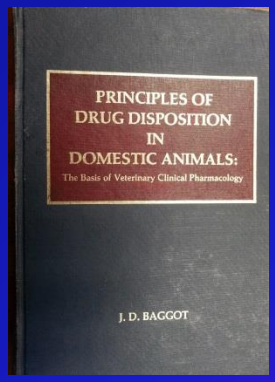
Food Safety: The Intersection of Pharmacometrics and Veterinary Medicine

Jim E. Riviere, DVM, PhD, DSc (hon)
MacDonald Endowed Chair in Veterinary Medicine
Kansas Biosciences Eminent Scholar
University Distinguished Professor

Director, Institute of Computational Comparative Medicine
-A Certara Phoenix Center of Excellence-
Kansas State University
Manhattan, Kansas USA

Veterinary Medicine

- Pharmacokinetic has been a component of veterinary medicine since the 1970's.
- Applications and endpoints are very similar to human practice: pharmacodynamic and toxicodynamic approaches.
- Animal Health encompasses dogs, cats and other small pets, horses, zoo animals, wildlife and the major food producing species: cattle, swine, poultry and farm-raised fish.



The difference is that in food producing animals, we eat our patients or their products!

Overview

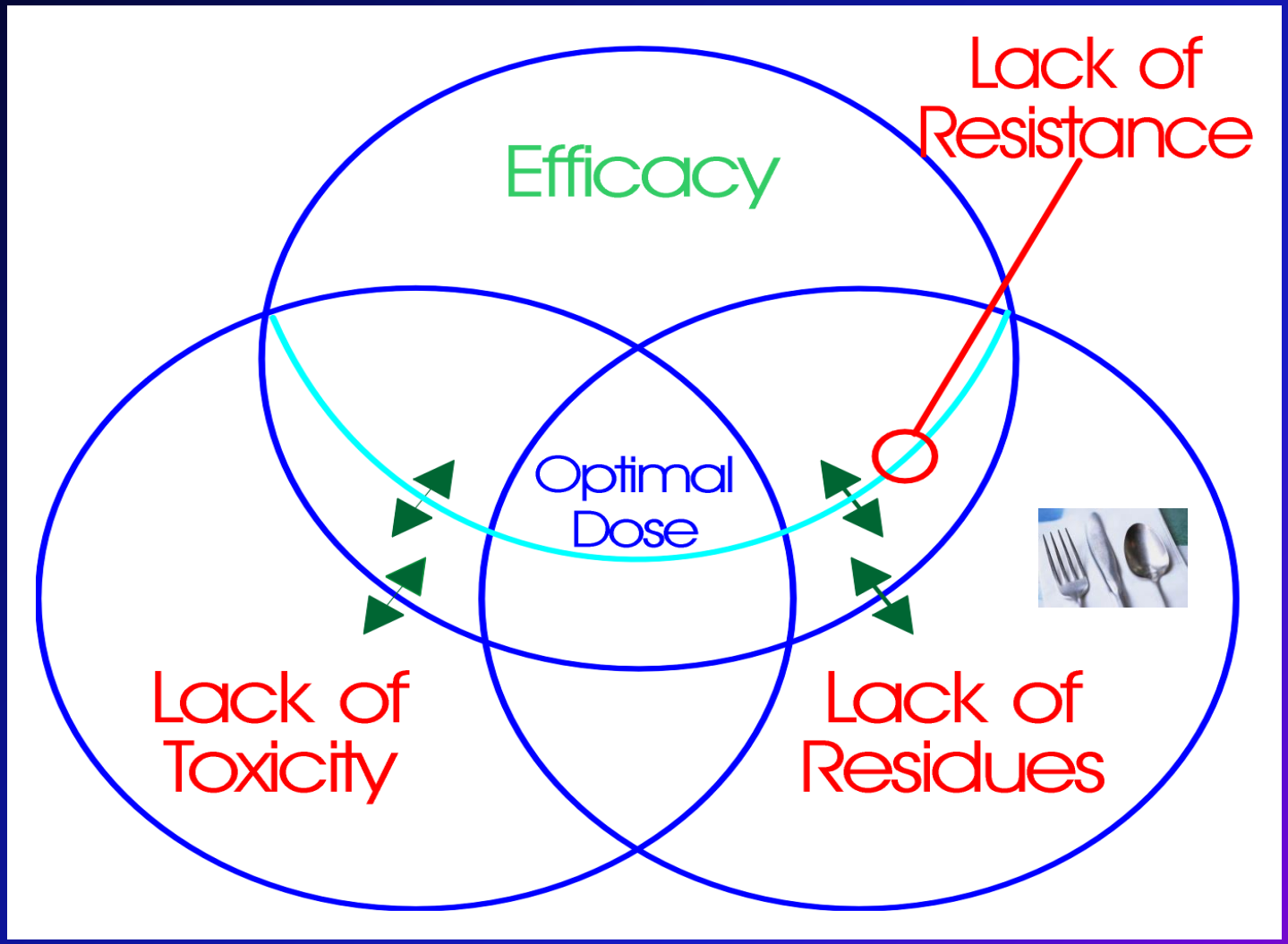
- Presentation will focus on food animal pharmacology because of its implications to human food safety.
- Present some concepts of tissue residue PK gleaned from 3 decades of dealing with field cases of drug and chemical residues handled by the U.S. FARAD risk-management program; an academic consortium of Kansas State University, University of California-Davis, University of Florida-Gainesville and North Carolina State University.

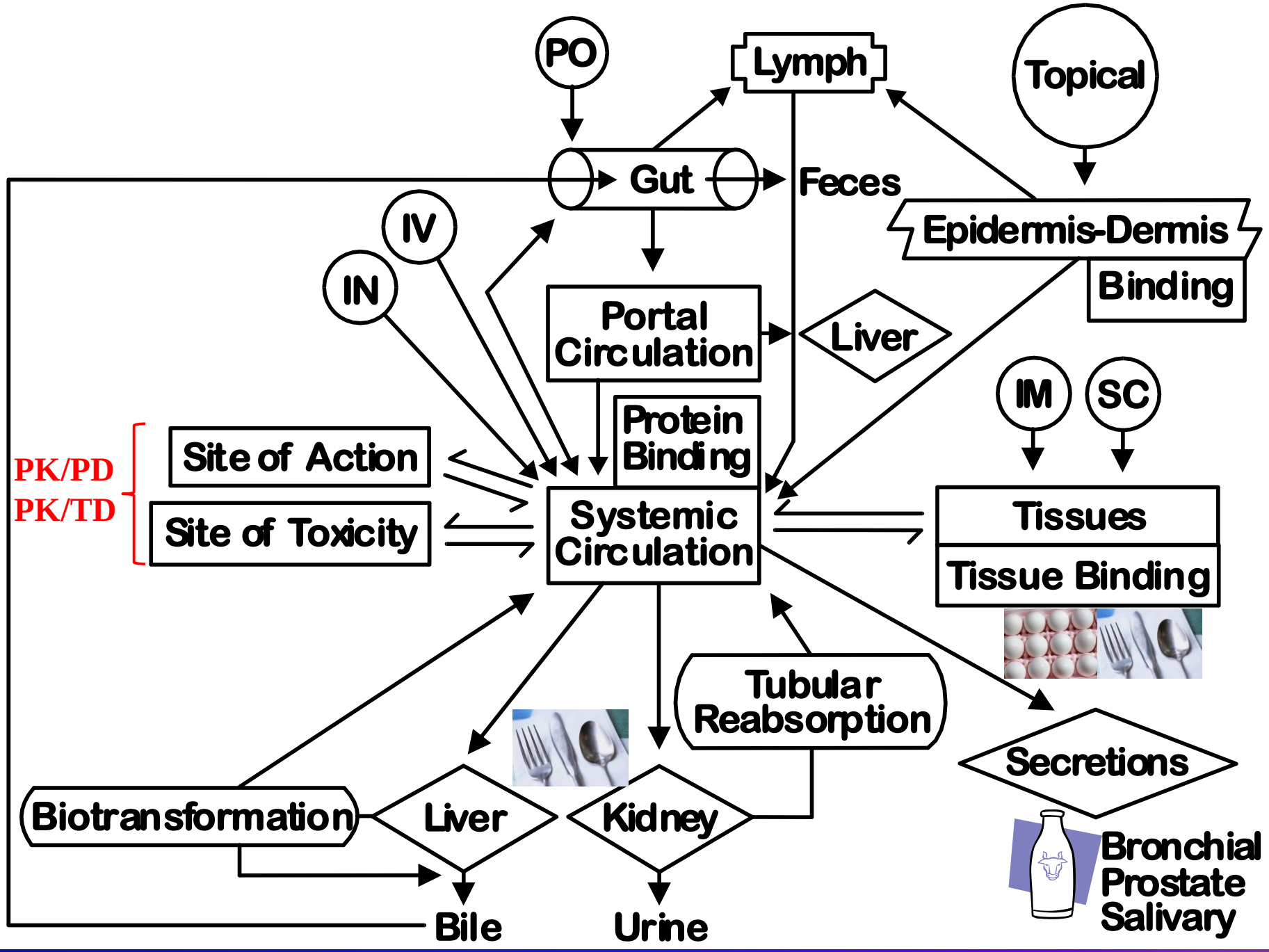
FARAD™

Food Animal Residue Avoidance and Depletion Program
1982 – 2013

www.FARAD.org

Food Animal Antimicrobial Pharmacometric Endpoints

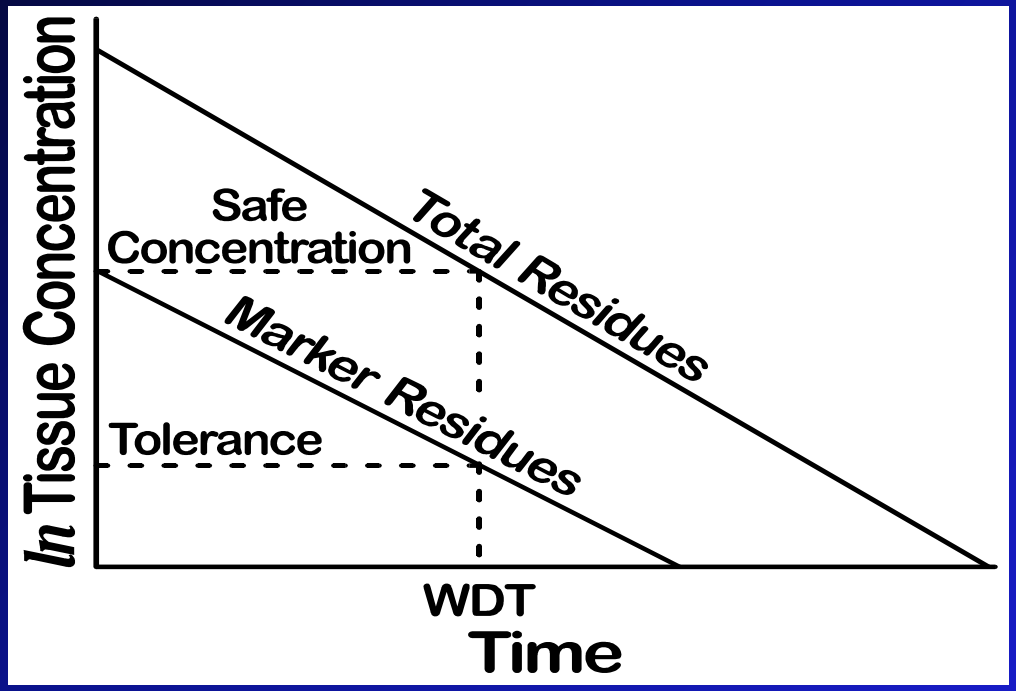



PO
Lymph
Topical
Gut
Feces
IV
IN
Epidermis-Dermis
Binding
Portal Circulation
Liver
IM
SC
Protein Binding
PK/PD
PK/TD
Site of Action
Site of Toxicity
Systemic Circulation
Tissues
Tissue Binding
Tubular Reabsorption
Secretions
Biotransformation
Liver
Kidney
Bronchial
Prostate
Salivary
Bile
Urine

Concept of Withdrawal Time

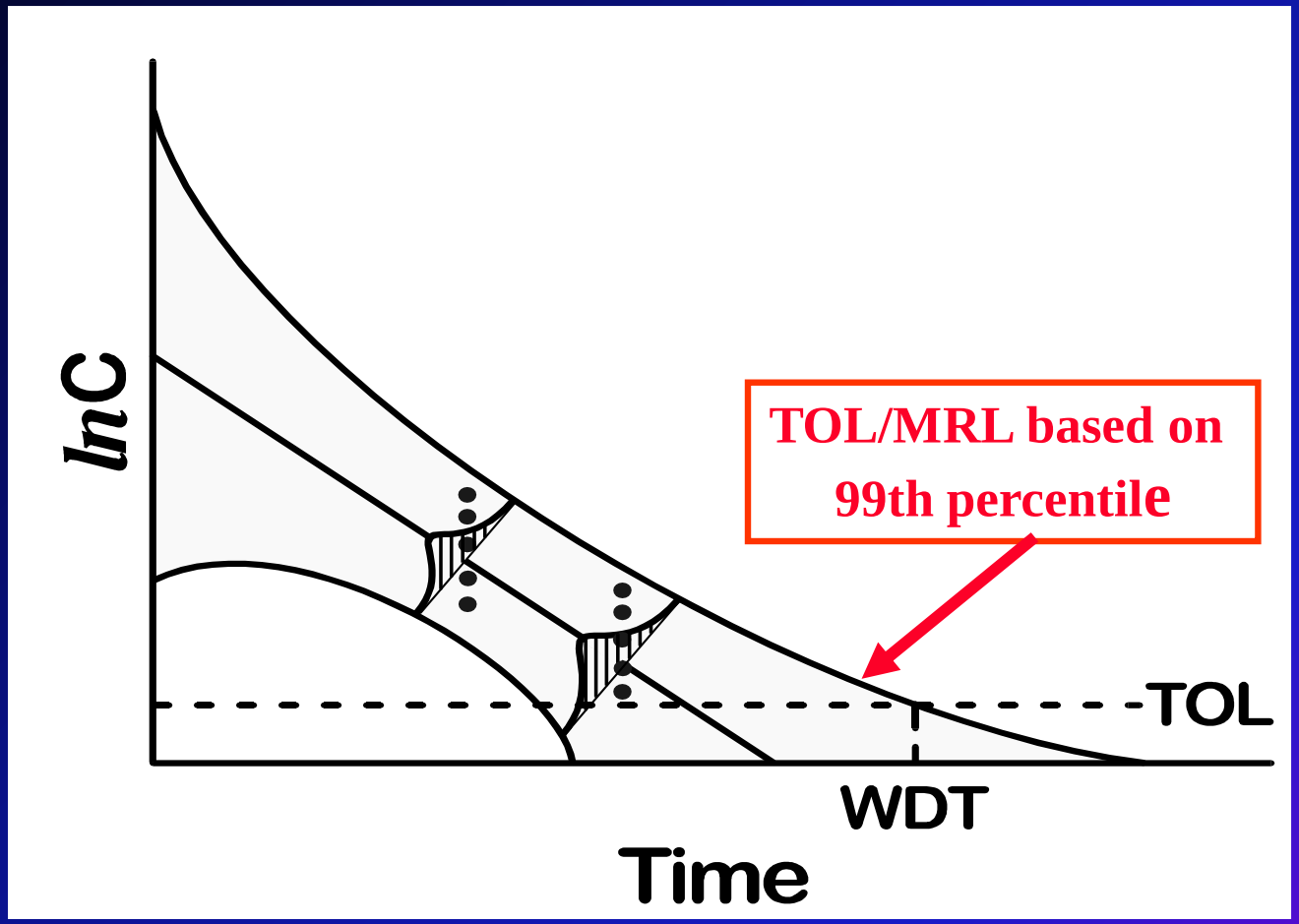
- After dosing, a withdrawal time must be selected to ensure residue in edible meat, milk or egg product is below a safe threshold (ppm, ppb).
- Safe concentrations in edible tissue (meat, liver, kidney, milk, egg) for human consumption are determined from toxicological analysis based on allowable daily intake (ADI) and various regulatory "jurisdiction-dependent" safety and food consumption factors.
- This results in a Tolerance (TOL) in US and Maximum Residue Level (MRL) in Europe for assayable target tissues collected at slaughter or harvest (milk and eggs).
- From a pharmacometric perspective, this is a fixed value and error is a function of sampling and analysis.

TOL/MRL based on parent drug and metabolite



These studies are done in small homogeneous groups of healthy animals, slaughtered at increasing time points, modeled using linear regression of logarithm of chemical concentrations vs time in each target tissue. Essentially a naive pooled data analysis. **Totally independent of any systemic disposition processes.**

The calculated WDT is based on the 99% confidence interval determined with p<0.05.



Designed to provide a conservative estimate to cover variability in disposition

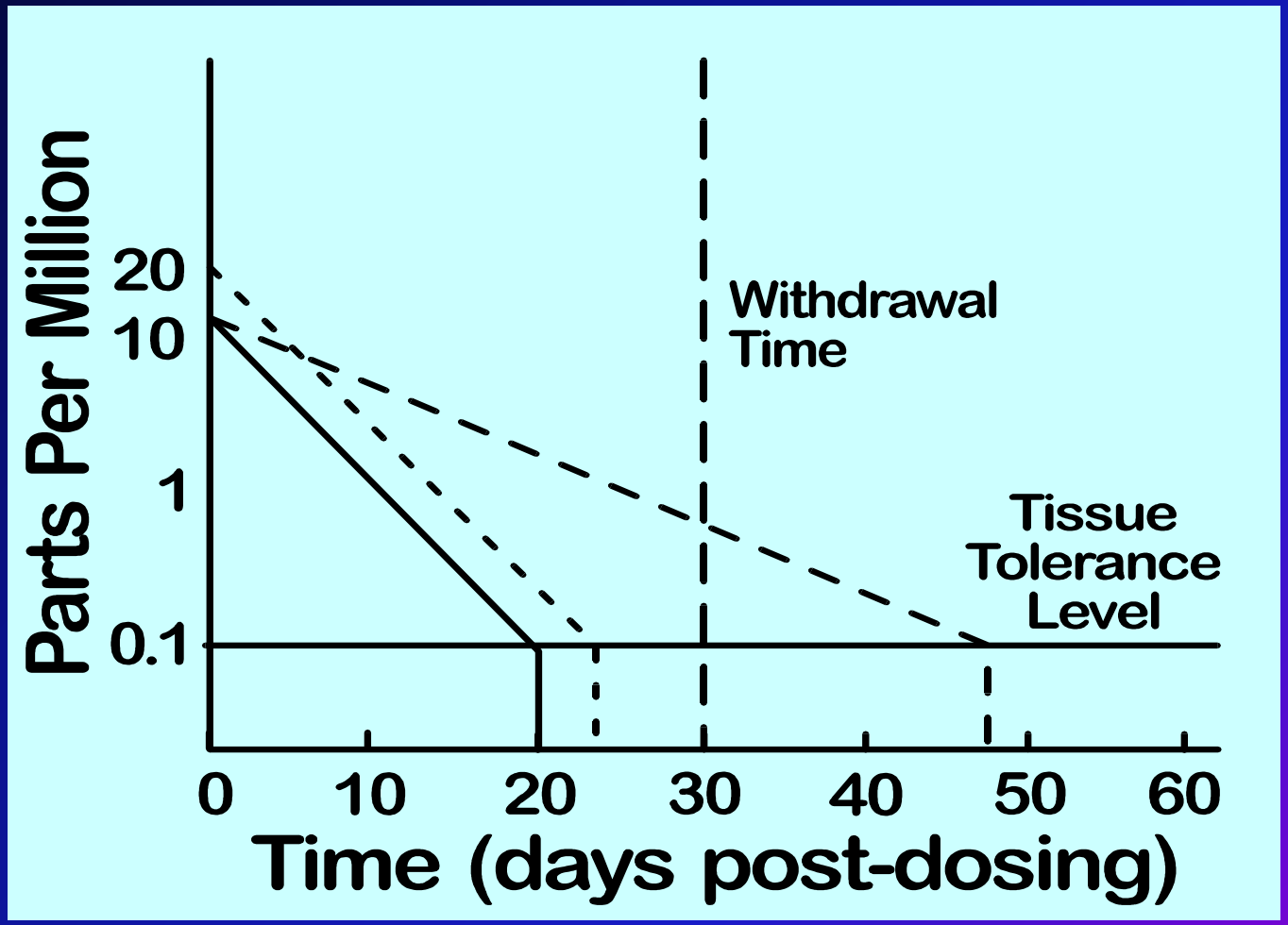
Pharmacokinetics of Tissue Residue Depletion

- This approach has resulted in regulatory endpoints with a reasonable degree of experimental reproducibility relative to the withdrawal time metric. Great for harmonization activities.
- However, it lacks any direct relationship to accepted drug disposition processes seen in clinical populations of animals actually treated for the disease approved on the label.
- Changes in systemic deposition will modulate drug delivery to the target tissues and hence influence target tissue levels.

Estimating withdrawal intervals is a daily task of the US FARAD consortium

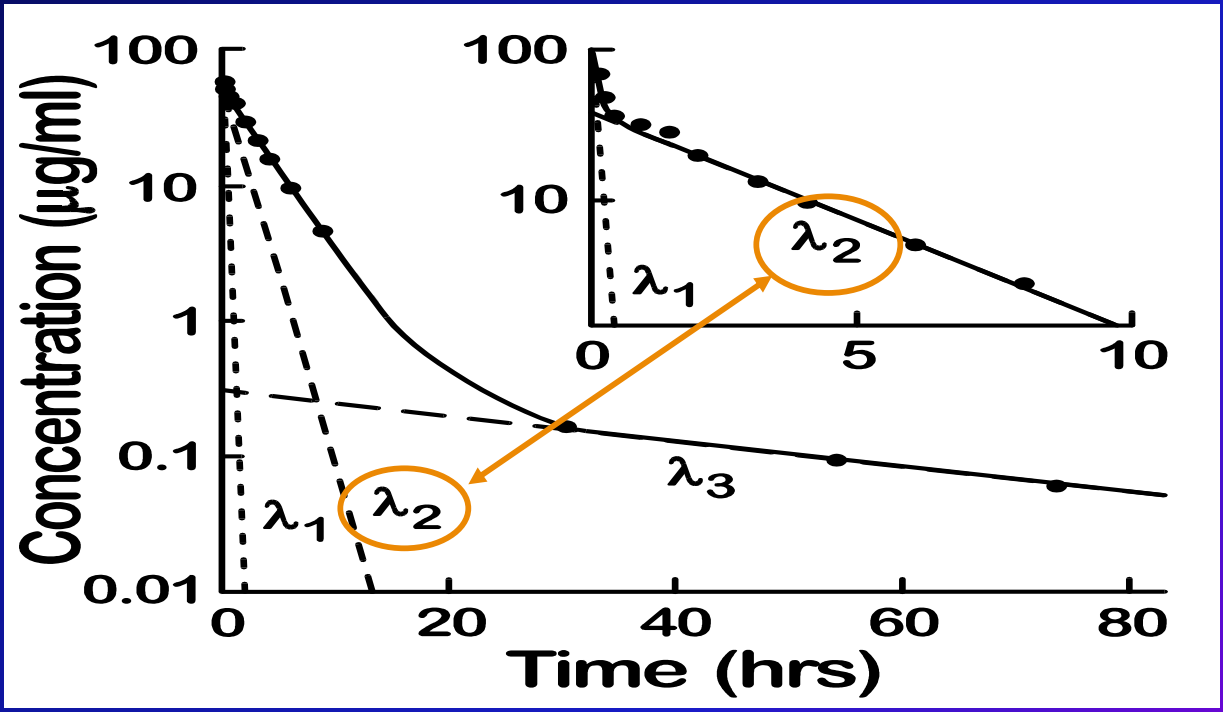
- Estimates WDTs after accidental exposure (pesticides, contaminants) or extra-label drug use (ELDU) in US.
- Insures safety of ELDU (Increased dose) which is often done to avoid induction of bacterial resistance and assure effective dose given in face of diseases altering disposition.
- Veterinarians request support through web submission and telephone hot-line to FARAD sites.
- Becoming more of an issue since food safety is increasingly in public eye and analytical chemistry outpaces pharmacology and toxicology.

WDT is sensitive to change in applied dose or altered disposition (Cl, Vd) from age or disease

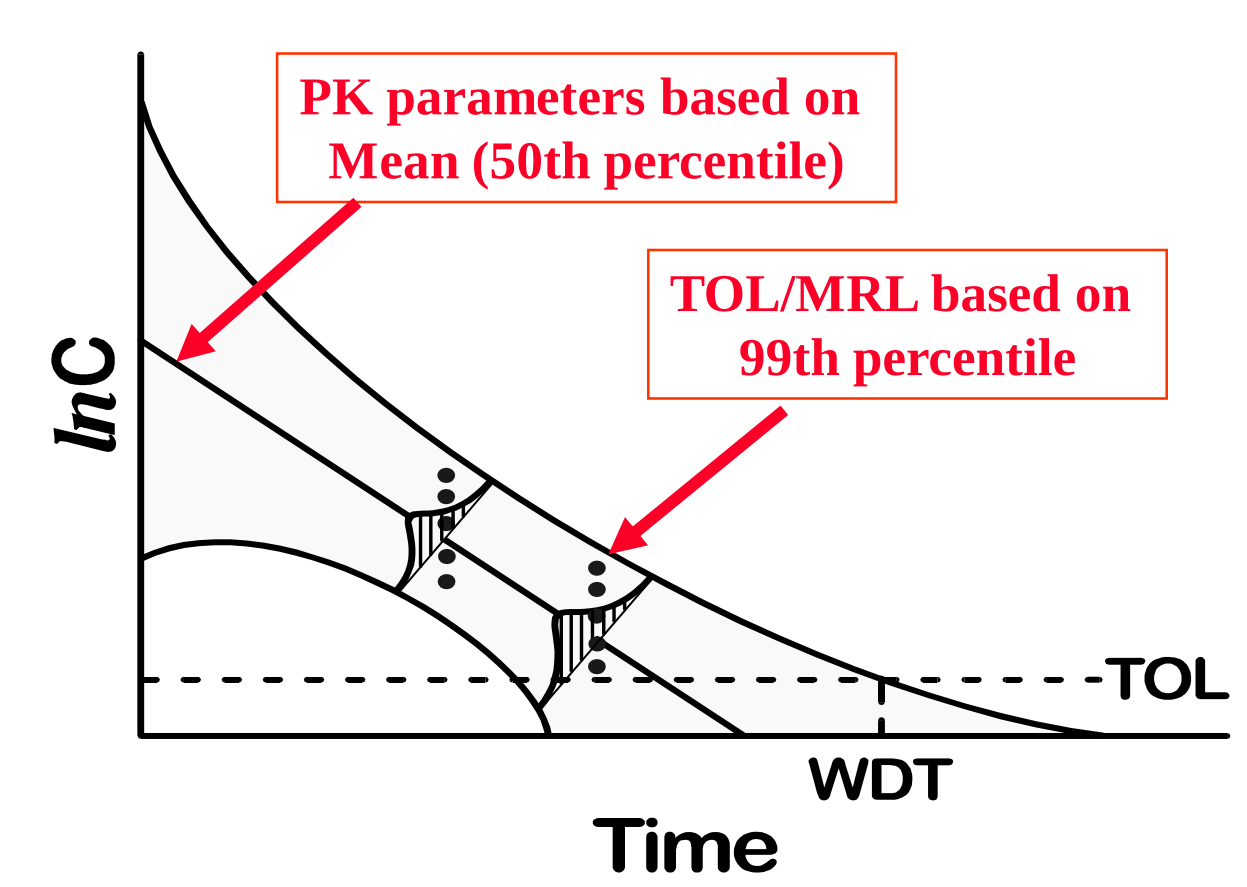


Residue Concentrations << Therapeutic Meaningful Concentrations

- Factors that influence tissue concentrations are generally irrelevant to either therapeutic efficacy or toxicological potential
- Even more true as analytical methods improve

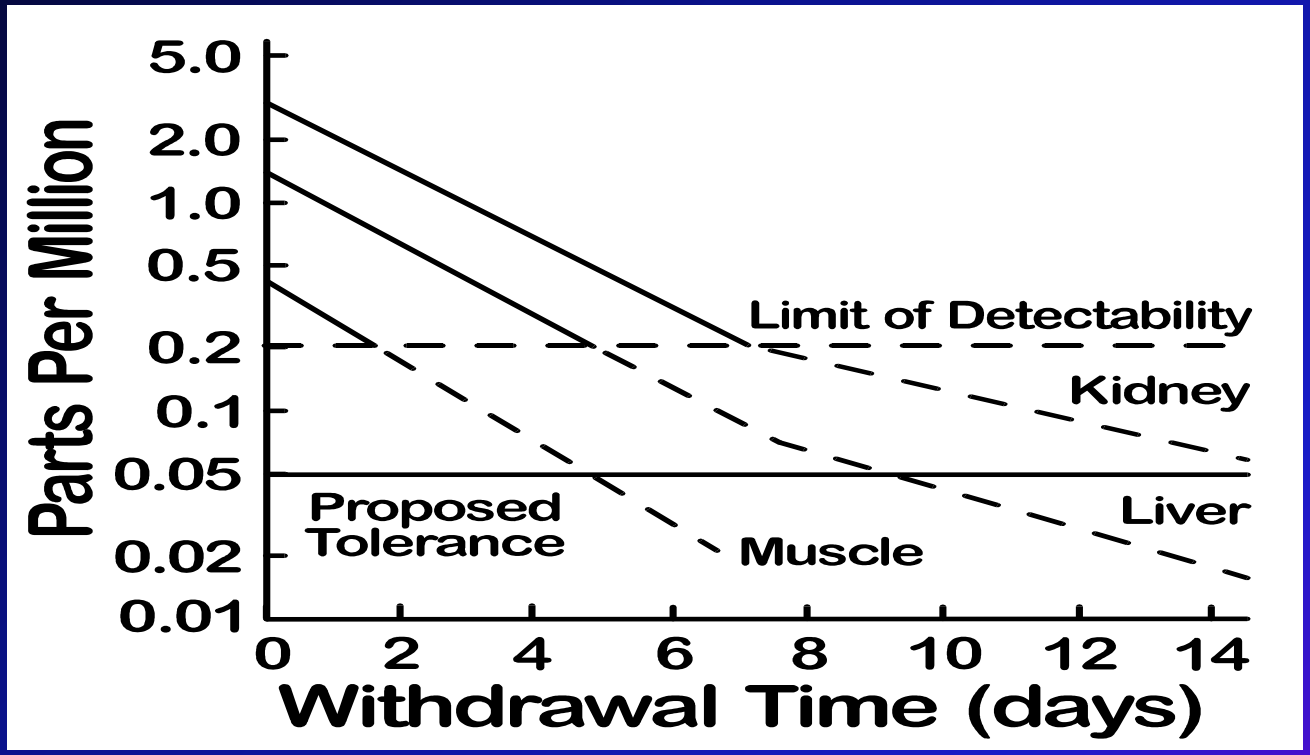


WDT is based on 99% CI determined with p<0.05



Published PK data are generally based on means of studies with small sample sizes, without statistical power to estimate tails of distributions.

Relation between WTD and Tolerance

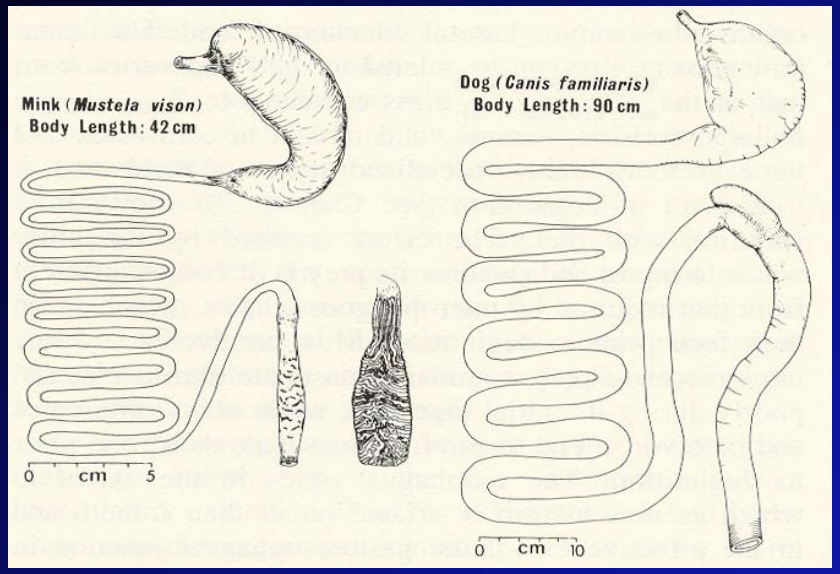


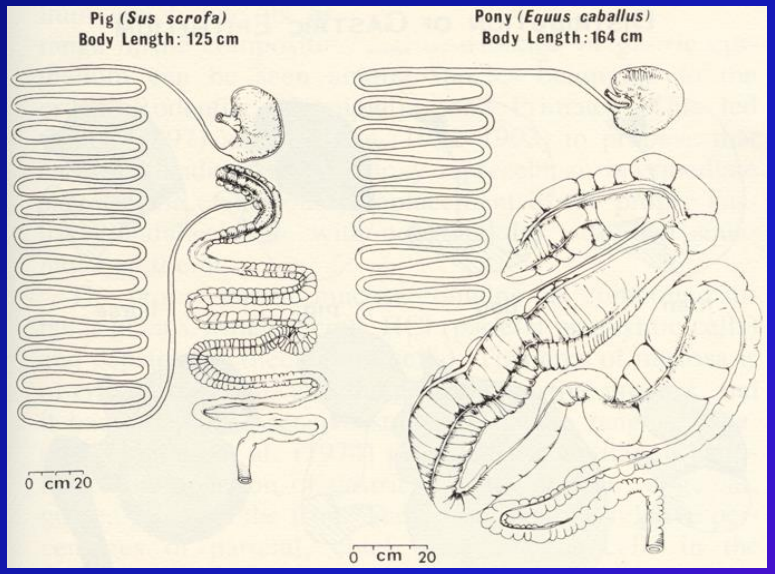
Tissue depletion profiles are often non-linear as concentration drops.

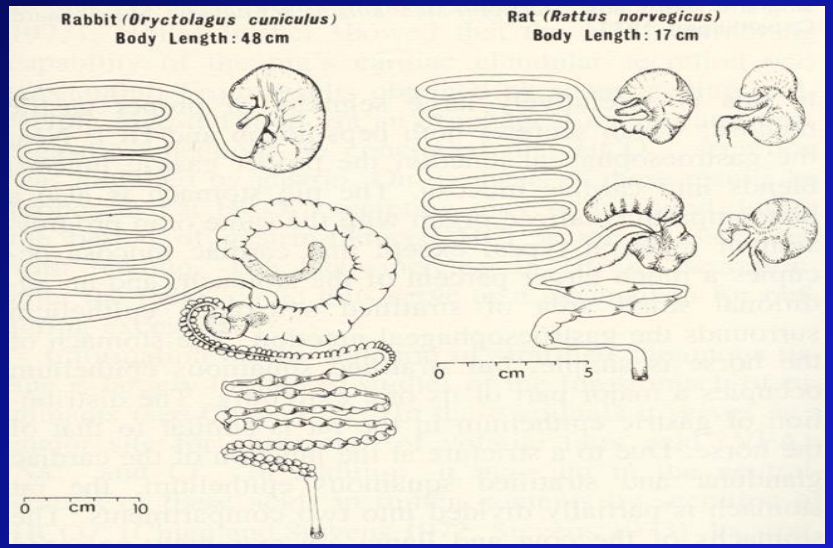
Now that we know the endpoints, what are the factors contributing to enhanced variability

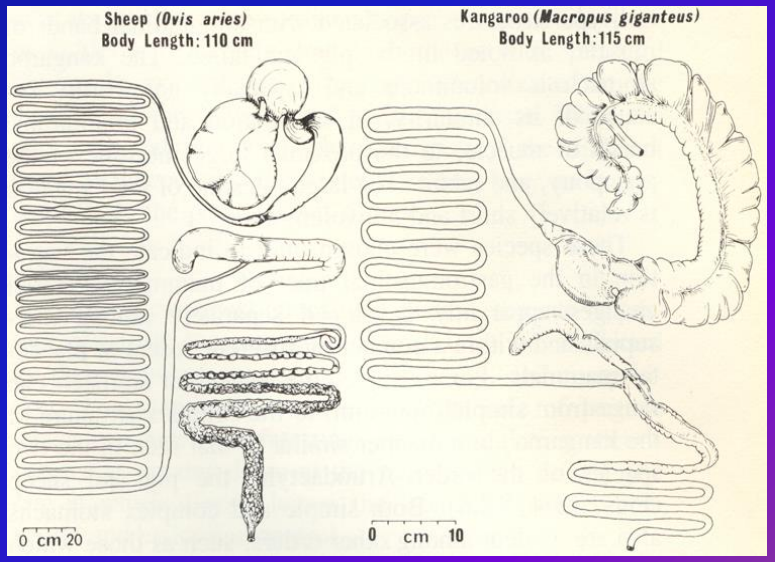
- Anatomical diversity and species differences in general
  - Will not discuss metabolism further as these concerns are shared with human drug development, and encountered when extrapolating from pre-clinical rodent or dog trials.
- Different routes of administration
- Large variability in dose delivery
- Populations are often dosed
- Lack of control of environmental variables
- Nutrition, age, breed differences
- Difficulty in obtaining necessary samples due to fundamentally different economic models

Large anatomical diversity in structure of gastrointestinal tracts



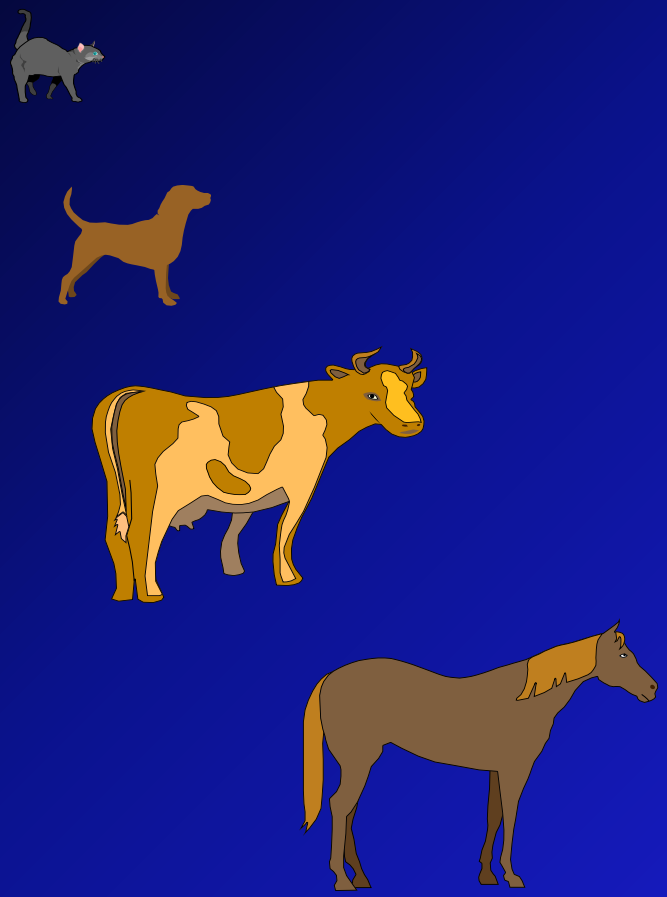


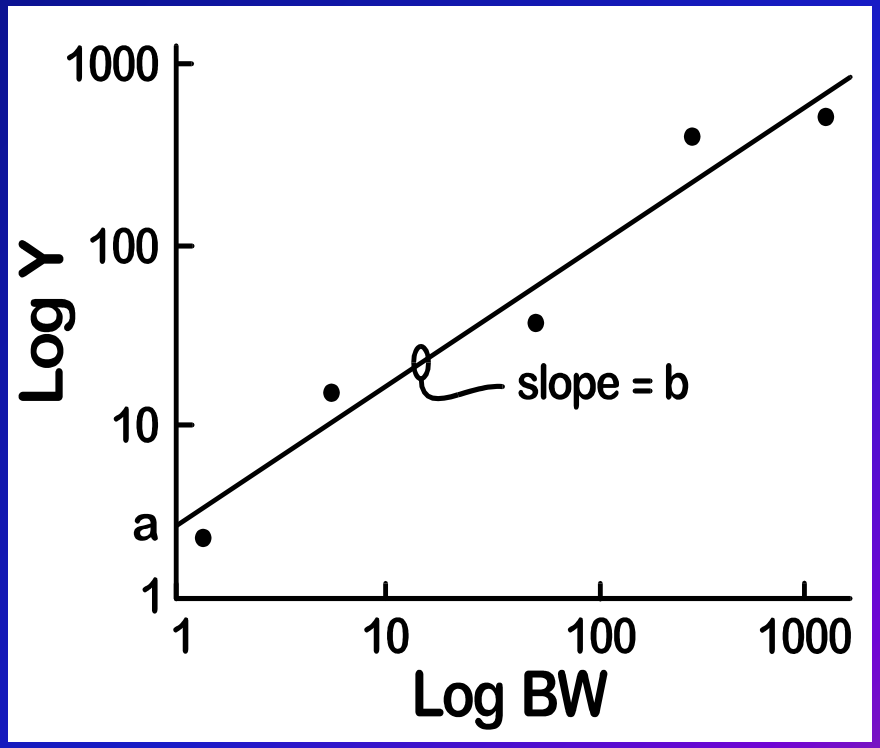




INTERSPECIES EXTRAPOLATIONS ARE POSSIBLE USING PRINCIPLES OF ALLOMETRY

$$Y = a\,(BW)^{b}$$





Allometric Analysis of 44 Drugs

- Data obtained from FARAD PK database
- Data on 44 drugs with a minimum of 4 species analyzed with single dose, IV kinetics
- Half-life analyzed to determine fit to $Y = a (BW)^b$
- A group of 11 drugs had statistically significant ($p<0.05$) allometric relationships. Lack of significance could be due to physiology or insufficient data.
- Assessed by weight ratio, # species, # repeats
- This means 33 drugs didn't work!

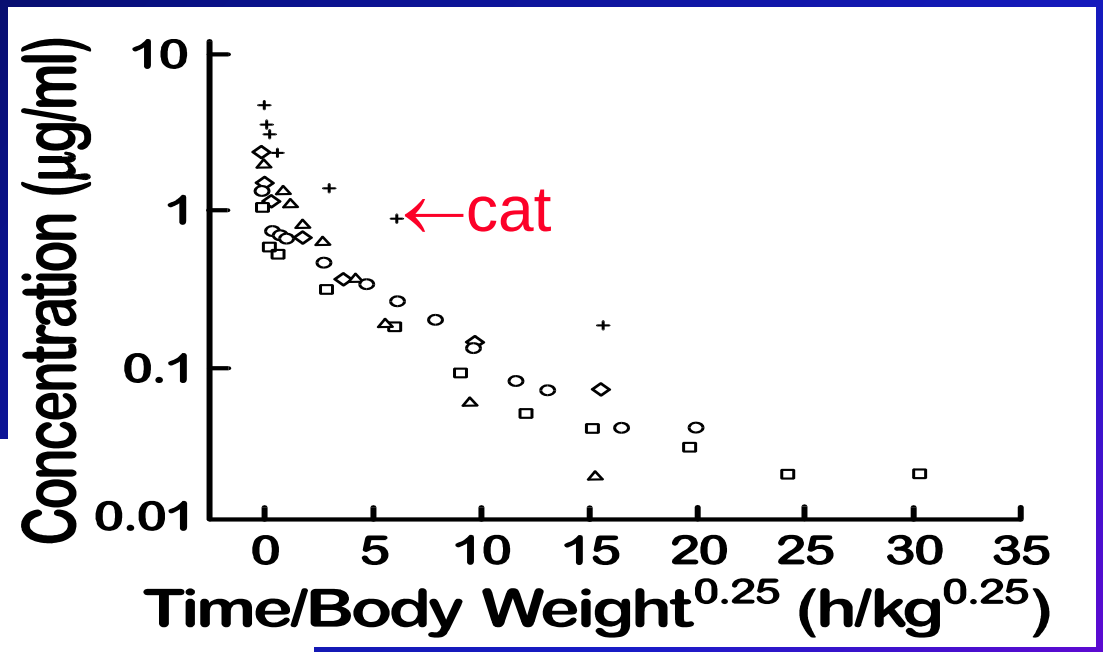
Riviere JE, Martin T, Sundlof S, Craigmill AL: J. Vet. Pharmacol. Therap 20: 453 - 463, 1997.

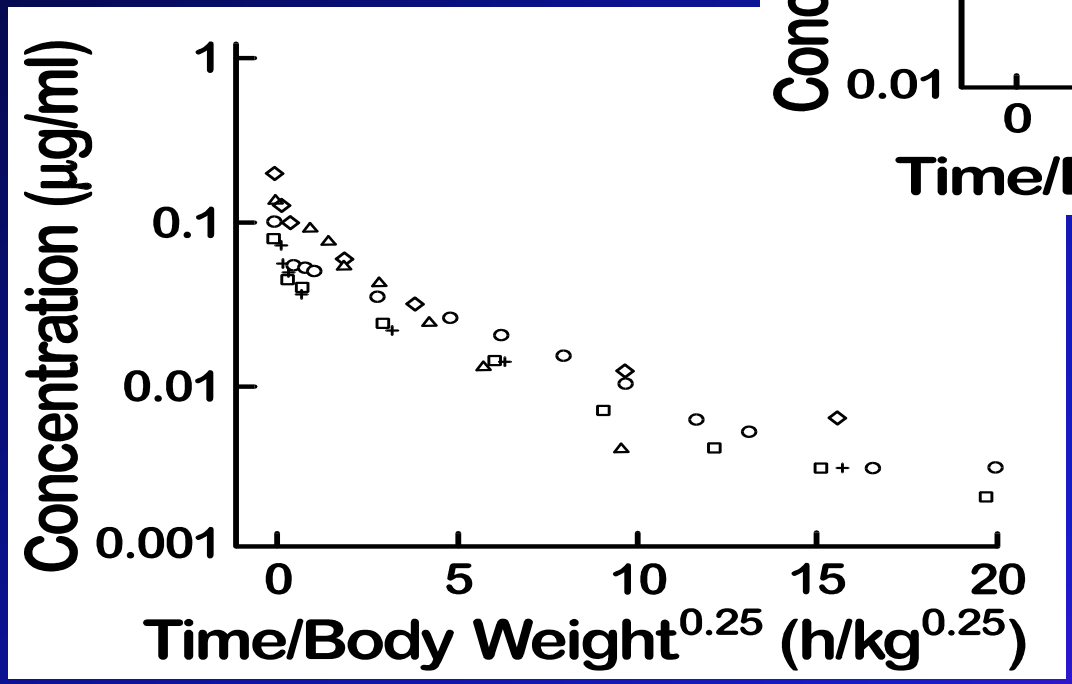
Allometric Analysis

- 11 drugs had average b = 0.24 (± 0.09) with a r^2 = 0.87 (± 0.09)
- Tetracycline, Erythromycin, Oxytetracycline, Cephapirin, Diazepam, Apramycin, Chlortetracycline, Gentamicin, Prednisolone, Carbenicillin, Ampicillin
- Mean log body weight ratio was 8.1 (± 2.1) with data on 6.2 (± 2.2) species and 10 citations.
- Other drugs didn't scale (not enough data, "problems"

Interspecies Doxycycline Dedrick Plot - Total vs FreeDrug

(Cats, Dogs, Pigs, Angus Calves, Holstein Calves)





Routes of Administration

- Different administration techniques in addition to those shared with human and small animal medicine
  - Intrarumenal – think “submarine”
  - Intravaginal
  - Intramammary
- Large variability in dose delivery to animal
  - Water administration – temperature dependent
  - Feed administration – issues of behavioral dominance
  - Dips for topical administration – homogeneity

Large Variability from Population Dosing

- Large animal medicine has also been termed “herd-flock” medicine
- Individuals are not treated, populations are.
- Drug delivery is not uniform to all individuals
  - Reduced delivery could lead to lack of efficacy or induction of resistance for antimicrobials
  - Increase delivery could result in toxicity, but with wide margins of safety employed, more likely violative tissue residues

Disease effects drug pharmacokinetics

- Little debate that disease may alter PK properties
- Classic study by Nouws in 1977 showed most residue violations were in culled dairy cows.
- Lees and Higgins in 1984 showed NSAID flunixin can accumulate at inflammation site by binding to receptors present in diseased animals.
- Flunixin is the *residue issue de jour* in 2013. We reported on actual flunixin PK in normal and mastitic cows (n=10).

  Healthy: Cl = 120 ± 31 ml/h/kg; T ½ = 3.7 ± 1.9 h

  Mastitis: Cl = 67 ± 47 ml/h/kg; T ½ = 4.4 ± 1.8 h

  At established WDT, 8/10 diseased cows had violative residues of 5-OH flunixin (marker residue)

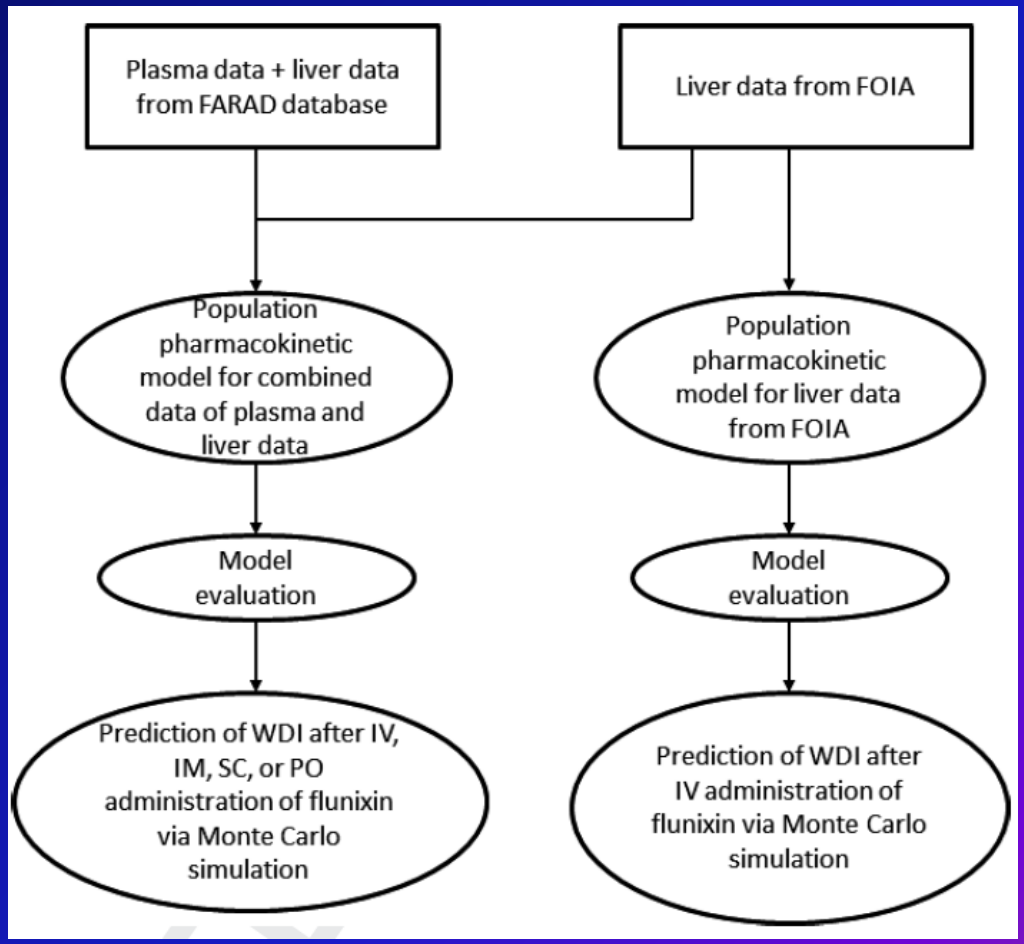
- In many cases, interactions are drug specific
  - Cytokine alterations in biotransformation

Population pharmacokinetics has been the primary tool to investigate and quantitiate these issues

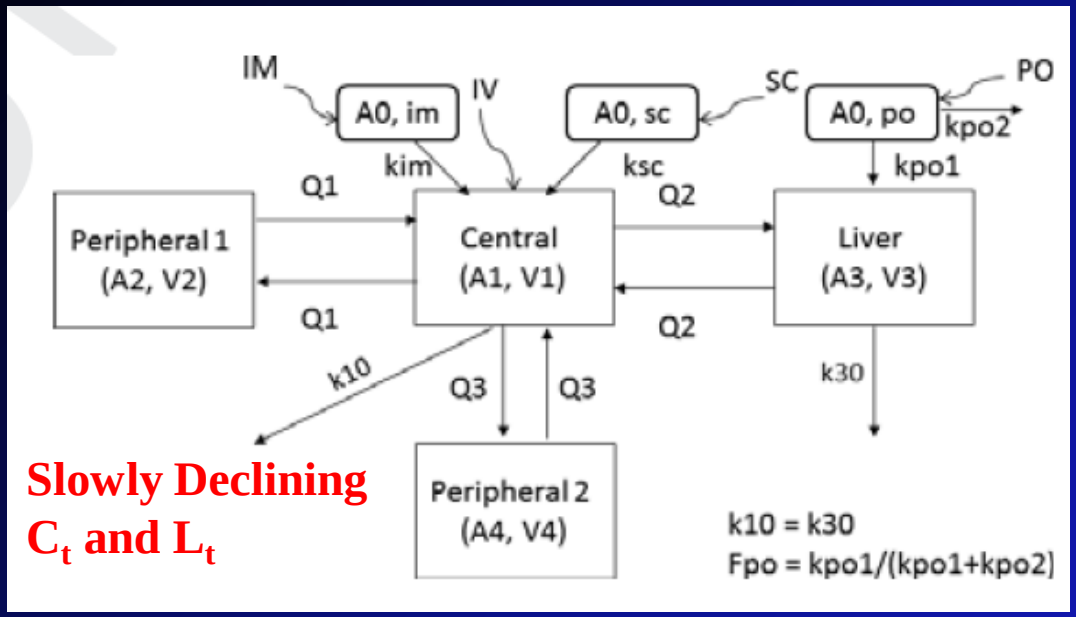
- Mixed-Effect Modeling (MEM)
- "*Traditional*" pharmacokinetics does not allow for variability to be easily integrated into an analysis.
- Requires less samples per individual but more individuals can be included.
- Allows use of covariates which can be used to predict disease effects.

Illustrate with flunixin studies in cattle: Needed to determine why violations were occurring

- Used data from six published studies of flunixin plasma deposition using various dosage regimens to a diverse groups of cattle
- Liver data obtained from one publication and US FDA FOIA from sponsor submission.
- **Need to link plasma to liver data to capture disease effects on Cl !**



Wu, Baynes, Leavens, Tell, Riviere. J. Vet. Pharmacol. Therap. 36: 248-257, 2013



Slowly Declining C_t and L_t

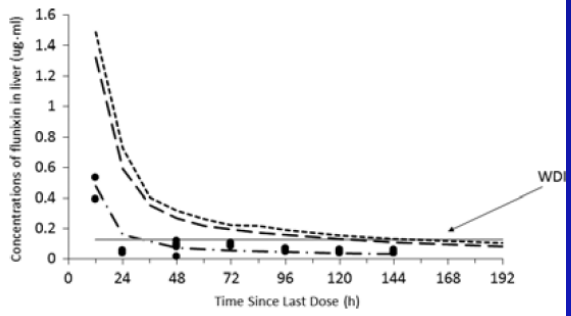
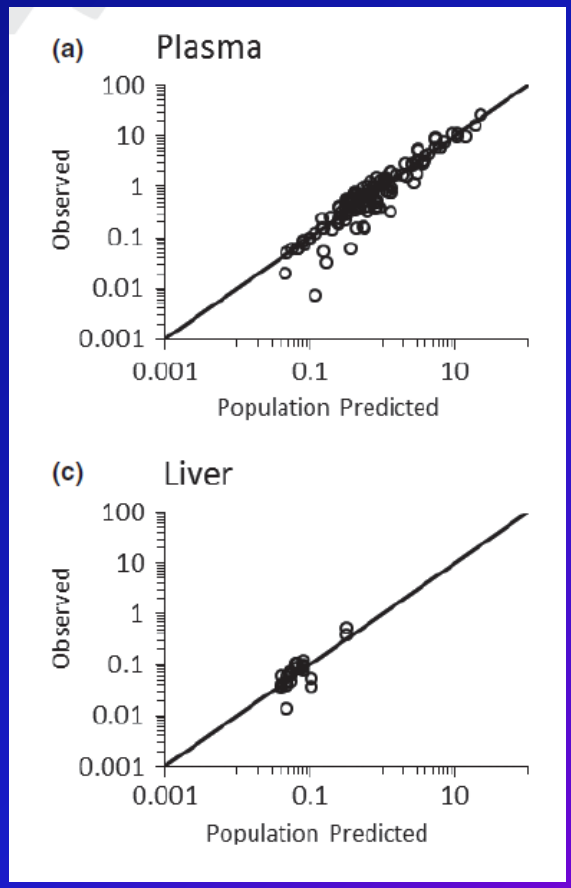


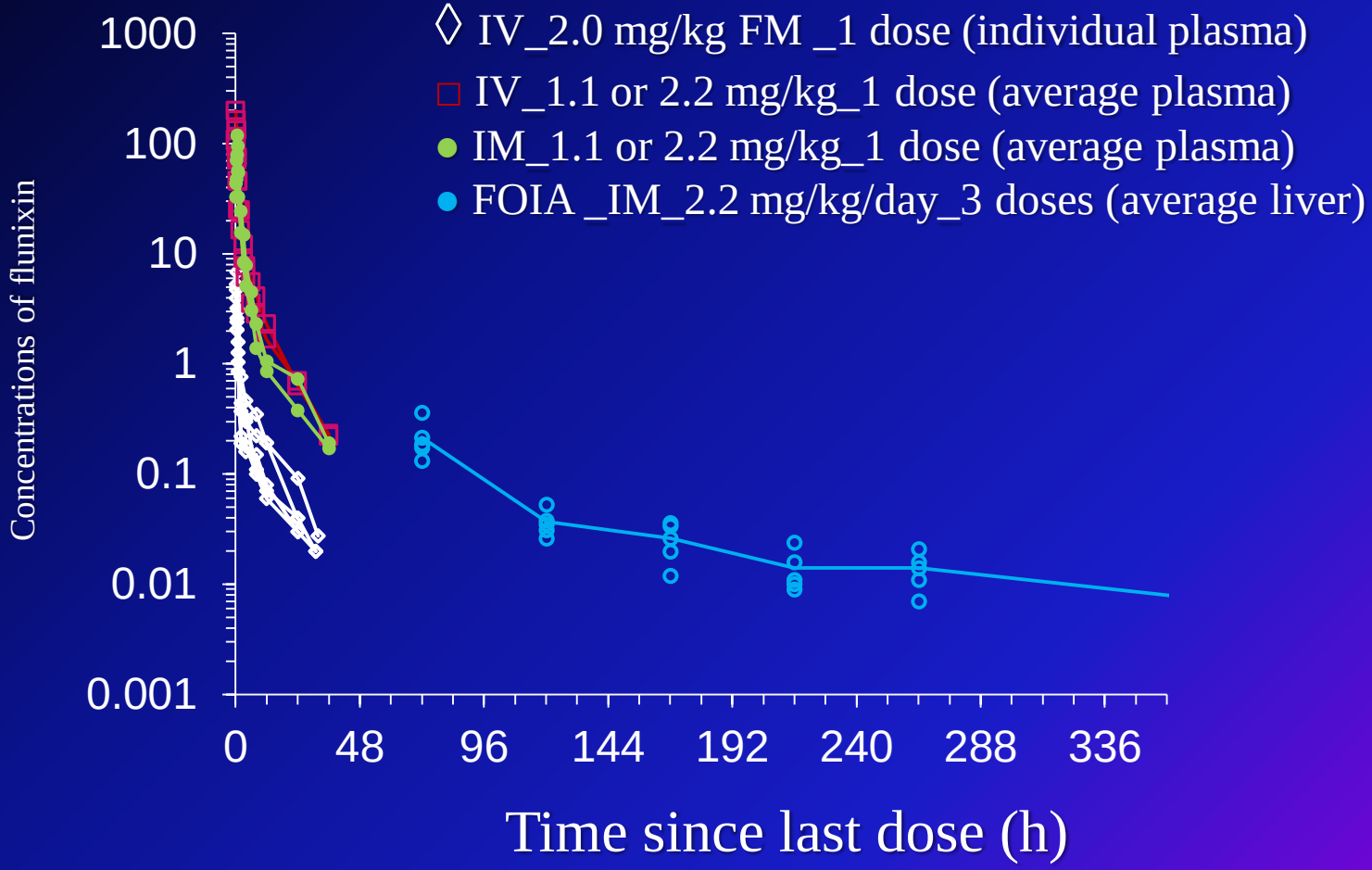
Fig. 5. Simulated data for the tissue residue of flunixin in liver. The upper limit of 95% confidence interval for 99 percentile (– – –) of the simulated flunixin residue in liver was used to determine the withdraw interval following dosing of flunixin in cattle. The 99 percentiles of the simulated flunixin residue in liver are represented by a dashed line (— — —). The 50 percentiles of the simulated flunixin residue in liver are represented by a dashed line (— · —). The observed concentrations of flunixin in liver are represented by closed circles. The solid gray line is the tolerance of flunixin. The dosing schedule is a dose of 3.6 mg/kg once daily for 3 days by intravenous (IV) administration.



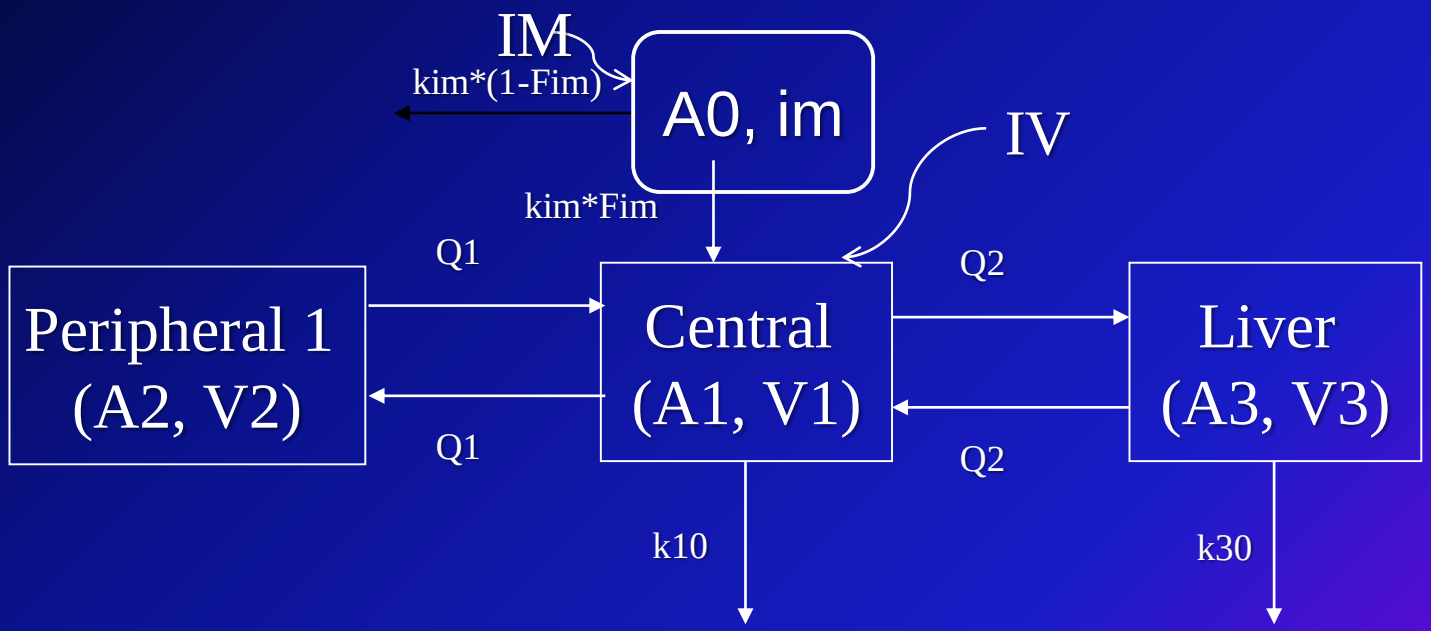
Findings from the Flunixin study

- **Withdrawal times estimated using plasma data, reflective of true treated populations, were longer than those determined from control residue trial (*Note same WDI was found using PopPK or FDA Tolerance Method with same data)***
- **Withdrawal times varied by route and disease, this was confounded with length of sampling across studies. Reduced clearance was predicted by model and later confirmed.**
- **Major need is more data from**
  - **Field conditions**
  - **Diseased animals**
  - **Longer plasma collection to match withdrawal times**
  - **Plasma and target liver tissues collected in same study- USDA Agriculture Research Service is now collaborating to conduct such a study**

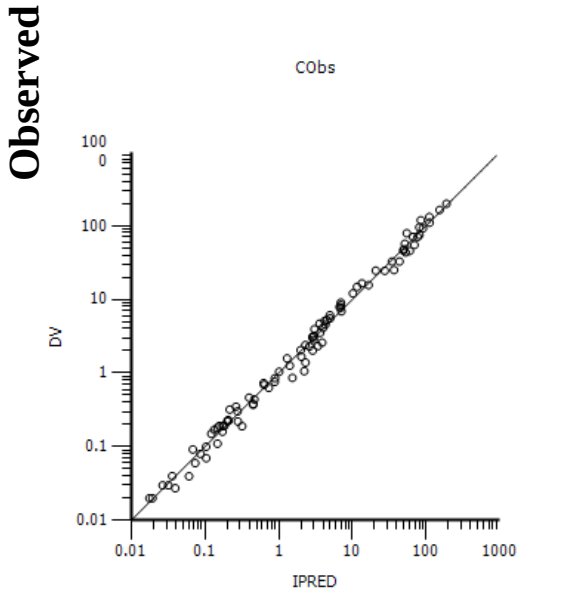
Porcine Model of Flunixin Concentrations in Plasma and Liver



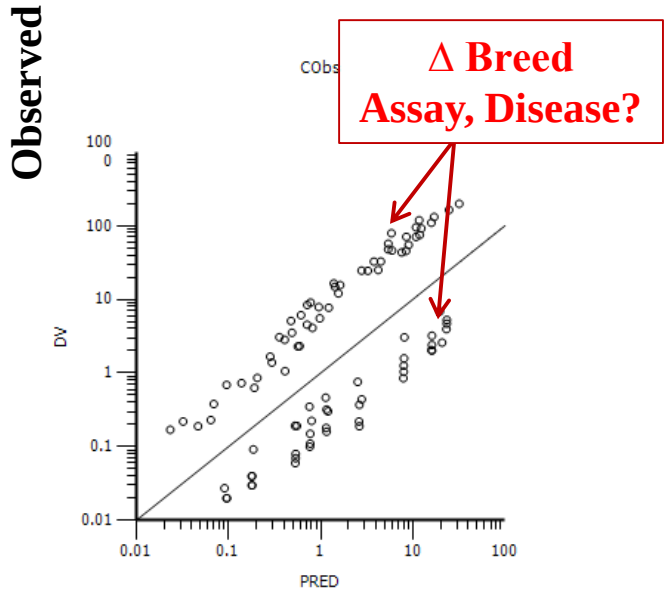
PK Model Structure for Confluent Plasma and Liver Data



Goodness-of-fit for Plasma Data and WDI Estimates



Individual predicted



Population predicted

Used PopPK to estimate how large of a change in elimination rate constant (k) is needed for the median of 95 and 99 percentile of tissue residue predictions to be the above tolerance limit.

	95 percentile	99 percentile
Flunixin in Cattle	30%	20%
Penicillin in Swine	30%	20%
Penicillin in Cattle	30%	20%

These are NOT large changes!

Physiological-Based Pharmacokinetic Model-PBPK

- A second more mechanistic approach used by FARAD
- Models are constructed based on actual disposition of drug in the body using organs and blood flows
- Equilibrium ratios (R_t) are constructed relating concentrations in blood to tissues
- Mass balance equations are written in terms of blood flow to specific organs and extraction ratios

$$dC/dt = (\text{Rate In}) - (\text{Rate Out}) = Q_t [C_p - C_t/R_t] / V_t$$

- Long history of PBPK models

1924 – Haggard (anesthetic movement into brain)

1937 – Teorell (general application to xenobiotics)

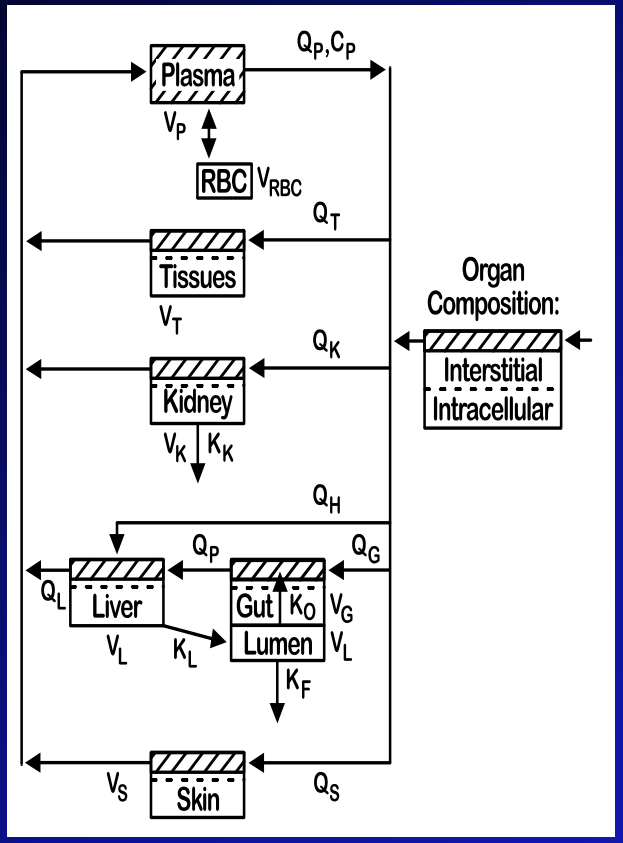
1970's - Bischoff and Dedrick (PBPK with therapeutic focus)

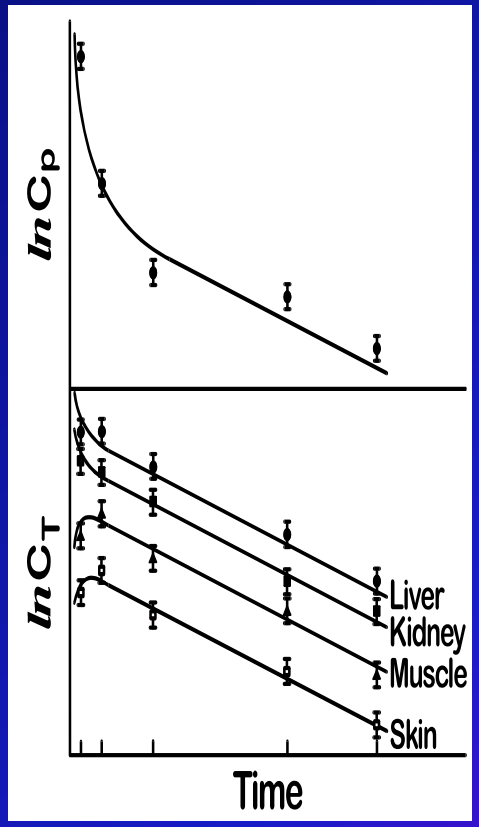
1980's – use in toxicology for exposure assessment

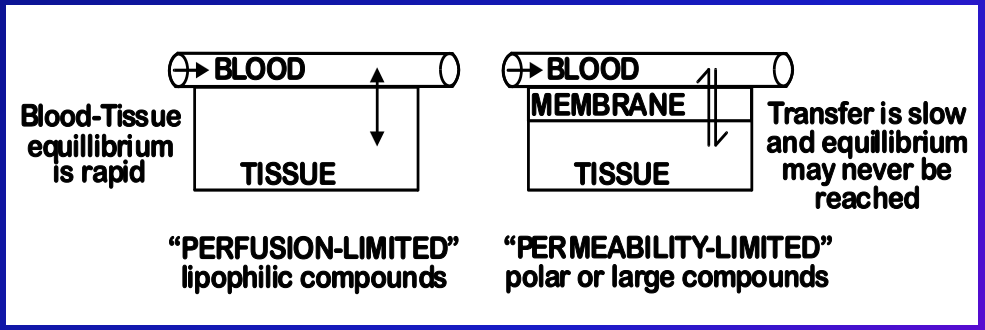
1990's – mechanistic PBPK models for risk assessment

2000 – revival of PBPK models for therapeutic drugs

Physiological-Based Pharmacokinetic Modeling







Physiological-Based Pharmacokinetic Model

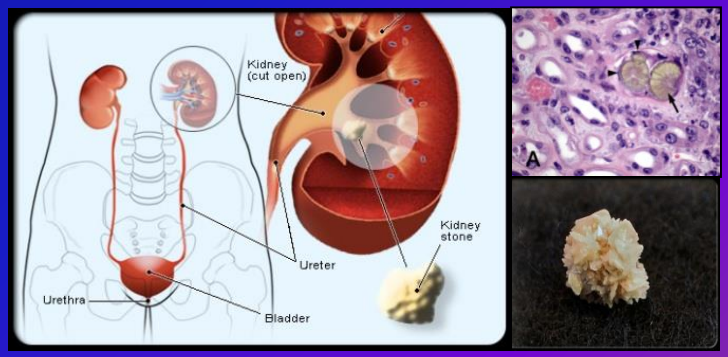
- Easy to directly incorporate *in vitro* data
- Ideally suited for interspecies extrapolations
- Gastro-Plus is a good example of PK applications
- Easy to incorporate *in vitro* models
  - dissolution data into oral absorption models
  - biotransformation models
  - tissue transporters

BUT

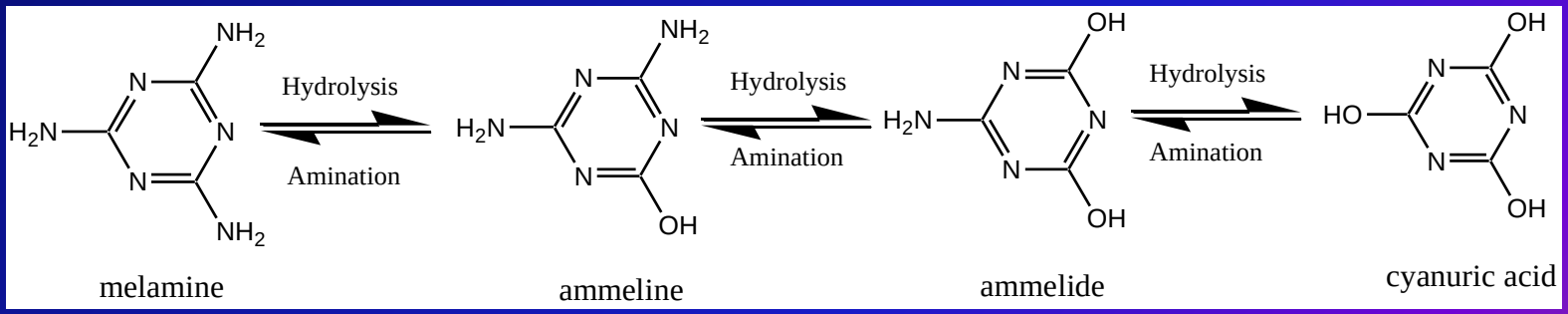
- Difficulties with mathematical identifiability of models
- Variance structure is not well described
- Statistical approaches to use such models in data extrapolation need to be explored

First Example: Melamine Food Contamination

- Cause of global pet food crisis and human renal failure in China.
- Primarily an economic adulterant used to supply nitrogen to “trick” nitrogen-based protein assays.
- Source was byproduct of plastic industry.
- Found to be widely used for this purpose for many years, including “energy drinks.”
- Once studied as legitimate nitrogen source for ruminants.
- Disposition is primarily to kidney



- Melamine spiked “protein mixes” went into other food: milk formula in China, dog food in US
- Widely used and probably not an issue if diluted and not always present. However supply lines concentrated into a few sources, all contaminated.
- Acute, widespread outbreaks triggered further investigation.
- Pure melamine was studied by NIEHS National Toxicology Program two decades ago. Not an acute problem. **Pre-crisis, melamine was not on anyone’s screening list.**
- Problem is when cyanuric acid is present.

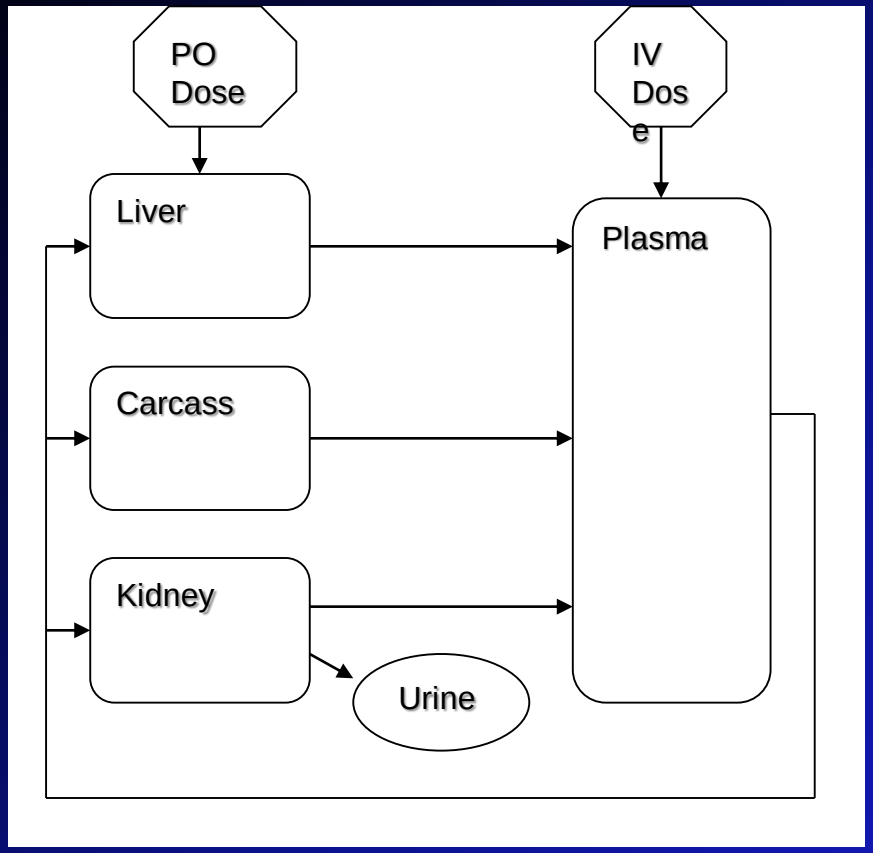


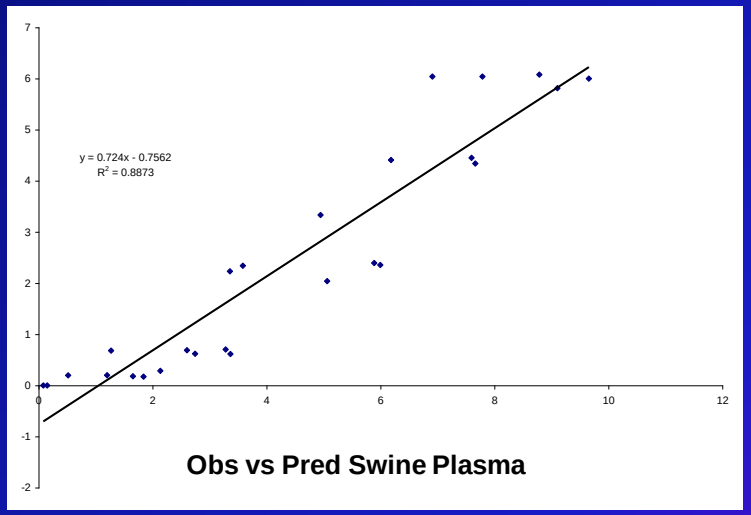
Melamine Pharmacokinetics and Withdrawal Times after Feeding Pigs Contaminated Feed

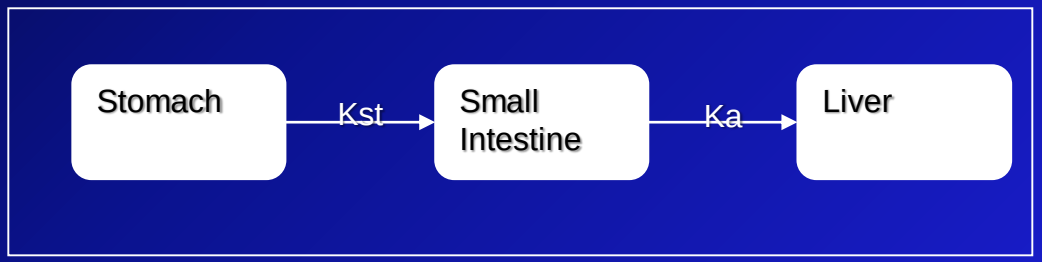
- FARAD was faced with swine producers feeding melamine contaminated dog food to 20,000 swine. What is WDT? No time to do a 10 year study!
- Initially used a classic IV one compartment PK model in pigs to assess clearance (0.11 ± 0.01 L/h/kg) and T ½ (4.04 ± 0.37 h).

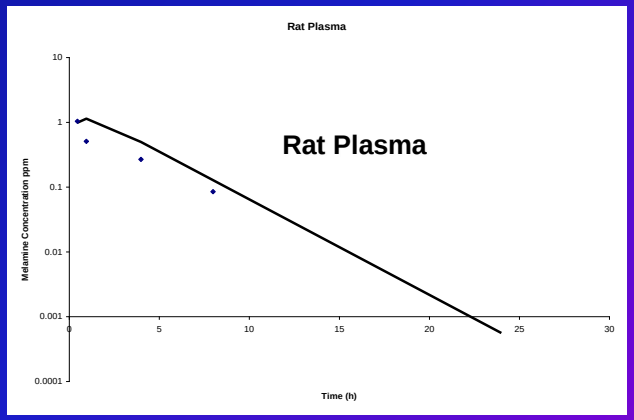
  (*Baynes, Smith, Mason, Barrett, Barlow, Riviere. Food Chem. Toxicol. 46: 1196, 2008*)
- Used these data and literature values for rat disposition study to parameterize a PBPK model to estimate tissue withdrawal times based on using available data.

Melamine PBPK Model



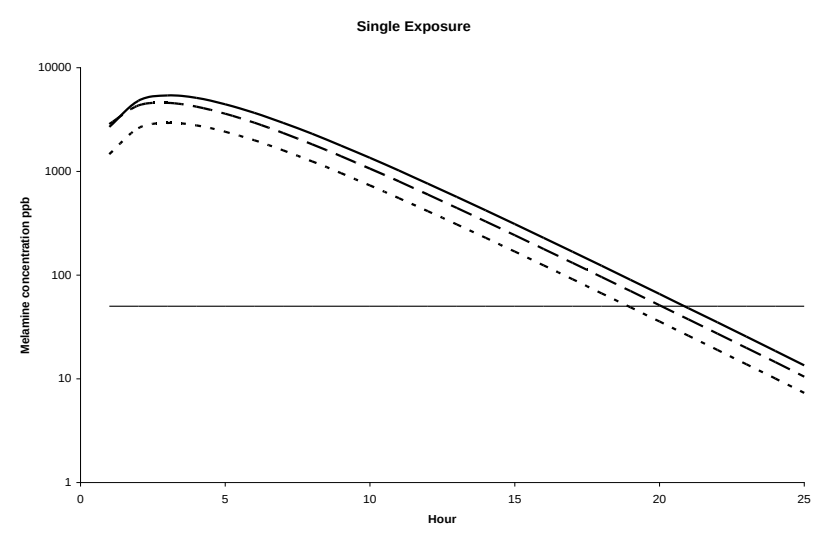


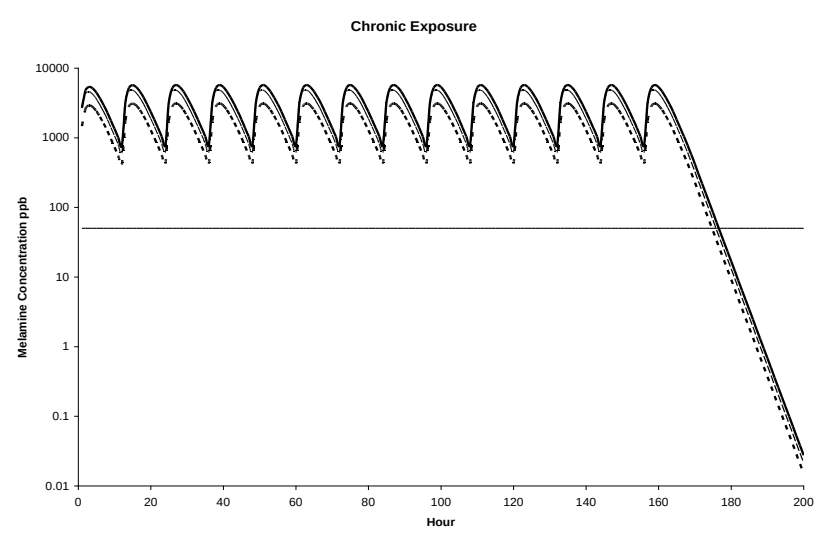




Buur, Baynes, Riviere. Reg. Toxicol. Pharmacol. 51: 324-331, 2008

Estimated WDT of 19-21 hrs after single dose oral exposure to 3-5 mg/kg melamine in feed. WDT of 20-21 hrs estimated after repeated dosing. Disposition largely restricted to kidney.





This nicely illustrates use of PBPK model simulations in context of FARAD task of estimating withdrawal times when complete studies cannot be performed and "label" doses do not exist.

Tulathromycin PBPK Model in Goats

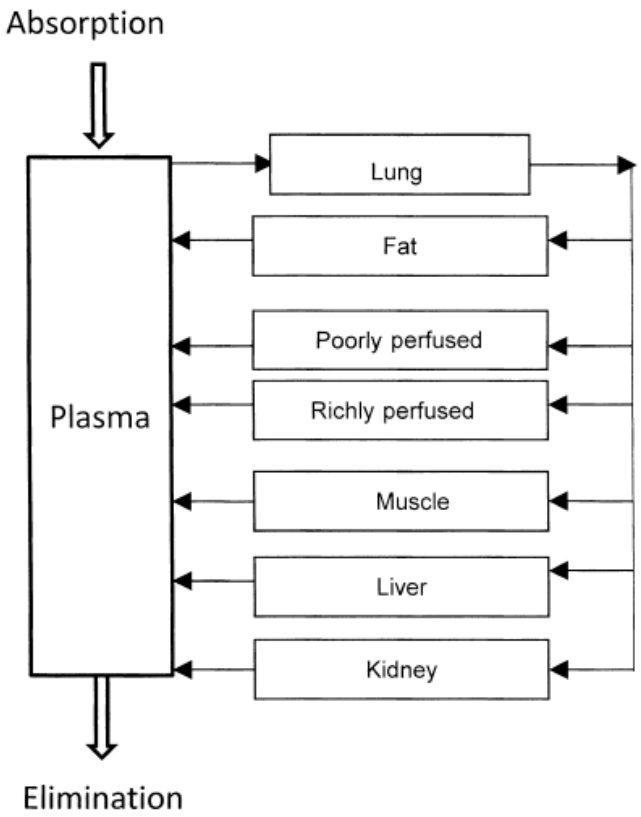


Fig. 1. Schematic of physiologically based pharmacokinetic model.

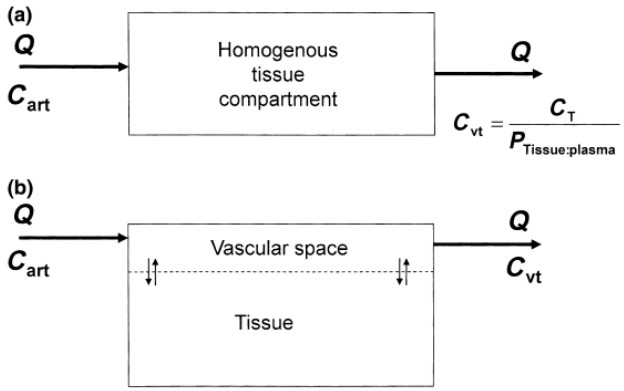


Fig. 2. (a) Model for blood-flow-limited distribution, (b) Model for diffusion-limited distribution.

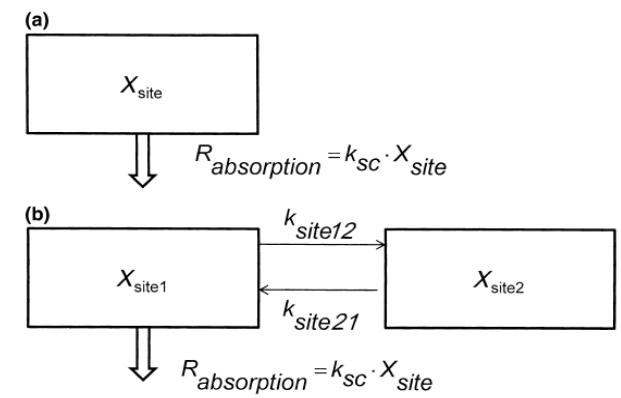
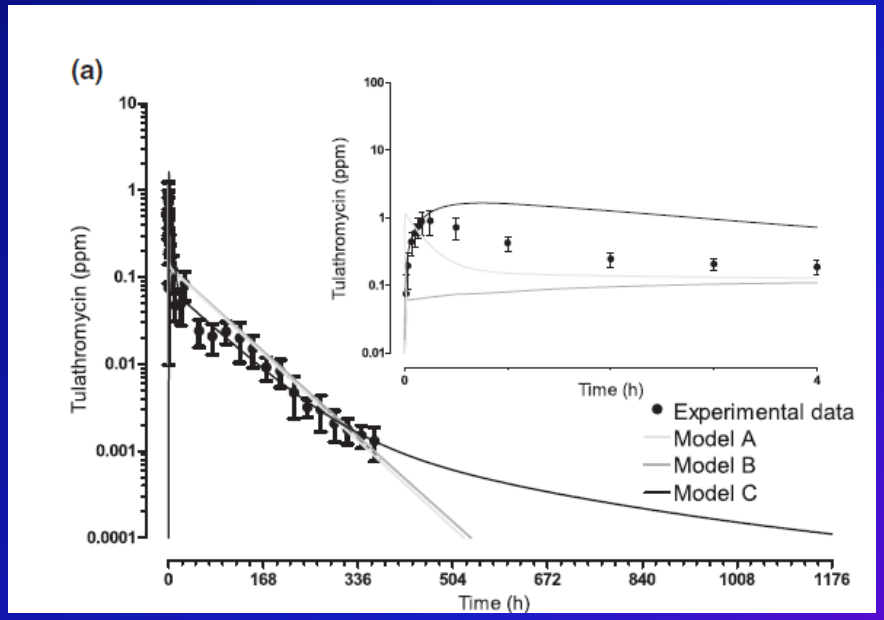


Fig. 3. Model diagrams for (a) 1st order absorption from a single injection site compartment and (b) 1st order absorption from a two compartment injection site.

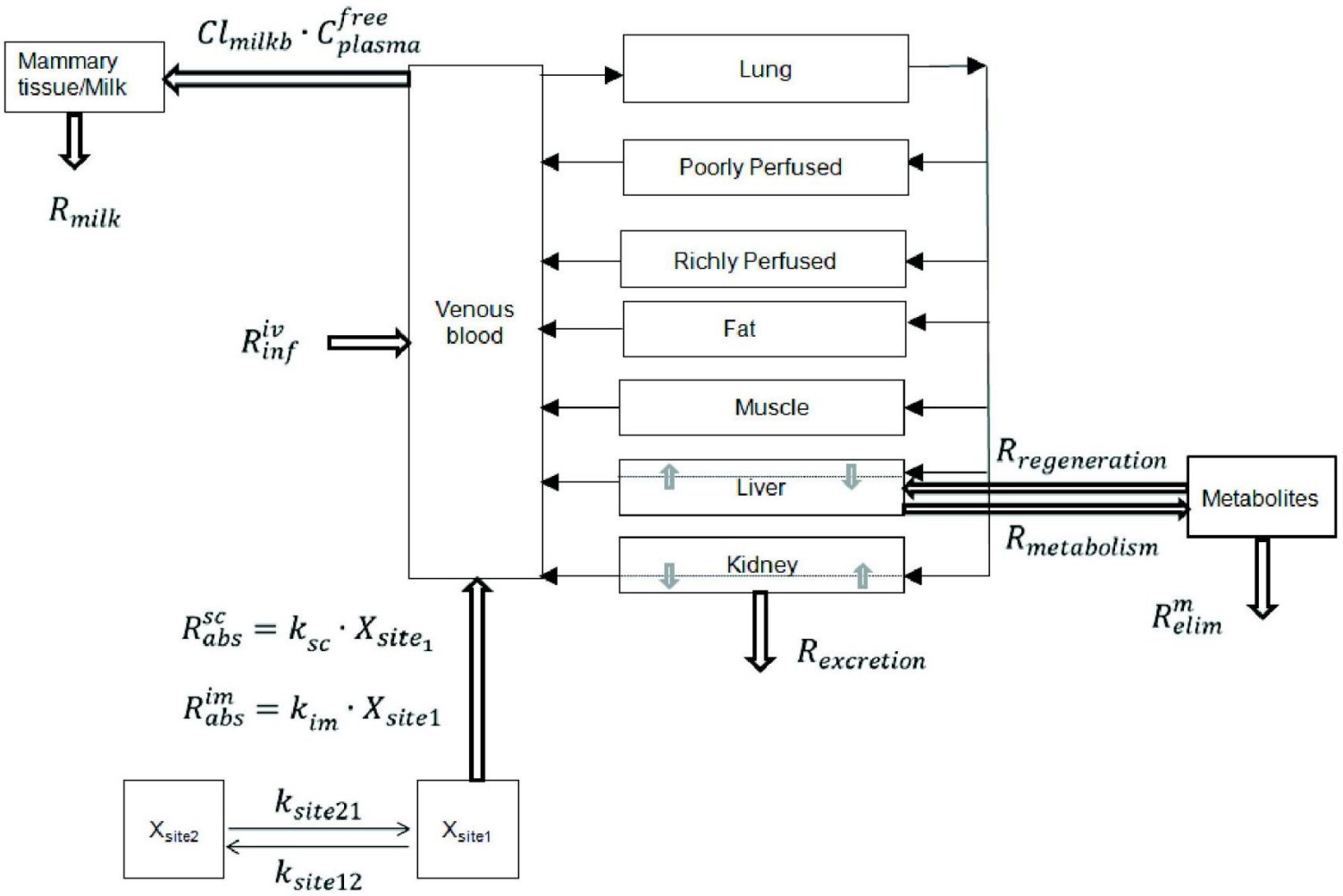
Leavens, Tell, Clothier, Griffith, Baynes, Riviere. J. Vet. Pharmacol. Therap. 35: 121-131, 2012

Tulathromycin PBPK Goat Results

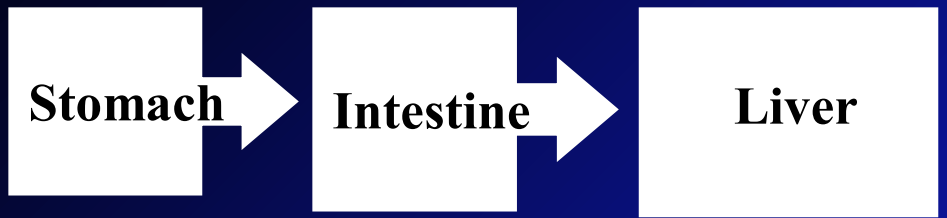
- Diffusion limited tissue uptake was needed to predict later time points
  - Water soluble, 806 MW – limited diffusion across lipid membranes
- Applied to market age and juvenile goats
- Now studying meat versus milk goats



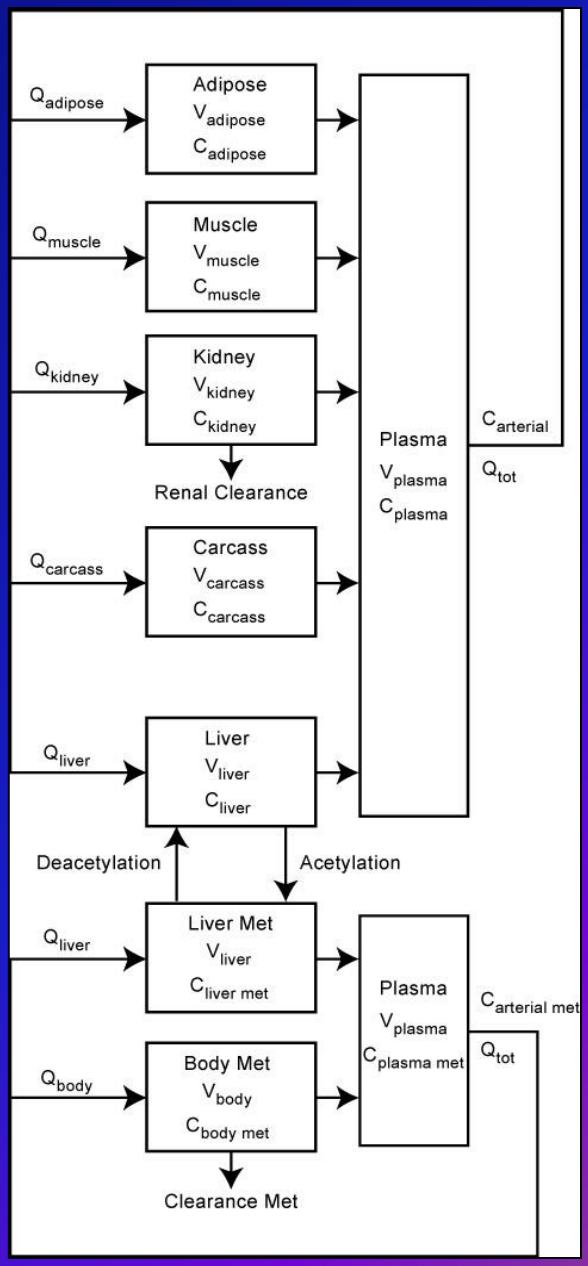
A Flunixin PBPK Example: Work in Progress



Sulfamethazine Swine PBPK Model



- Entry into intestine is governed by gastric emptying time (Kst)
- Entry into liver is governed by rate of absorption (Ka)



Burr, Baynes and Riviere, Am.J.Vet.Res. 66:1686-93, 2005

Sulfamethazine Swine PBPK Model

$$\frac{dC_{muscle}}{dt} = \frac{[C_{arterial} - (C_{muscle}/P_{muscle})] \bullet Q_{muscle}}{V_{muscle}}$$

$$\frac{dC_{adipose}}{dt} = \frac{[C_{arterial} - (C_{adipose}/P_{adipose})] \bullet Q_{adipose}}{V_{adipose}}$$

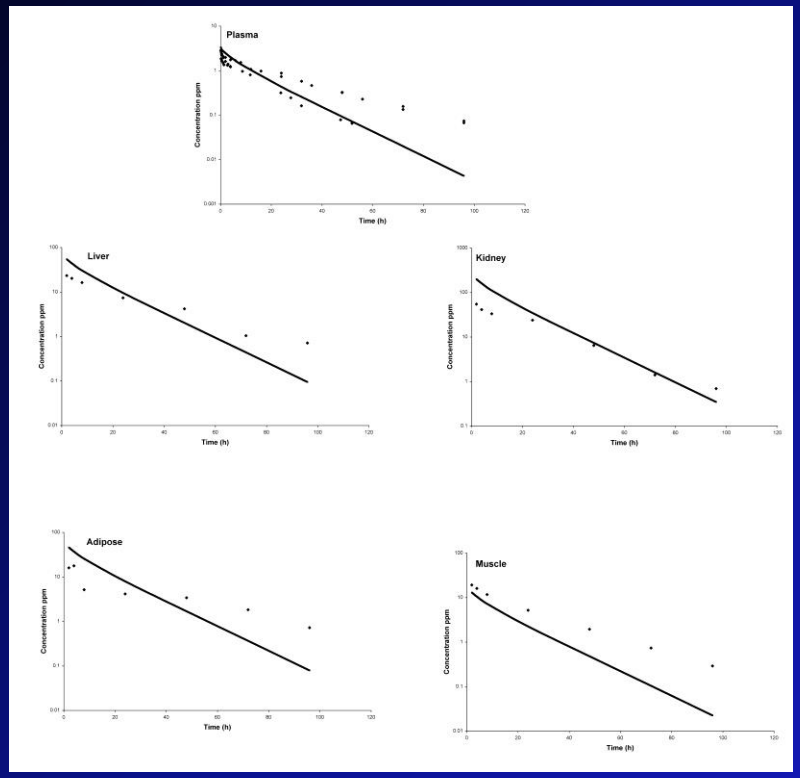
$$\frac{dC_{carcass}}{dt} = \frac{[C_{arterial} - (C_{carcass}/P_{carcass})] \bullet Q_{carcass}}{V_{carcass}}$$

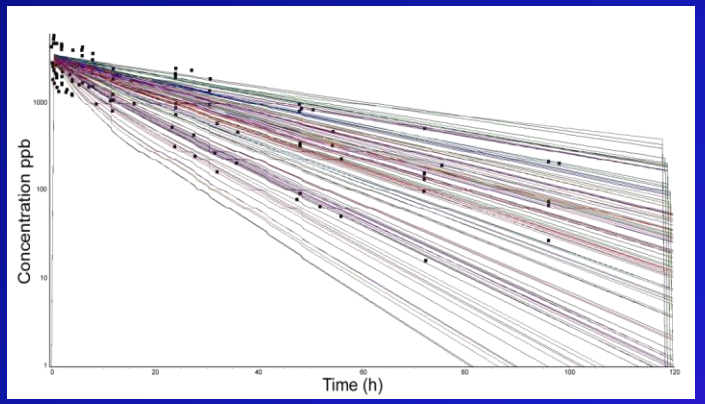
$$\frac{dC_{kidney}}{dt} = \frac{[C_{arterial} - (C_{kidney}/P_{kidney})] \bullet Q_{kidney} - (C_{arterial} \bullet CL_{renal})}{V_{kidney}}$$

$$\frac{dC_{liver}}{dt} = \frac{[C_{arterial} - (C_{liver}/P_{liver})] \bullet Q_{liver} - (C_{liver} \bullet CL_{acytlation}) + (C_{liver\ metabolite} \bullet CL_{deacytlation})}{V_{liver}}$$

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

Added population variation in Monte Carlo 1000 animal simulation





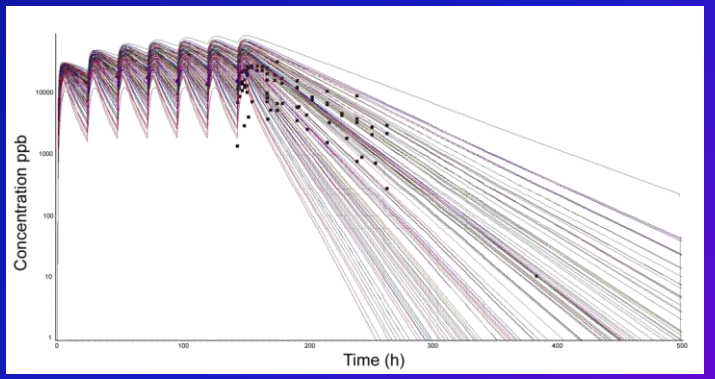


TABLE 2. Final distributions for sensitive parameters used in the Monte Carlo analysis

Parameter (units)[a]	Mean	Mean (transformed)	Variance	Lower bound[d]	Upper bound[d]	References
K_a (1/h)	0.1	−1	0.88	0.0682	3.01	24, 34, 39
K_{st} (1/h)	0.1	−1	0.4	0.0183	1.05	19, 24
CL hepatic[b] (ml/min/kg)	0.39	−0.4	0.32	0.05	1.5	29, 30, 39, 44
P binding SMZ[c] (%)	0.42	−0.38	0.1	0.37	0.99	25, 26, 29, 30, 31
P binding met[c] (%)	0.35	−0.45	0.11	0.34	0.92	25, 26, 29, 30, 31

Burr, Baynes, Smith and Riviere, Antimicrob Agents Chemother 50: 2344-2351, 2006

Simulation then assessed potential residue violations based on population data.

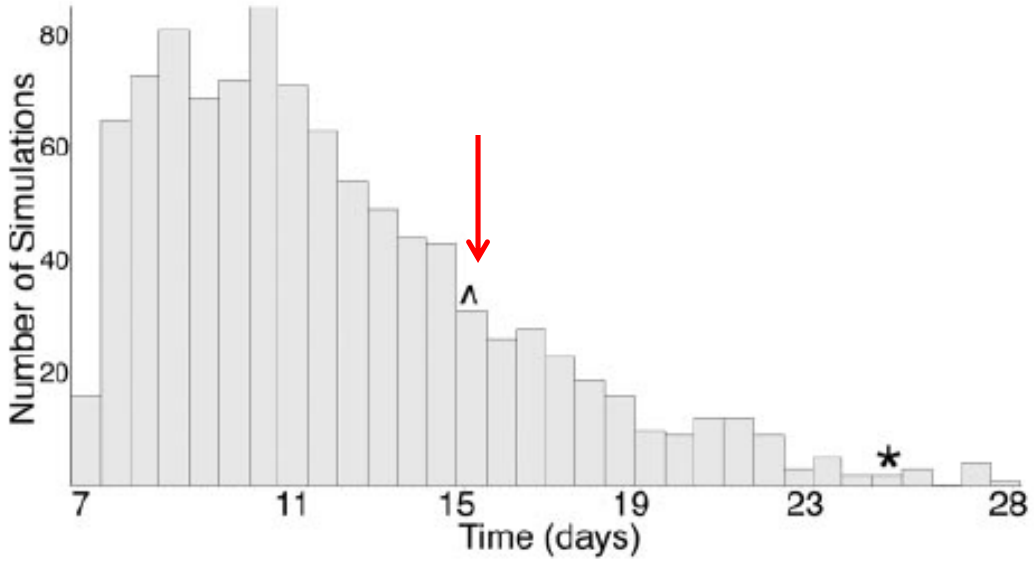
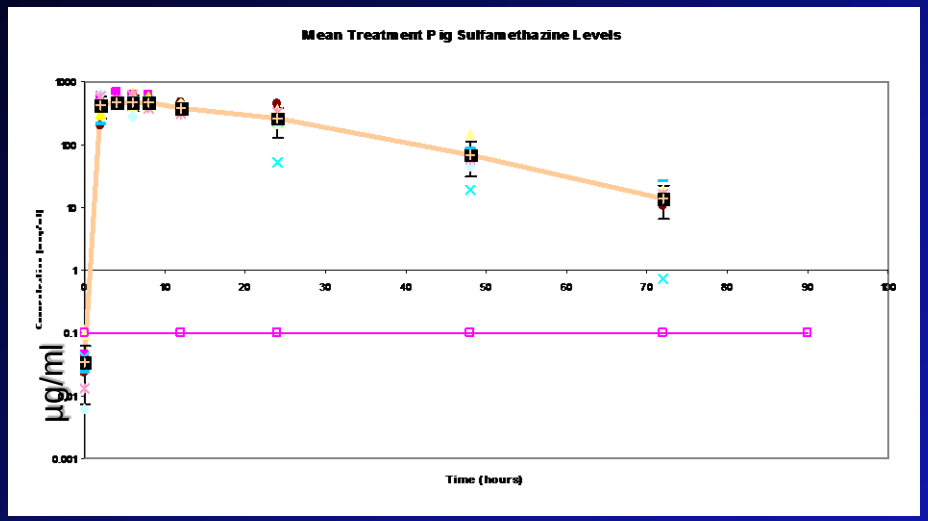
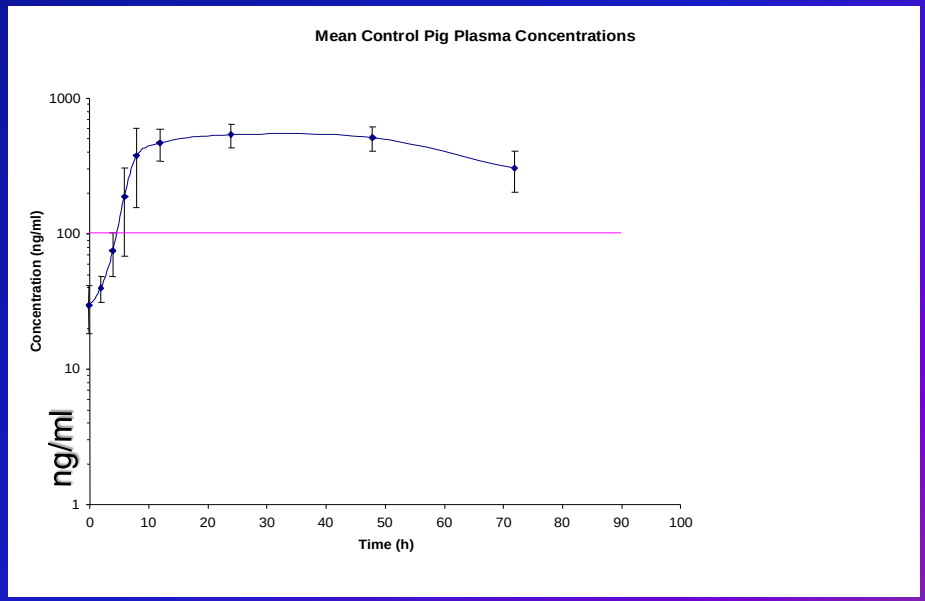


FIG. 5. Representative distribution of the time that it takes for sulfamethazine concentrations to fall below the tolerance of 0.1 ppm in kidney tissue from a Monte Carlo run of 1,000 simulations. *, 99th percentile of the distribution; ^, current withdrawal time of 15 days.

Sulfamethazine Cross-Pen Water Contamination in Pig Production Unit



Used SMZ PBPK model to estimate dose required to match observed plasma levels (ng/ml) in **untreated** pen mates. Very low levels driven by analytical methods.

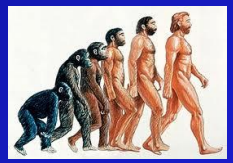


0.15 mg/kg followed by continuous lower exposure at 0.059 mg/kg matched observed exposure.

Mason, Baynes, Buur, Riviere, Almond. J. Food Prot. 71, 584-589, 2008

Discussion

- Fundamental scientific issue is that tissue residues are very low concentration, “down-stream” from systemic circulation, and thus affected by factors modulating plasma disposition.
- Numerous factors (production class, age, disease, housing) may influence systemic PK and the observed tissue residue profile.
- Modern 21st century pharmacometric techniques can be used to estimate withdrawal times using sound science and statistical inference in more realistic field use scenarios, providing insight into where problems may occur.
- How do regulatory methods evolve to keep pace with modern science (or at least remain only a few decades behind!)


Discussion

- Pharmacometrics is desperately needed in veterinary medicine because of the large biological variability coupled with the reduced ability to do large trials. We must simulate to figure out what is going on!
- Population databases of animals exist. How can we use these data to more precisely model inference space?
- One could propose using PK models to predict withdrawal times based upon mechanistic models and relevant exposure scenarios and then validate them using trials acceptable to regulatory agency
- Human food safety concerns and increased improvements in analytical chemistry will continue to drive this field.

Animal Health Modeling & Simulation Society

- AHM&S is a new association founded in 2012 that aims to promote the development, application, and dissemination of modeling and simulation techniques in the field of Veterinary Pharmacology and Toxicology.
- The objective of AHM&S is to use in silico/mathematical model-based approaches for a better integration and understanding of quantitative pharmacology in veterinary and comparative biomedicine.

Animal Health Modeling & Simulation Society

- Examples of issues to be dealt with are applications of quantitative pharmacology, dosing regimen determination, withdrawal time determination, trial design and analysis and disease modeling.
- AHM&S held its first live meeting at the University of Strathclyde, Glasgow, Monday in conjunction with this PAGE meeting.

The End