a scientifically accurate estimate of drug residues in tissues at specific time points after administration. When there is a lack of data on depletion of drug concentrations in tissues, the recommended withdrawal times provided by FARAD are based instead on depletion curves of plasma concentrations, which are the only data available to scale systemic drug exposure between label and extralabel dosing. In these cases, data would be unavailable to directly correlate drug depletion to tolerance concentrations allowed in tissues.[5,6]

Physiologic-based pharmacokinetic (PBPK) models are used to describe and predict the kinetics

Received January 21, 2005.
Accepted March 4, 2005.
From the Food Animal Residue Avoidance Databank, Center for Chemical Toxicology Research and Pharmacokinetics, Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606 (Buur, Baynes, Riviere); and the Food Animal Residue Avoidance Databank, Environmental Toxicology Extension, Department of Environmental Toxicology, College of Agriculture and Environmental Sciences, University of California, Davis, CA 95616 (Craigmill).
Supported in part by USDA-Cooperative State Research, Education and Extension Service grant No. 2002-45051-01362.
Address correspondence to Dr. Buur.

of xenobiotics on the basis of physiologic mechanisms by linking physiologic tissue blocks together via a communal plasma compartment. These models have been used in human medicine to predict therapeutic doses for chemotherapeutics or for drug development, and in toxicologic studies, they have been used for development of reference concentrations and doses.[7-10] They have also been used in conjunction with a pharmacodynamic study[11] to investigate mechanisms of action in novel or toxic compounds for which classical testing is insufficient. In veterinary medicine, PBPK models have been described for sulfathiazole administration to swine[12] and oxytetracycline administration to sheep and fish.[13,14]

The objectives of the study reported here were to develop and validate a PBPK model and to estimate sulfamethazine concentrations after IV administration in swine. We also applied the model to the prediction of extended withholding intervals for use in accordance with AMDUCA.

Materials and Methods

Development of a PBPK model—A flow-limited PBPK model was developed for predictive purposes. For predicting sulfamethazine concentrations, it consisted of tissue compartments for edible tissues (blood, muscle, adipose, liver, and kidney) and a single compartment representing the remainder of the carcass. Additional compartments for the N-acetyl metabolite were created and included compartments for the liver and blood specifically and a generalized compartment representing the remainder of the body. No concentration-versus-time data were available for the carcass compartment or for tissue concentrations of the N-acetyl metabolite. Thus, a model with 9 compartments was developed (**Figure 1**).

Physiologic constants of organ volume, tissue blood volume, and blood flow for market-weight pigs were obtained from published reports (**Appendix 1**).[11,15-17] The density of plasma was assumed to be 1 g/mL. Physicochemical constants of tissue-to-blood partition coefficients were calculated from published values.[18-21] Hepatic blood flow was modeled as the combination of hepatic arterial and portal circulations. Other biological constants were also included in the model.

Values for renal and hepatic clearance were based on kinetic data published elsewhere.[19,22,23] The main pathway of metabolism in pigs is direct acetylation of sulfamethazine to the N4-acetyl metabolite. Michalis-Menton kinetics were not incorporated into this model because it has been reported[24] that clinically relevant doses do not saturate the hepatic acetyl-transferase enzyme; thus, zero-order kinetics are not needed. Therefore, hepatic clearance was modeled by use of a linear excretion constant. Enterohepatic recycling of the parent compound was considered insubstantial and not included in the model. Because the deacetylation of the N-acetyl metabolite can increase plasma concentrations of the parent compound,[25] we incorporated this aspect into the model. Compartments for the N-acetyl metabolite were linked to the model for the parent compound through the liver because these reactions mainly take place in hepatocytes. Renal clearance is mainly through filtration. Because there is no evidence of active secretion, renal clearance was modeled by use of a first-order excretion constant. Starting values for hepatic and renal clearance were the mean of the published values.

Protein binding was incorporated into the model by the inclusion of a mass-balance equation within the blood compartment. The fraction of unbound drug was calculated and then applied to the total mass of drug within the plasma compartment. Binding of drug within tissues was not incorporated into the model.

A homogenization term was created that combined tissue concentration with tissue blood concentration. This represented the process used in quantitative analysis of tissue samples whereby the total drug within the sample is directly related to the proportion of tissue blood and tissue within the sample. The amount of drug within the vascular space was

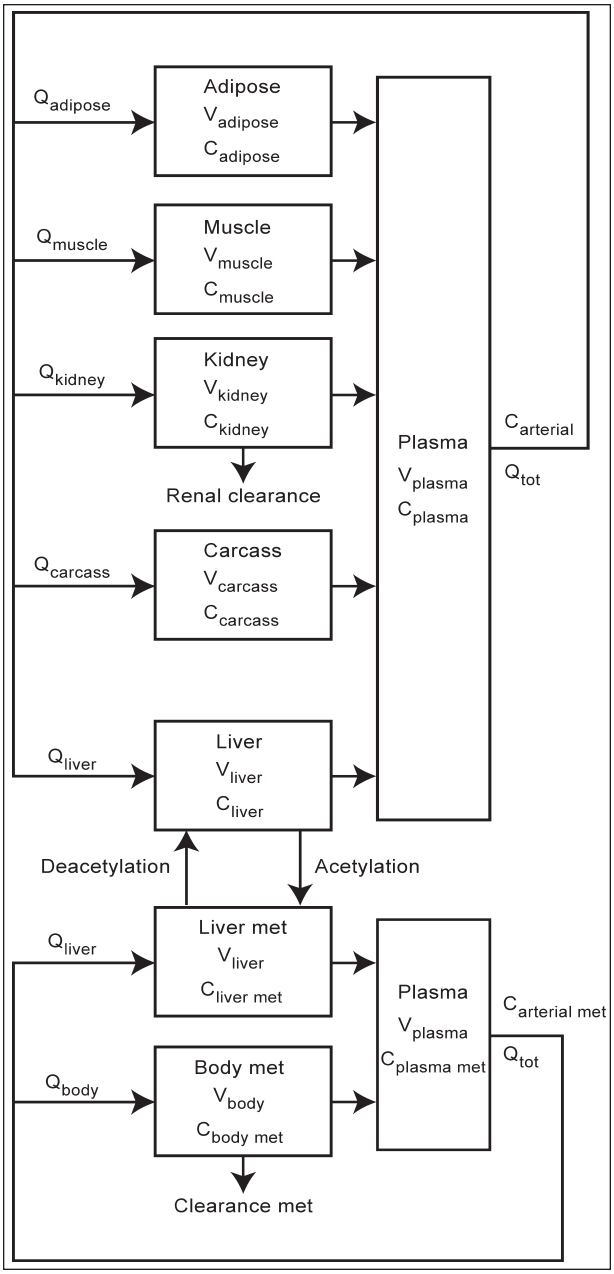

Figure 1—Schematic representation of a physiologic-based pharmacokinetic model for use in determining concentrations after IV injection of sulfamethazine in swine. Notice the tissue volume ($V_{adipose}$, V_{muscle}, V_{kidney}, V_{plasma}, $V_{carcass}$, V_{liver}, and V_{body}, respectively) and tissue concentration ($C_{adipose}$, C_{muscle}, C_{kidney}, C_{plasma}, $C_{arterial}$, $C_{carcass}$, C_{liver}, $C_{plasma\ met}$, $C_{liver\ met}$, and $C_{body\ met}$, respectively) within each tissue compartment and tissue blood flow ($Q_{adipose}$, Q_{muscle}, Q_{kidney}, $Q_{carcass}$, Q_{liver}, and Q_{body}, respectively) for each compartment. Q_{tot} = Cardiac output. Met = Metabolite.

Table 1—Starting and final values and upper and lower limits for constants of a model used to determine concentrations after IV administration of sulfamethazine to pigs.

Constant	Lower limit	Starting value	Upper limit	Final value
Hepatic clearance (mL/[min • kg])	0.05	0.078	3	0.62
Renal clearance (mL/[min • kg])	0.01	0.59	4	0.03
Protein binding (%)	0.5	0.64	1	0.57
Tissue-to-blood partition coefficient				
Adipose	0.01	0.1	5	0.336
Kidney	0.01	0.38	5	1.68
Liver	0.01	0.4	5	0.378
Muscle	0.01	0.17	5	0.08
Blood flow (% cardiac output)				
Liver	0.01	0.24	0.5	0.38
Kidney	0.01	0.1	0.5	0.1188
Muscle	0.01	0.25	0.5	0.25
Adipose	0.01	0.08	0.5	0.08
Acetyl metabolite protein binding (%)	0.5	65	1	0.57
Acetyl metabolite liver tissue-to-blood partition coefficient	0.01	0.23	5	0.079
Acetyl metabolite body tissue-to-blood partition coefficient	0.01	1	5	1.297
Rate of deacetylation (/h)	0.01	0.357	5	3.66
Acetyl metabolite clearance (mL/[min · kg])	0.01	0.5	5	2.558

then calculated. The concentration of the total homogenized sample concentration was expressed by use of the following equation:

$$C_{tissue\text{-}hs} = (Vbc_{tissue} \times Ctb) + ([1 - Vbc_{tissue}] \times C_{tissue}),$$

where $C_{tissue\text{-}hs}$ is the concentration of sulfamethazine in the homogenized sample, Vbc_{tissue} is the volume of the vascular space for a given tissue (reported as a percentage of organ weight), Ctb is the concentration of sulfamethazine in the tissue blood, and C_{tissue} is the concentration of sulfamethazine in the tissue.

Model simulations were solved by use of a commercially available computer program[a] that was equipped with a graphic modulator for model development and an optimizer for sensitivity, determination of constants, and prediction analysis. Differential equations were used to describe the rate of change in mass in each compartment (**Appendix 2**).

Sensitivity analysis and optimization of constants—Data used for optimization of the N-acetyl metabolite distribution were obtained from 2 studies.[18,22] Data used for sensitivity and optimization of sulfamethazine were obtained from a single study.[22] Optimization of constants was limited to mean (n = 12) plasma concentration data. Sensitivity analysis was performed for several constants, including hepatic clearance, renal clearance, protein binding, tissue-to-blood partition coefficients (muscle, adipose, liver, and kidneys), and blood flow (muscle, adipose, liver, and kidneys). Final determination and optimization of constants were accomplished for blood flows to compartments (liver, kidneys, muscle, and adipose), hepatic clearance, renal clearance, protein binding, and partition coefficients (muscle, liver, kidneys, and adipose) for sulfamethazine. Determination and optimization of constants were accomplished for N-acetyl protein binding, N-acetyl deacetylation rate, renal clearance, and partition coefficients (liver and body). Model constants were adjusted to best fit the curve by use of a maximum-likelihood estimation algorithm. Limits were set to ensure biologically plausible values.

Model validation—The model was validated by comparison with an external data set created from published studies and estimates included in the FARAD database. Studies were excluded from comparison on the basis of assay methods and physiologic status of the pigs. Excluded studies incorporated colorimetric analysis of drug concentrations, general anesthesia, and experimentally infected pigs. The resulting data set encompassed 3 studies[18,19,23] performed separately by 3 research groups. Pigs ranged in weight from 18 to 32 kg. Dosages ranged from 20 to 50 mg/kg. Sulfamethazine concentrations were reported for plasma, muscle, kidney, liver, and adipose tissues. Datum points represented the means of values reported in studies and were obtained by use of a data extraction program.[b] Mean values in our study were calculated from 6, 7, and 3 samples/data point for each of the 3 studies, respectively.[18,19,23]

Table 2—Results for model validation accomplished by comparison of predicted concentrations to observed concentrations by use of an external data set.

Tissue	Slope (m)	Intercept (b)	R^2
Plasma	0.7358	5.498	0.9286
Kidney	0.2469	4.8166	0.9422
Liver	0.4082	2.1849	0.9792
Muscle	1.4278	1.0028	0.9945
Adipose	0.3338	1.3145	0.8554

Slope (m) and intercept (b) for the regression line y = mx + b.
R^2 = Correlation coefficient.

Predicted values for tissue concentrations were reported as a combination of tissue and tissue blood samples that would mimic the homogenization process used in analysis of tissue samples. Simulated predicted values were compared with values for observed data points, and regression lines were plotted. Linear regression correlation values were calculated. Residual plots were also created and evaluated for spread and distribution. All simulations were adjusted on the basis of dosage.

Application of model to determine interval to prevent violative residues in tissues—The optimized model was then used to determine a reasonable tissue-withhold interval after IV administration of sulfamethazine at a dosage of 50 mg/kg. This was compared with the value currently provided by FARAD to practitioners. Currently, FARAD estimates a withhold interval by use of 10 times the plasma half-life of sulfamethazine, an approach that is appropriate for many drug classes.

Results

Because sulfamethazine is a small polar molecule with small tissue-to-blood partition coefficients, a dif-

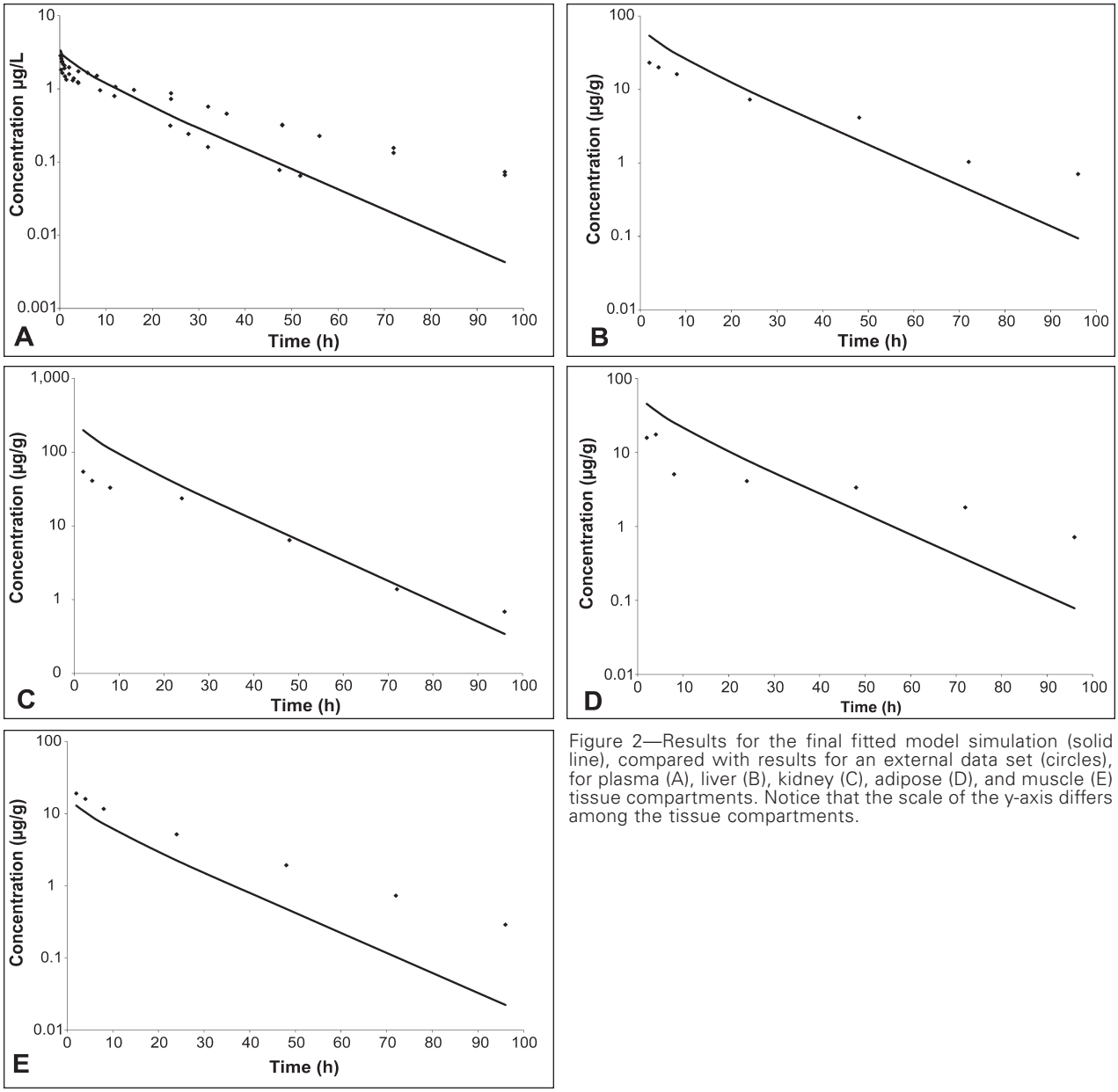

Figure 2—Results for the final fitted model simulation (solid line), compared with results for an external data set (circles), for plasma (A), liver (B), kidney (C), adipose (D), and muscle (E) tissue compartments. Notice that the scale of the y-axis differs among the tissue compartments.

fusion-limited model was also created (data not shown) to determine whether blood flow or permeability would be rate limiting. The diffusion-limited model did not significantly increase the predictive power of the model; therefore, the simpler flow-limited model was accepted.

Sensitivity analysis revealed that hepatic clearance, renal clearance, and protein binding of sulfamethazine were the most important constants included in the model. Because of the lack of available tissue data, it is quite possible that the partition coefficients did not reveal their true sensitivity and importance within this model. Results of the optimization for blood flows, renal clearance, hepatic clearance, protein binding, and tissue-to-blood partition coefficients for sulfamethazine and its N-acetyl metabolite were determined (**Table 1**).

When compared with means of external data sets, the model had correlations for plasma, kidney, liver, muscle, and adipose tissues of 0.93, 0.94, 0.99, 0.99, and 0.86, respectively. Results for validation of the model were determined (**Table 2**). Residual analysis revealed a pattern of overprediction of values at early time points in all tissues with the exception of muscle, in which the model underpredicted values at all time points. However, good accuracy was found at the terminal time points. Results of the resulting simulations were plotted (**Figure 2**). Results of the residual analysis and validation procedure were also plotted (**Figures 3 and 4**).

Depletion curves of edible tissues after IV administration of sulfamethazine at a dosage of 50 mg/kg, in relation to the tolerance value of 0.1 µg/g, were plotted (**Figure 5**). These depletion curves represented the 50th percentile of the population, not the upper limit of the 95% confidence interval of the 99th percentile of the population needed to satisfy regulatory agencies such as the FDA–Center for Veterinary Medicine. The

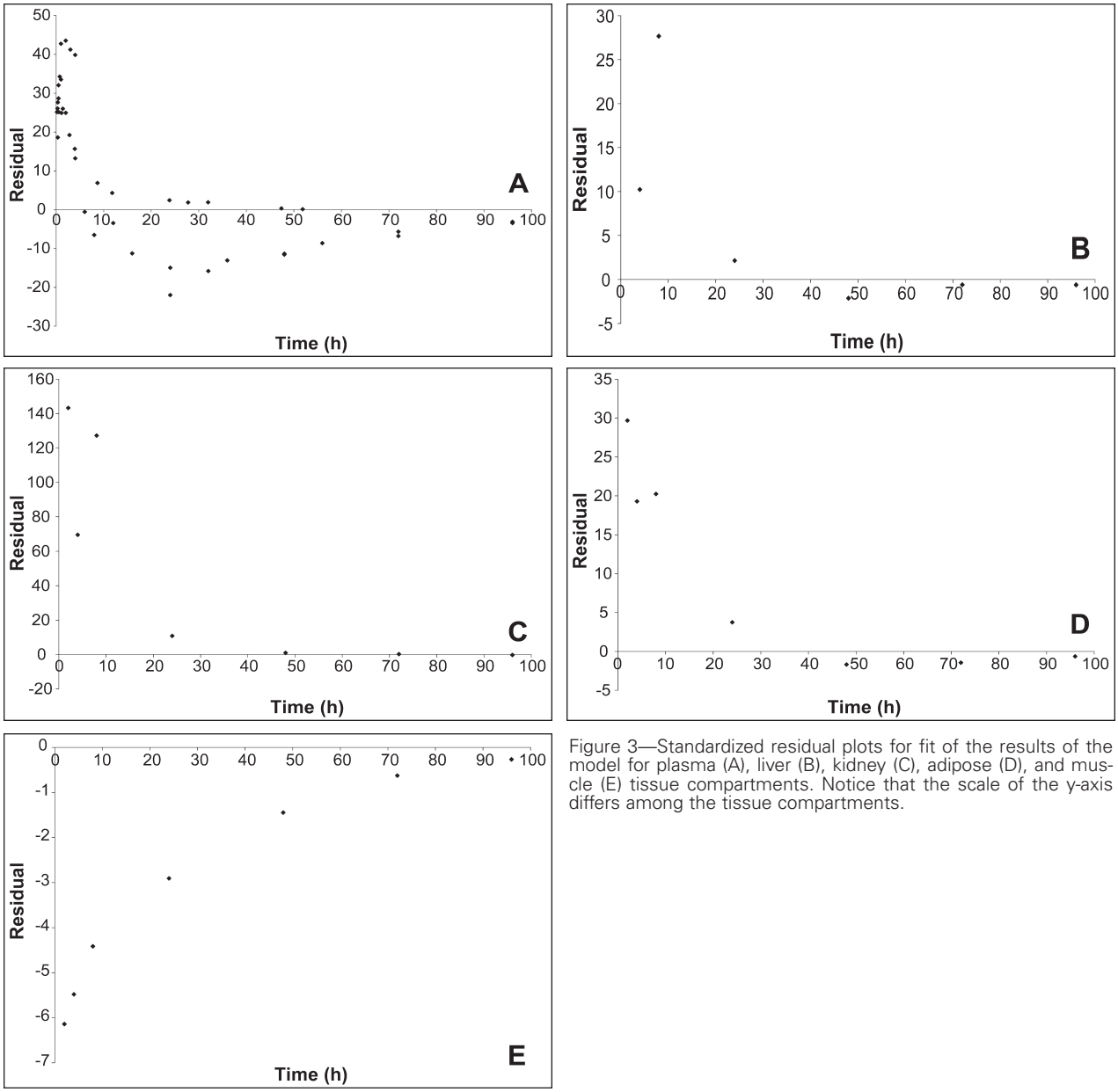

Figure 3—Standardized residual plots for fit of the results of the model for plasma (A), liver (B), kidney (C), adipose (D), and muscle (E) tissue compartments. Notice that the scale of the y-axis differs among the tissue compartments.

model was used to predict that all tissue concentrations would be below the tolerance value of 0.1 µg/g by 120 hours after injection and that plasma concentrations would be below the tolerance value by 109 hours after injection. The longest duration for sulfamethazine concentrations above the tolerance value was seen for kidney tissues. Because this would constitute an extralabel use of sulfamethazine, there currently is no withdrawal time listed on an FDA-approved product. The current FARAD recommendation is 100 hours and is determined on the basis of a value that is 10 times the plasma half-life.

Discussion

We developed and validated a model for plasma and tissue pharmacokinetics of sulfamethazine after IV administration to swine. To increase the accuracy and robustness of the model, several techniques were used.

Addition of the N-acetyl metabolite and its deacetylation back to the parent compound was incorporated in the model, which was found to increase the accuracy of concentrations at later time points. The importance of this metabolic pathway differs among sulfonamides. In 1 study,[25] investigators documented that this is an important metabolic pathway for sulfamonomethoxine and sulfamethazine but that the deacetylation pathway has less importance for sulfadiazine. Another model for sulfathiazol did not include the deacetylation metabolic pathway.[12] However, it is unknown whether the pharmacokinetics of sulfathiazol are influenced by the deacetylation pathway.

A homogenization term was used to calculate the concentration of sulfamethazine in specific tissues that accounted for the techniques used experimentally to determine those specific tissue concentrations. Techniques used to calculate tissue concentrations

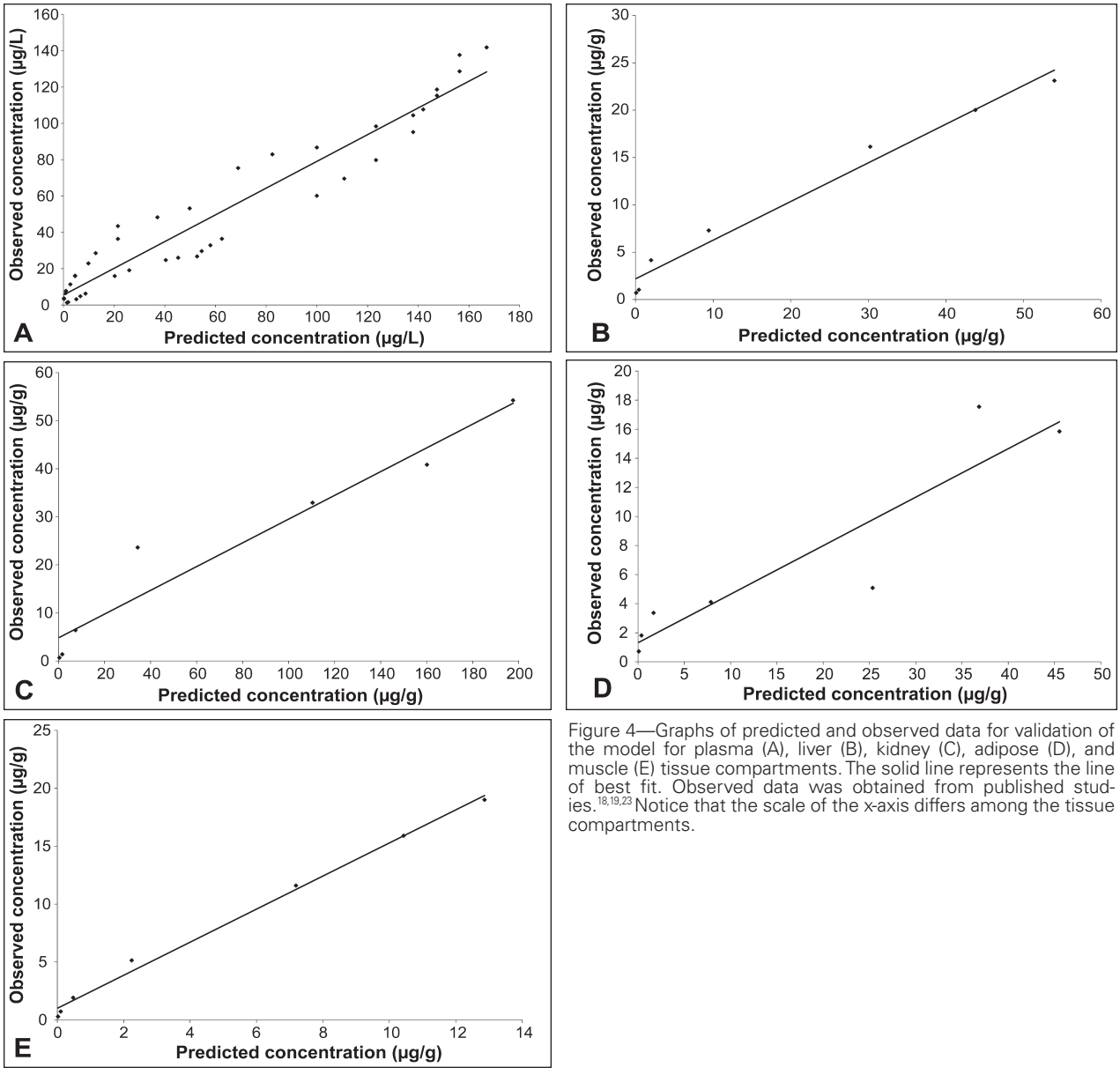

Figure 4—Graphs of predicted and observed data for validation of the model for plasma (A), liver (B), kidney (C), adipose (D), and muscle (E) tissue compartments. The solid line represents the line of best fit. Observed data was obtained from published studies.[18,19,23] Notice that the scale of the x-axis differs among the tissue compartments.

require the homogenization of tissue blood along with cellular tissue. Because of the relatively larger mass of drug found in blood relative to the cellular tissues themselves, artificially increased tissue concentrations may result for drugs with small partition coefficients.[26] Inclusion of the homogenization term increased correlation values and the resulting fit of data (data not shown), especially at terminal end points at which more drug is contained in the plasma compartment than in the cellular tissue matrix.

Protein binding was an important aspect of drug distribution in this model. Generally, when a drug is not considered to be highly protein bound (binding > 90%), this aspect is neglected to simplify the model.[27] In another study,[28] investigators also stated that changing the free fraction of a drug does not result in a change of the free concentration of drug when the drug is at a steady state. However, the model described here does not consider the drug at a steady state. It was seen

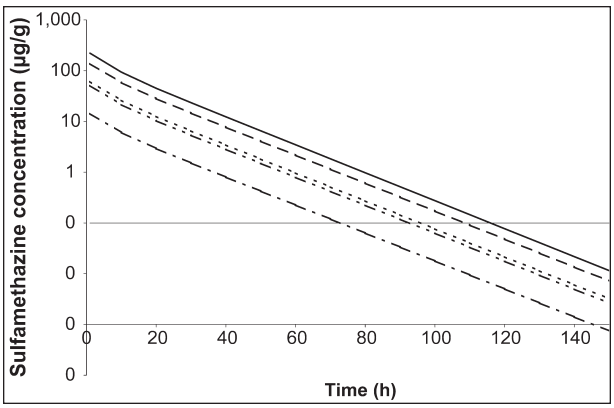

Figure 5—Predicted depletion curves for tissue concentrations of drug in adipose (dot-dash-dot line), kidney (solid line), liver (dashed line), muscle (dot-dot-dash line), and plasma (dotted line) tissue compartments after IV administration of sulfamethazine at a dosage of 50 mg/kg in swine. The horizontal line represents the tolerance limit of 0.1 µg/g.

in the results for our model that moderate protein binding of 57% can have an influence on the kinetics of sulfamethazine, which may prolong tissue concentrations and increase the likelihood for violative residues in tissues (data not shown). This phenomenon deserves additional study, and these findings deserve further study and confirmation.

The sensitivity analysis and optimization of constants were based on plasma concentrations rather than tissue concentrations. This was attributable to a lack of available tissue data. Because we did not optimize constants for each specific tissue compartment, sensitivity of the model to partitioning coefficients was not observed. Instead, the analysis focused on constants (ie, protein binding and clearance) that would greatly change plasma concentration. It could be expected that with more tissue data, the partitioning coefficients could be further refined without much change to the overall plasma concentrations. This, in turn, could help with accuracy within the tissue compartments.

All tissue correlations were excellent. Only adipose tissue had a correlation coefficient < 0.9. The accuracy of this model could be improved by use of individual data points rather than use of means for comparisons. This was not done because the individual data points were not reported in any of the studies used for optimization or validation. Unfortunately, SD values were also not reported in those studies. Thus, individual variability was essentially ignored in the observed-to-predicted comparisons. Also, pigs used in the studies were not of market weight, and the model was designed for use in market-weight swine. This would change relative body mass and blood flow distributions. However, sensitivity and optimization of blood flows did not reveal any substantial inaccuracies in these measurements. Age changes could also alter the relative clearance of sulfamethazine. The acetylation-deacetylation metabolic pathway could be changed by induction of enzymes or increases in intrinsic enzymatic activity. Changes in renal clearance could develop over time because of changes in glomerular filtration rate. With the high sensitivity of the model to changes in clearance, age-related changes in clearance could impact the accuracy of the model.

Further refinement of partitioning coefficients could also improve accuracy. However, that would require a more robust data set for tissue concentrations that is not available in the published studies. We chose not to include the only set of tissue data in the optimization process so that the validation would reflect a purely external data set. Even with these limitations, the model reported here could be used to accurately predict plasma and tissue concentrations after administration of several doses.

The withhold time predicted by use of the model is greater than what would currently be recommended by FARAD because the model takes into account specific tolerance concentrations and tissue concentrations rather than extrapolating to 99.98% excretion by use of 10 times the plasma half-life. Because the kidneys actually retain sulfamethazine longer than does plasma, predicting a withhold time on the basis of plasma concentrations would underpredict the true distribution of sulfamethazine within a pig. Studies such as this are required to determine those drugs for which simple rules of thumb or other half-life multipliers are applicable.[6]

In the study reported here, we developed a PBPK model for sulfamethazine after IV injection in pigs. We used this model to accurately predict an appropriate extended withdrawal interval to prevent violative residues in edible tissues. In contrast to compartmental pharmacokinetic models for sulfamethazine, the model reported here can be used to provide predictions for a range of doses and yields mechanistic information relating to drug distribution to tissues and pharmacokinetics. Use of this model emphasized the relationship between drug distribution in tissues and protein binding as well as the acetylation-deacetylation metabolic pathway. This model also provided information about pharmacokinetics for specific tissues that are often not included in compartmental or population pharmacokinetic models. This model can be further refined to include long-term oral administration and more specific protein-binding kinetics. As more population information becomes known, statistical inferences can be made by use of Monte Carlo analysis or bootstrapping techniques that would parallel the power inherent in population pharmacokinetic models yet still provide physiochemical mechanistic information. The model can then be used to study additional physiologic mechanisms and drug distributions that may explain the reason that we still have instances of violative residues in edible tissues.

a. ACSLxtreme, version 1.4, Aegis Technologies Group Inc, Huntsville, Ala.
b. UN-SCAN-IT, version 6.0, Silk Scientific Inc, Orem, Utah.

References

1. Entriken TL. Product monographs. In: Valcarcel M, eds. *Veterinary pharmaceuticals and biologics*. 12th ed. Lenexa, Kan: Veterinary Healthcare Communications, 2001;2114–2115.

2. FDA. Title 21 Code of Federal Regulations 556.670.

3. USDA, Food Safety Inspection Service. Section 3. *2000 FSIS national residue program data*. Washington, DC: USDA, 2001;14-27. Available at: www.fsis.usda.gov/OPHS/red2000. Accessed June 12, 2002.

4. Martín-Jiménez T, Baynes RE, Craigmill A, et al. Extrapolated withdrawal-interval estimator (EWE) algorithm: a quantitative approach to establishing extralabel withdrawal times. *Regul Toxicol Pharmacol* 2002;36:131–137.

5. Damian P, Craigmill A, Riviere J. Breaking new ground. *J Am Vet Med Assoc* 1997;210:633–634.

6. Gehring R, Baynes RE, Craigmill AL, et al. Feasibility of using half-life multipliers to estimate extended withdrawal intervals following the extralabel use of drugs in food-producing animals. *J Food Prot* 2004;67:555–560.

7. Gentry PR, Covington TR, Clewell HJ III. Application of a physiologically based pharmacokinetic model for reference dose and reference concentration estimation for acetone. *J Toxicol Environ Health A* 2003;66:2209–2225.

8. Clewell HJ III, Gentry PR, Gearhart JM. Investigation of the potential impact of benchmark dose and pharmacokinetic modeling in noncancer risk assessment. *J Toxicol Environ Health* 1997;52:475–515.

9. Charnick SB, Kawai R, Nedelman JR, et al. Perspectives in pharmacokinetics. Physiologically based pharmacokinetic modeling as a tool for drug development. *J Pharmacokinet Biopharm* 1995;23:217–229.

10. Grass GM, Sinko PJ. Physiologically-based pharmacokinetic simulation modeling. *Adv Drug Deliv Rev* 2002;54:433–451.

11. Kawai R, Lemaire M, Steimer JL, et al. Physiologically based pharmacokinetic study on a cyclosporin derivative, SDZ IMM 125. *J Pharmacokinet Biopharm* 1994;22:327–365.

12. Duddy J, Hayden TL, Bourne DWA, et al. Physiological model for distribution of sulfathiazole in swine. *J Pharm Sci* 1984;73:1525–1528.

13. Craigmill AL. A physiologically based pharmacokinetic model for oxytetracycline residues in sheep. *J Vet Pharmacol Ther* 2003;26:55–63.

14. Brocklebank JR, Namdari R, Law FC. An oxytetracycline residue depletion study to assess the physiologically based pharmacokinetic (PBPK) model in farmed Atlantic salmon. *Can Vet J* 1997;38:645–646.

15. Tranquilli J, Parks CM, Thurman JC, et al. Organ blood flow and distribution of cardiac output in nonanesthetized swine. *Am J Vet Res* 1982;43:895–897.

16. Lundeen G, Manohar M, Parks C. Systemic distribution of blood flow in swine while awake and during 1.0 and 1.5 MAC isoflurane anesthesia with or without 50% nitrous oxide. *Anesth Analg* 1983;62:499–512.

17. Pond WG, Mersmann HJ. *Biology of the domestic pig*. Ithaca, NY: Cornell University Press, 2001.

18. Nouws JFM, Vree TB, Baakman M, et al. Pharmacokinetics, renal clearance, tissue distribution, and residue aspects of sulphadimidine and its N4-acetyl metabolite in pigs. *Vet Q* 1986;8:123–135.

19. Sweeney RW, Bardalaye PC, Smith CM, et al. Pharmacokinetic model for predicting sulfamethazine disposition in pigs. *Am J Vet Res* 1993;54:750–754.

20. Haasnoot W, Korsrud GO, Cazemier G, et al. Application of an enzyme immunoassay for the determination of sulphamethazine (sulphadimidine) residues in swine urine and plasma and their use as predictors of the level in edible tissue. *Food Addit Contam* 1996;13:811–821.

21. Mitchell AD, Paulson GD. Depletion kinetics of ^{14}C-sulfamethazine {4-amino-N-(4,6-dimethyl-2-pyrimidinyl)benzene[U-^{14}C]sulfonamide} metabolism in swine. *Drug Metab Dispos* 1986;14:161–165.

22. Nouws JFM, Mevius D, Vree TB, et al. Pharmacokinetics and renal clearance of sulphadimidine, sulphamerazine and sulphadiazine and their N4-acetyl and hydroxy metabolites in pigs. *Vet Q* 1989;11:78–86.

23. Yuan ZH, Miao XQ, Yin YH. Pharmacokinetics of ampicillin and sulfadimidine in pigs infected experimentally with *Streptococcus suum*. *J Vet Pharmacol Ther* 1997;20:318–322.

24. Mengelers MJB, Kleter GA, Hoogenboom LAP, et al. The biotransformation of sulfadimethoxine, sulfadimidine, sulfamethoxazole, trimethoprim and aditoprim by primary cultures of pig hepatocytes. *J Vet Pharmacol Ther* 1997;20:24–32.

25. Shimoda M, Okamoto K, Sikazwe G, et al. Deacetylation as a determinant of sulphonamide pharmacokinetics in pigs. *Vet Q* 1997;19:186–191.

26. Khor SP, Mayersohn M. Potential error in the measurement of tissue to blood distribution coefficients in physiological pharmacokinetic modeling. Residual tissue blood. I. Theoretical considerations. *Drug Metab Dispos* 1991;19:478–485.

27. Munsey T, Grigg RE, McCormack A, et al. Binding of sulphamethazine to pig plasma proteins and albumin. *J Vet Pharmacol Ther* 1996;19:135–141.

28. Toutain PL, Bousquet-Melou A. Free drug fraction vs free drug concentration: a matter of frequent confusion. *J Vet Pharmacol Ther* 2002;25:460–463.

Appendix 1

Organ weights and blood flow distributions for market-weight swine determined from published studies.

Organ	Blood flow[14,15] (percentage of cardiac output)*	Organ weight[16] (percentage of body weight)	Vascular space[11] (percentage of organ weight)
Adipose	0.08	0.340	0.040
Kidneys	0.10	0.004	0.105
Liver	0.24†	0.020	0.115
Muscle	0.25	0.400	0.026
Carcass‡	0.33	0.176	ND
Blood	1.00	0.060	ND

*Mean cardiac output determined in 3 studies[14-16] was 12 L/kg/h, and PCV was 33%.[15] †Value incorporates hepatic arterial and portal circulations. ‡Values calculated as 1 minus the sum of values for the other organs. ND = Not determined.

Appendix 2

Differential equations used to describe the rate of change of sulfamethazine in each tissue compartment.

Tissue compartment	Equation
Muscle	$dC_{muscle}/dt = ([C_{arterial} - \{C_{muscle}/P_{muscle}\}] \times Q_{muscle})/V_{muscle}$
Adipose	$dC_{adipose}/dt = ([C_{arterial} - \{C_{adipose}/P_{adipose}\}] \times Q_{adipose})/V_{adipose}$
Carcass	$dC_{carcass}/dt = ([C_{arterial} - \{C_{carcass}/P_{carcass}\}] \times Q_{carcass})/V_{carcass}$
Kidney	$dC_{kidney}/dt = ([C_{arterial} - \{C_{kidney} \times P_{kidney}\}] \times [Q_{kidney} - \{C_{arterial} \times Cl_{renal}\}])/V_{kidney}$
Liver	$dC_{liver}/dt = (([C_{arterial} - \{C_{liver}/P_{liver}\}] \times Q_{liver}) - (C_{liver} \times Cl_{acetylation}) + (C_{liver\ metabolite} \times Cl_{deacetylation}))/V_{liver}$
Plasma	$dC_{plasma}/dt = (([C_{muscle}/P_{muscle}] \times Q_{muscle}) + ([C_{adipose}/P_{adipose}] \times Q_{adipose}) + ([C_{kidney}/P_{kidney}] \times Q_{kidney}) + ([C_{liver}/P_{liver}] \times Q_{liver}) + ([C_{carcass}/P_{carcass}] \times Q_{carcass}) + IV\ dose - (C_{plasma} \times Q_{tot}))/V_{plasma}$

dC_{muscle}, $dC_{adipose}$, $dC_{carcass}$, dC_{kidney}, dC_{liver}, and dC_{plasma} = Change in concentration of sulfamethazine in each tissue compartment, respectively. dt = Change in time. $C_{arterial}$, C_{muscle}, $C_{adipose}$, $C_{carcass}$, C_{kidney}, C_{liver}, and C_{plasma} = Concentration of sulfamethazine in each tissue compartment, respectively. P_{muscle}, $P_{adipose}$, $P_{carcass}$, P_{kidney}, and P_{liver} = Tissue-to-blood partition coefficient for each tissue compartment, respectively. Q_{muscle}, $Q_{adipose}$, $Q_{carcass}$, Q_{kidney}, and Q_{liver} = Blood flow to each tissue compartment, respectively. V_{muscle}, $V_{adipose}$, $V_{carcass}$, V_{kidney}, V_{liver}, and V_{plasma} = Tissue volume for each tissue compartment, respectively. Cl_{renal}, $Cl_{acetylation}$, and $Cl_{deacetylation}$ = Clearance for each method (renal, acetylation, and deacetylation, respectively). $C_{liver\ metabolite}$ = Concentration of N-acetyl metabolite. IV dose = Total amount of drug injected. Q_{tot} = Cardiac output.