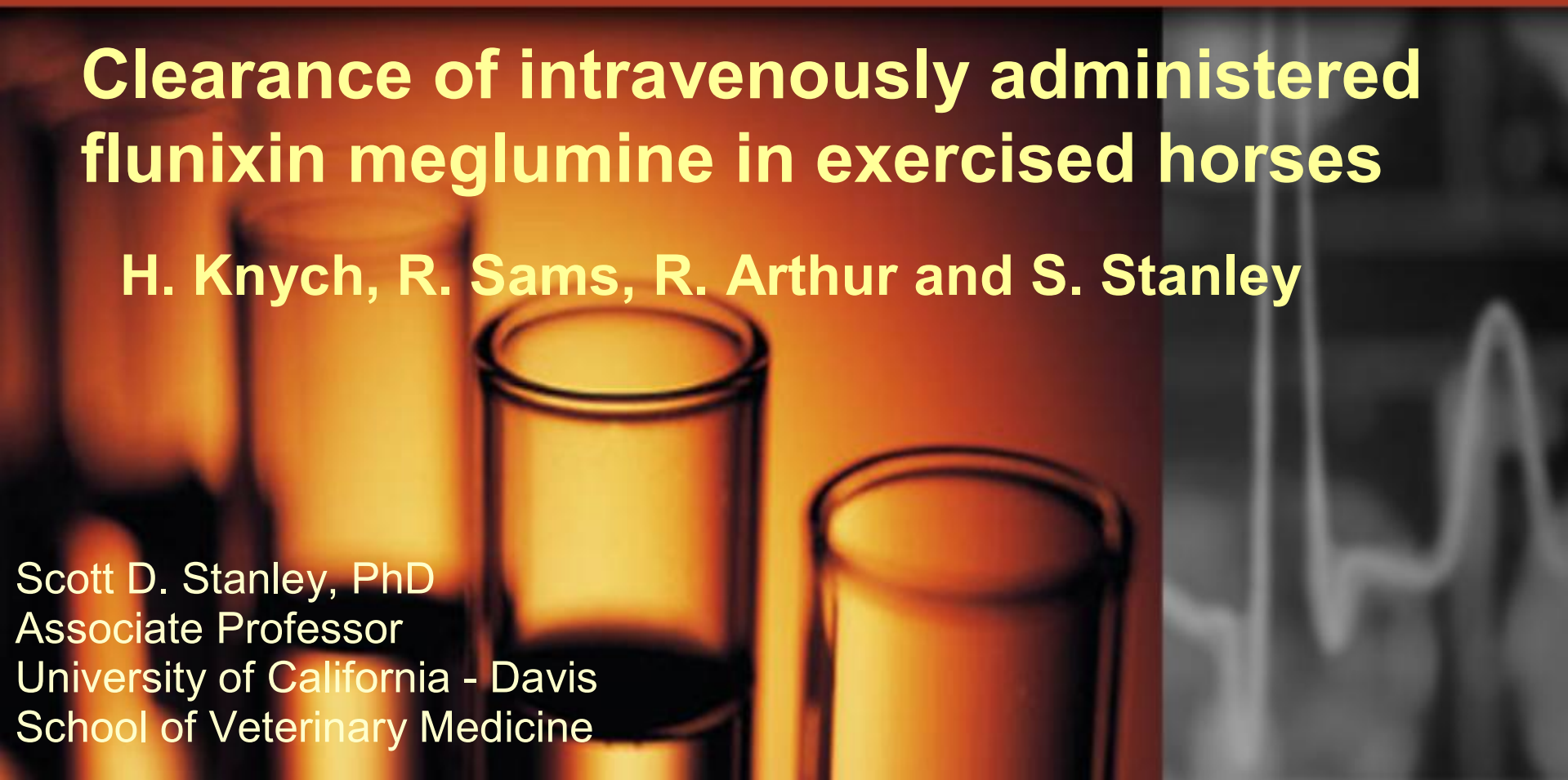
Regulating Therapeutic Drugs in California Thoroughbred Racing



Clearance of intravenously administered flunixin meglumine in exercised horses

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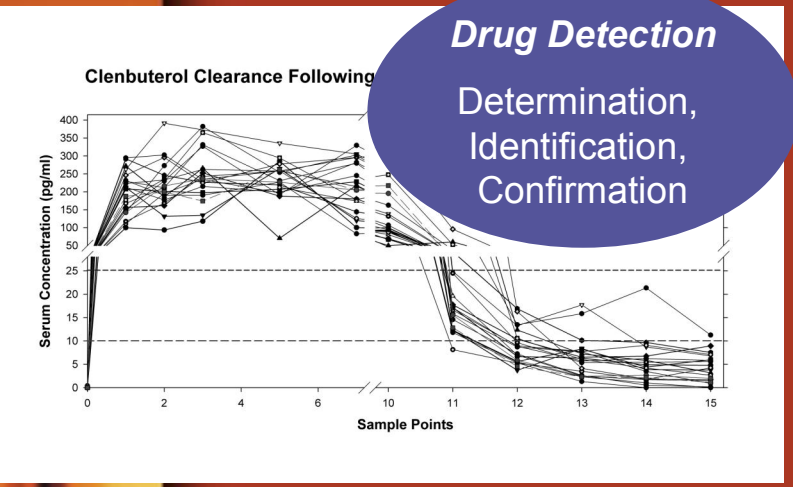
Equine Chemistry Research

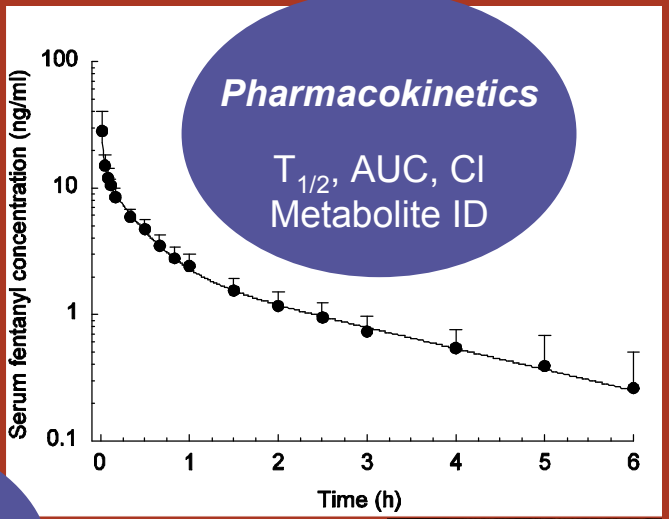
Drug Detection

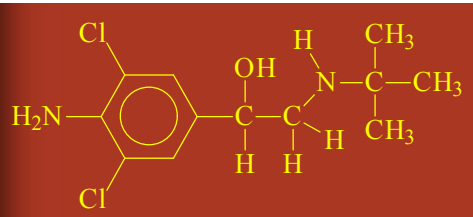
Determination,
Identification,
Confirmation



Pharmacokinetics

$T_{1/2}$, AUC, Cl
Metabolite ID

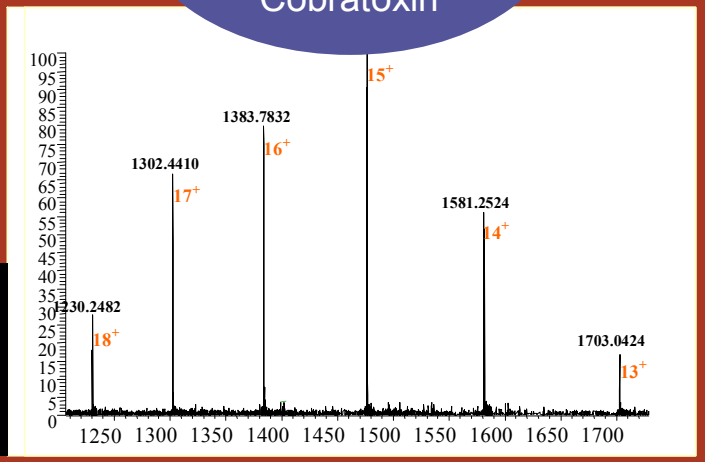




Clenbuterol m/z 240

Proteomics

Erythropoietin,
Growth Hormone,
Cobratoxin



Wha

THE AMERICAN
ASSOCIATION
FOR LABORATORY
ACCREDITATION

ACCREDITED LABORATORY

A2LA has accredited

EQUINE ANALYTICAL CHEMISTRY LABORATORY
Davis, CA

for technical competence in the field of

Chemical Testing

The accreditation covers the specific tests and types of tests listed on the agreed scope of accreditation. This laboratory meets the requirements of ISO/IEC 17025 - 1999 "General Requirements for the Competence of Testing and Calibration Laboratories" and any additional program requirements in the identified field of testing.

Presented this 13th day of September 2004.

President
For the Accreditation Council
Certificate Number 2205-01
Valid to September 30, 2006

For tests or types of tests to which this accreditation applies, please refer to the laboratory's Chemical Scope of Accreditation.

RMTC
Goals and Objectives

- **Uniform rules**
- **Coordinated national research program**
- **Uniform testing**
- **Uniform thresholds and withdrawal guidelines for therapeutic medications**
- **Improved security measures**

Model Policy

- Voluntary Administration of Furosemide
  - No exam requirement
- Urine Specific Gravity and Serum Thresholds to Control Furosemide Adm.
  - 1.010 specific gravity / 100 ng per ml
  - Administration guideline – max. dose of 500 mg and minimum of 150 mg, IV only, 4 hrs prior to post

Model Policy

- Use of 1 of 3 NSAIDs
  - PHENYLBUTAZONE
    - Plasma threshold - 5 μg/ml
  - Recommend administration of not more then 2.0 gram; 24 hours before post with dose administered intravenously

Model Policy

- Use of 1 of 3 NSAIDs
  - KETOPROFEN
    - Plasma threshold - 10 ng/ml
  - Recommend administration of not more then 2.2 mg/kg; 24 hours before post with dose administered intravenously

Model Policy

- Use of 1 of 3 NSAIDs
  - FLUNIXIN
    - Plasma threshold - 20 ng/ml
  - Recommend administration of not more then 500 mg; 24 hours before post with dose administered intravenously

What’s the problem!

CHRB Rule 1843…

- In 2005, the CHRB adopted the RMTC Model Rules for Race Day Medication and decreased the existing threshold for Flunixin.
- Flunixin is a therapeutic medication used routinely by CA equine practitioners for the treatment of musculoskeletal disorders in race horses.

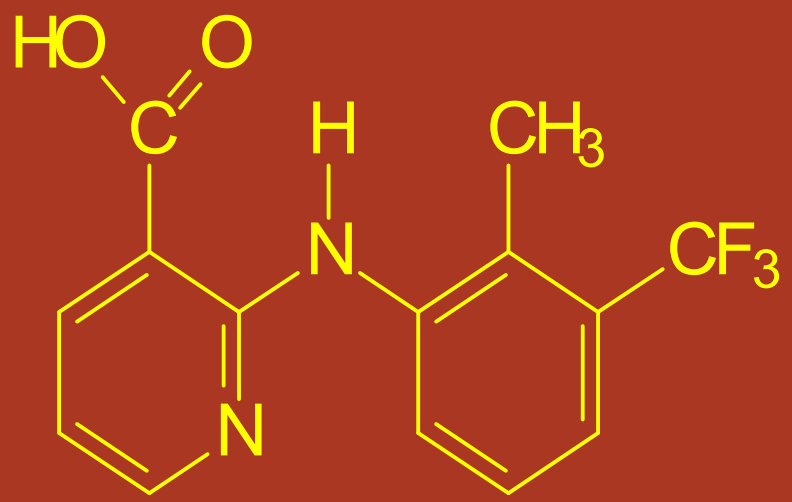
Study Objectives:

- Assess the RMTC recommended plasma threshold of 20 ng/ml following Flunixin administered intravenously at 24 hours
- Establish secondary plasma threshold for Flunixin following administered intravenously

Flunixin Meglumine

- Non-Steroidal Anti-Inflammatory Drug
- In horses, it is primarily used for its anti-inflammatory properties
- Several formulas are available
  - Flunixamine®, injectable preparation designed for intravenous administration
- Previously prohibited in most race horses in US
- Regulated via plasma at several concentrations
  - 20, 100, 500 & 1000 ng/mL

Flunixin



- Rapidly cleared from plasma
- Small volume of distribution
- Short half-life ~ 1-2 h
- Highly protein bound

Flunixin

- More rapid onset of analgesic effect than PBZ
- Effects last 24-36 hours
- Administered once daily
- Dose: <u>1.1 mg/kg orally or parenterally</u>

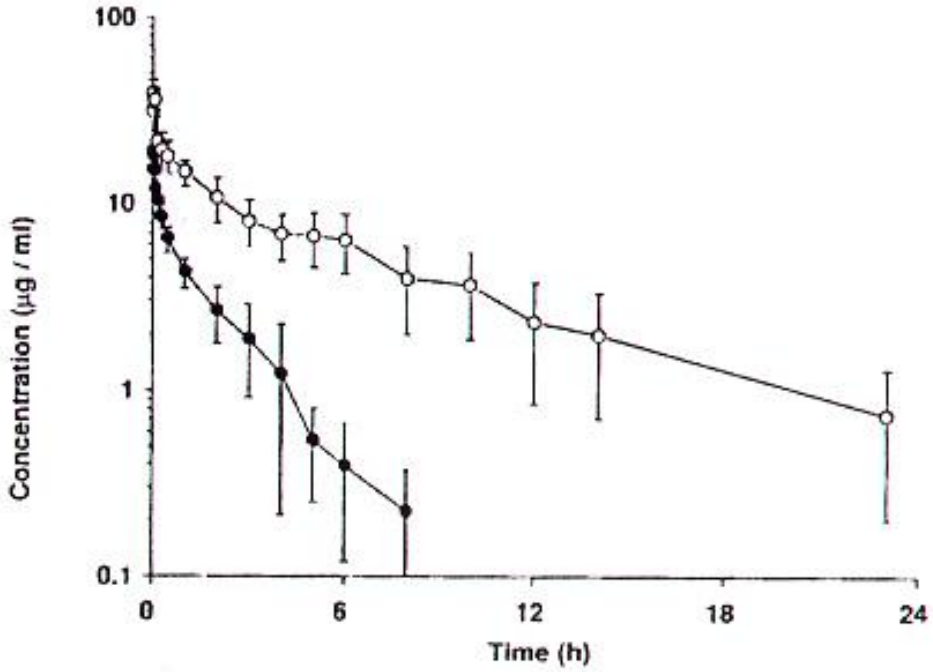


Fig. 2. The semilogarithmic plot of plasma time–concentration for phenylbutazone (○) and flunixin meglumine (●) after an intravenous administration of phenylbutazone (4 mg/kg) and flunixin meglumine (1 mg/kg) into the same five horses (mean ± SD).

J. vet. Pharmacol. Therap. **17**, 459–469, 1994

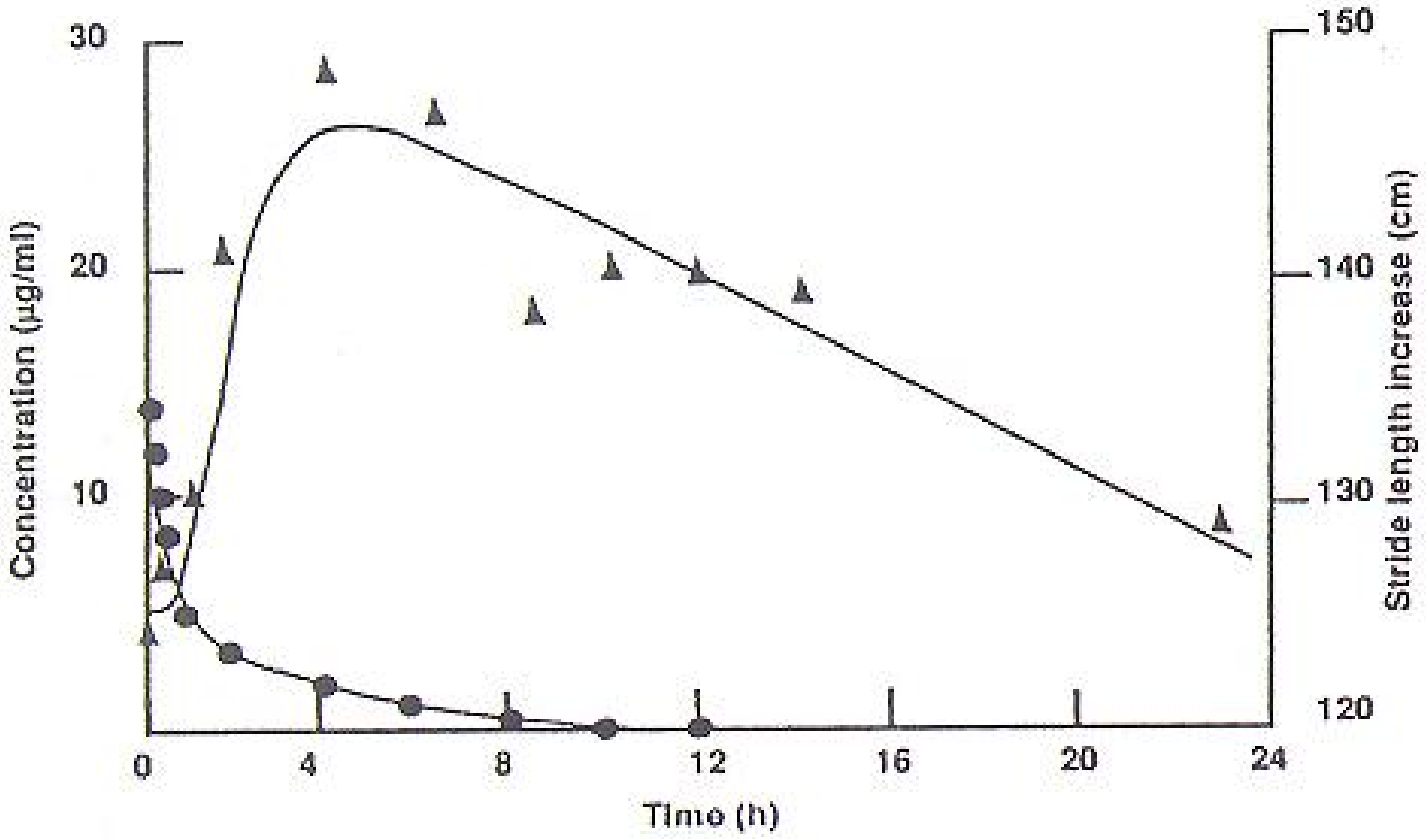


Fig. 5. Fit of the time course of flunixin meglumine (●) and stride length (▲) after an intravenous administration of flunixin meglumine (1 mg/kg) in a representative horse with adjuvant-induced carpitis.

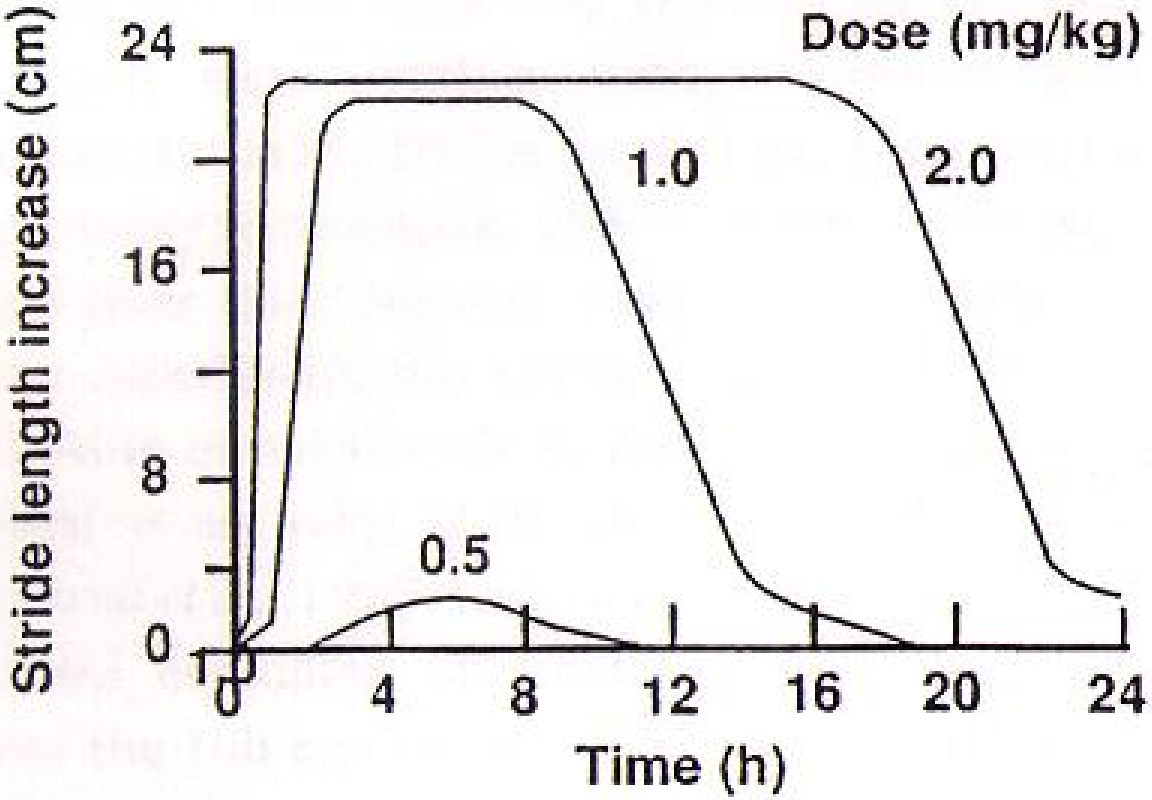
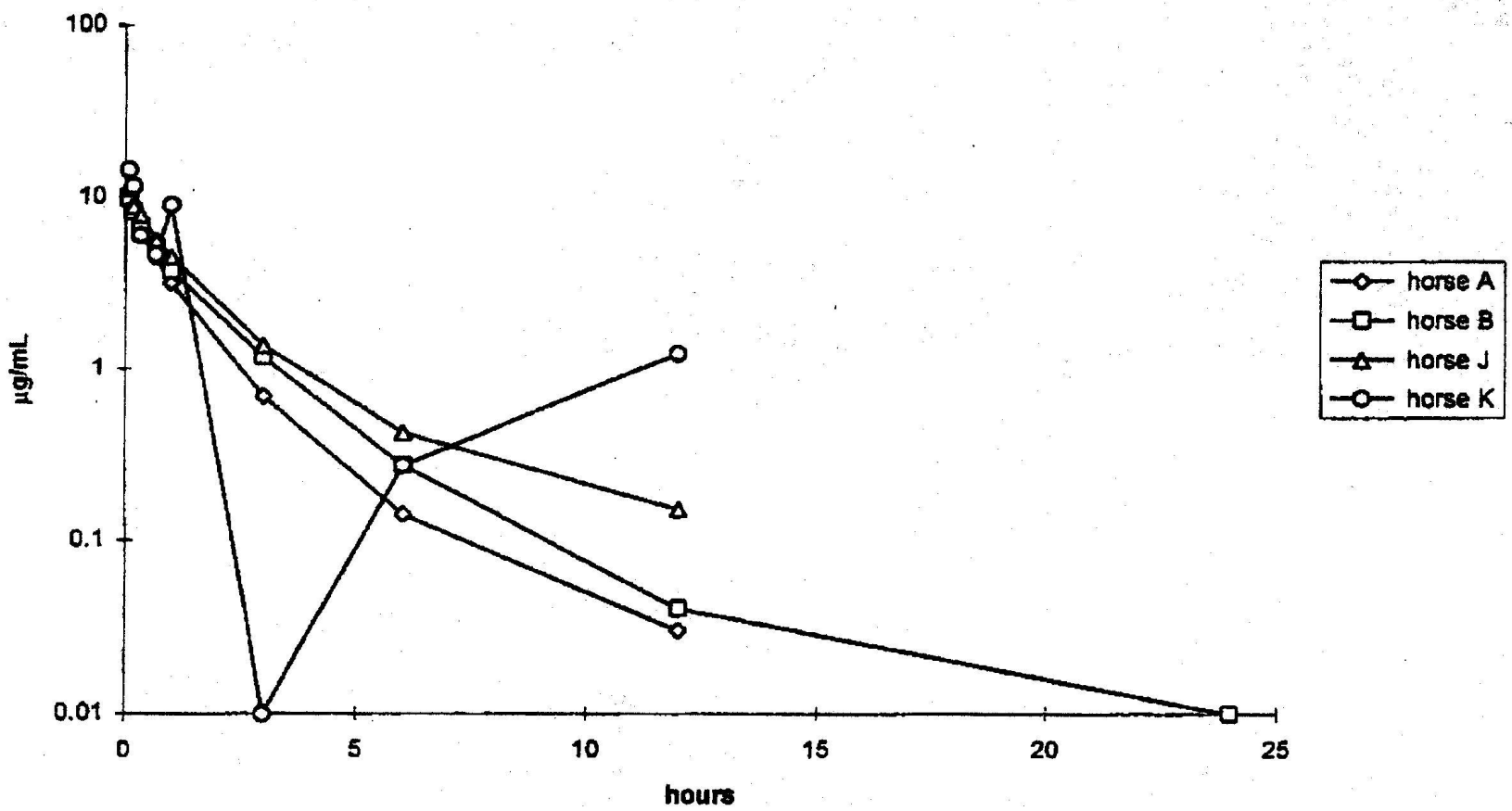


Fig. 7. Dose–effect relationship for flunixin meglumine, predicted by simulating a PK/PD model with mean pharmacokinetic and pharmacodynamic parameters obtained from five horses with adjuvant-induced carpitis.

Fig.5 Plasma flunixin concentrations following a single 500 mg IV administration



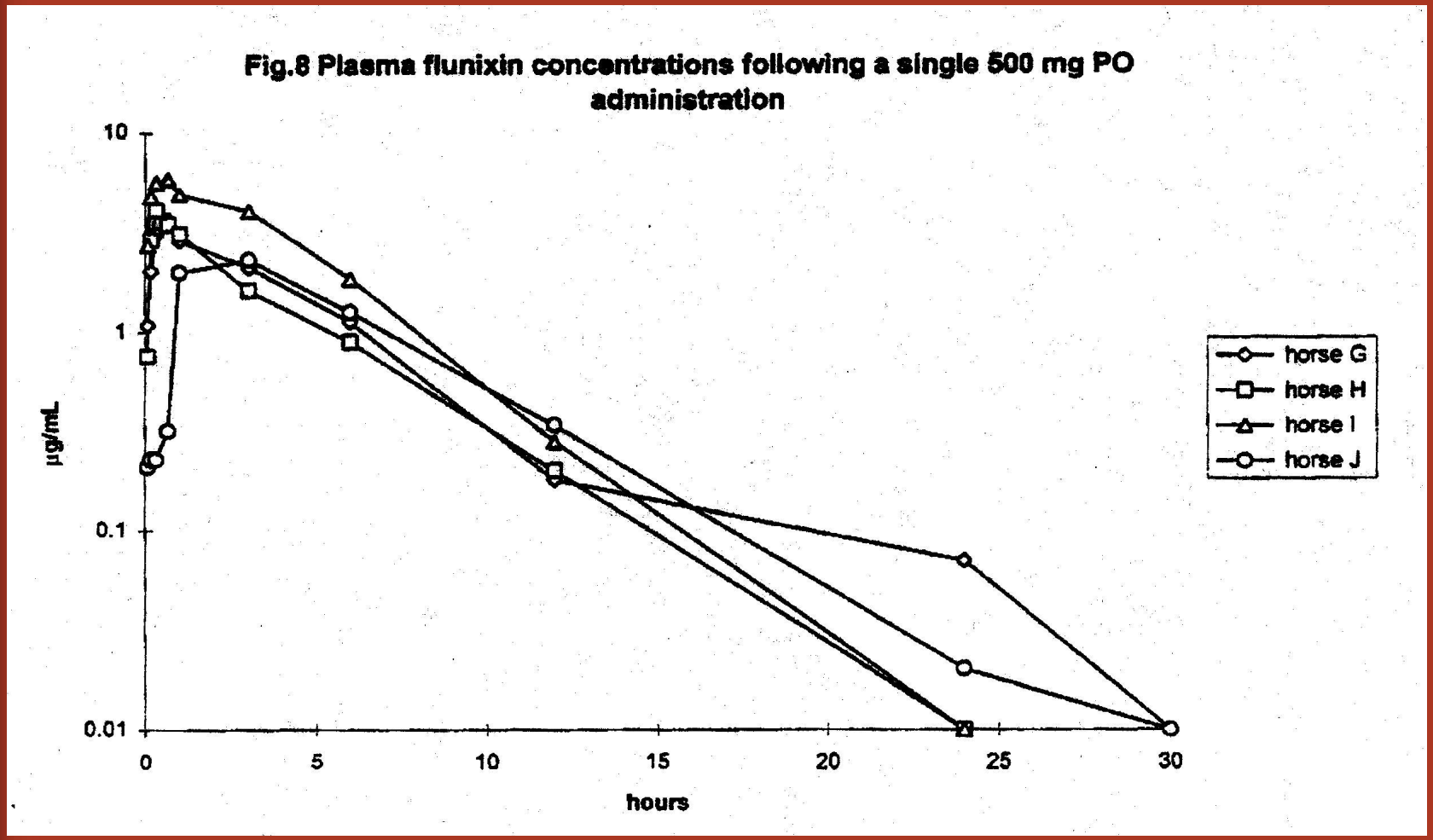


Fig.8 Plasma flunixin concentrations following a single 500 mg PO administration
10
1
0.1
0.01
µg/mL
0
5
10
15
20
25
30
hours
horse G
horse H
horse I
horse J

Study Design:
Phase 1

- Administered to 6 three years old TB, fasted for 12 h
  - IV catheter admin. of 500 mg of Flunixin
  - Assess post-administration plasma concentrations 2, 4, 6, 8, 12, 24 and 48 hrs
- Administered to 12 TB horses in training
  - 500 mg of Flunixin, IV administration
  - Assess post-administration plasma concentrations 6, 24 and 48 hrs

Phase 1: Analysis

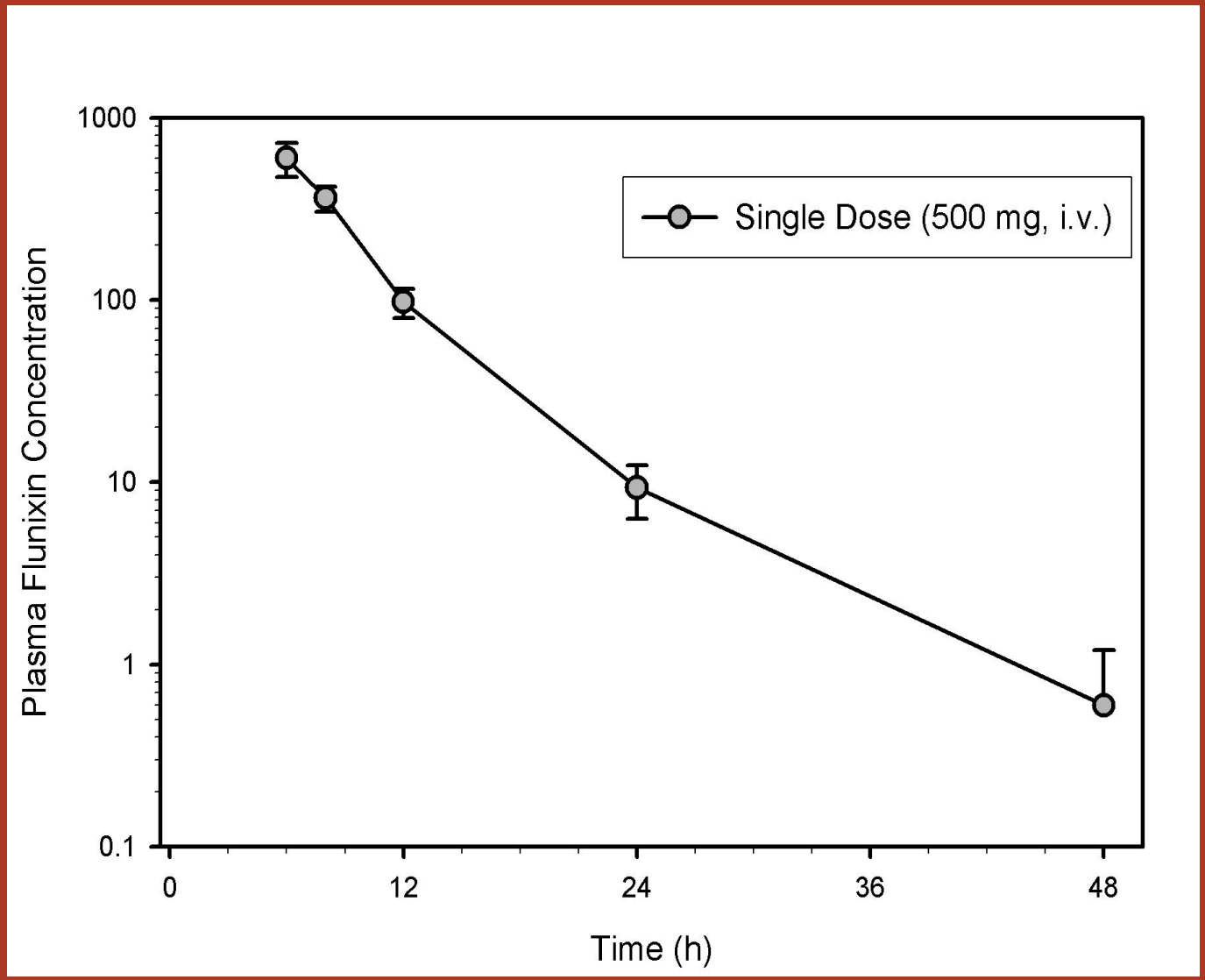
- Sample analysis
  - Plasma was collected at 0, 6, 8, 12, 24 and 48 hours after drug administration
  - Stored at –20°C until analysis by LC-MS for flunixin
- Pharmacokinetic analysis
  - Noncompartmental analysis
  - Bioequivalence testing, using C_{max}, AUC_t, and MRT_t

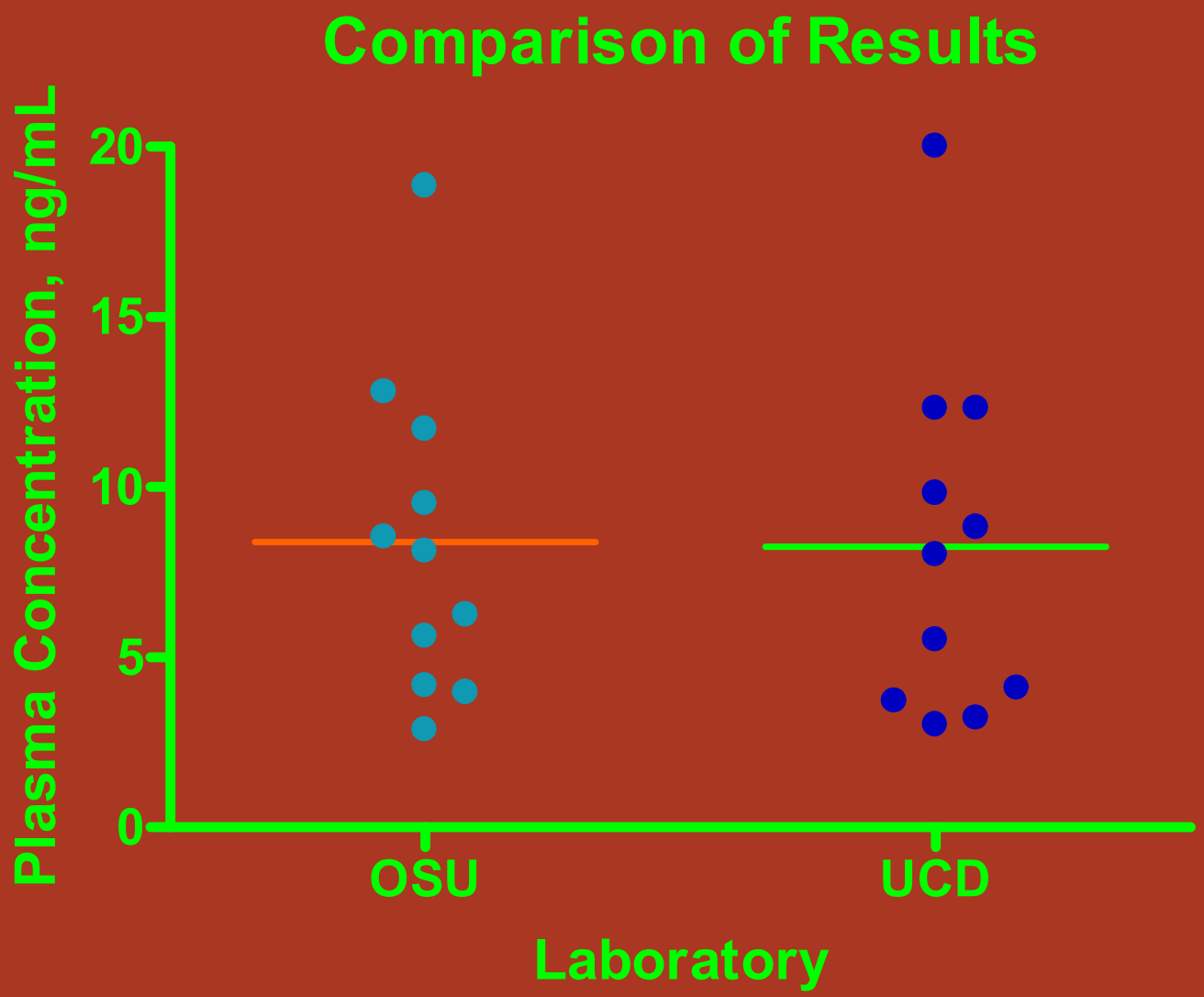
Pharmacokinetic Results

Parameter	Flunixin
C_{max} (ng/mL)	13.8 ± 1.1
T_{max} (h)	6.6 ± 0.8
$t_{1/2}$ (h)	2.2 ± 0.6
AUC_t (ng·h/mL)	12.5 ± 3.2
AUC (ng·h/mL)	15.0 ± 3.7
MRT_t (h)	1.5 ± 0.3
MRT(h)	6.6 ± 0.8

Flunixin Pharmacokinetics

- Verified the traditional studies of the kinetics of the disposition of plasma flunixin
- Plasma concentration versus time plots following single doses of flunixin


Plasma Flunixin Concentration
1000
100
10
1
0.1
0
12
24
36
48
Time (h)
Single Dose (500 mg, i.v.)


Comparison of Results
Plasma Concentration, ng/mL
20
15
10
5
0
OSU
UCD
Laboratory

Plasma Conc. Following a Single Dose of Flunixin

- Using the data for flunixin in plasma conc. at 24 hr are 9.3 ± 6.2 ng/mL (mean ± SD)
- The plasma conc. of 99.9% of the population are expected to fall within the range of 0 - 48 ng/mL by 24 hr after IV admin. of 500 mg of flunixin

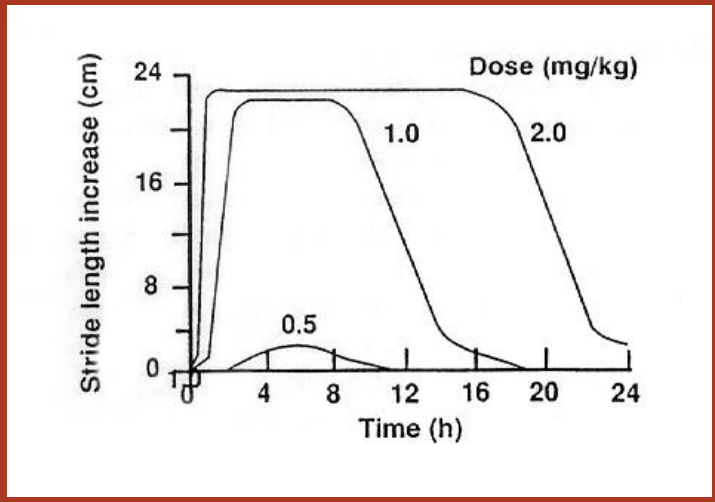
- Statistical Analysis (Type I drug):
  - Obtain plasma concentration versus time data at the target withdrawal time (recommend data from 20 horses as a goal).
  - Perform $\log_e$ transformation of data.
  - Calculate mean and standard deviation of log-transformed data.
  - Calculate mean + k x SD where k is a constant corresponding to the 95% confidence interval for the 95th percentile of the distribution of the data – non-central *t*-distribution.
  - Calculate the conc. corresponding to this value.
  - See following example for flunixin:

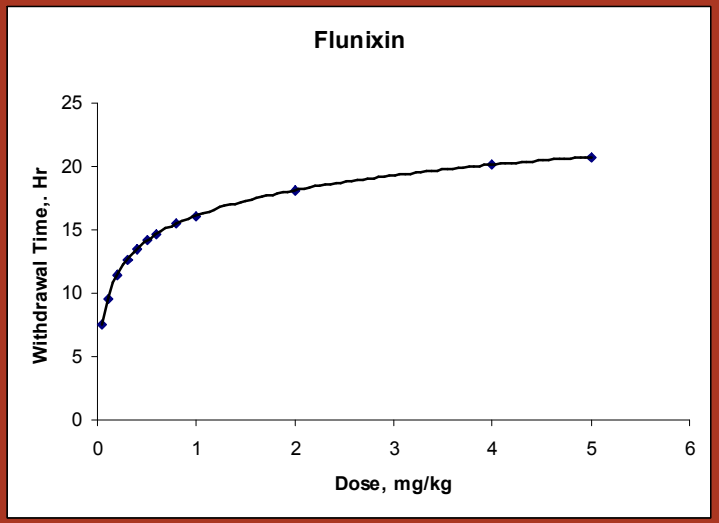
Type I Calculation (UCD)

	N = 12	N = 31
Mean	2.5849	2.3307
SD	0.6663	0.6324
Mean + k x SD	4.869	4.089
Concentration, ng/mL	47.4	40.9

Assessment of PK/PD Effects

- Examine PK/PD data to assess probability that a lower clinically effective dose could be administered within the withdrawal period.
- Note, the greatly diminished clinical effect of flunixin when administered at doses less than 1.0 mg/kg.





Establishing Thresholds

- European (Toutain) Model
- Sedentary Horse Model
- Racehorse Model

European (Toutain) Model

- Threshold determined by non-experimental approach:
  - Uses data in the literature (meta-analysis)
  - Plasma clearance is used to transform the effective dose into Effective Plasma Conc.
  - EPC is transformed into Irrelevant Plasma Conc. by applying a safety factor
  - Large measurement uncertainty (SF of 500; 50 for transformation x 10 for inter-individual variation)

European (Toutain) Model

- Assume drug disposition is linear
- $IPC_{Flunixin}$ = 764/500
- Predicted Threshold Value = 1.5 ppb
- Predicted Withdrawal Time – 4 days
- Inadvertent Positives - ???

Sedentary Model

- Threshold determined by using older non-exercised horses:
  - limited number (N = 4)
  - analytical method – direct measure of plasma conc.
  - no measurement uncertainty
- Probable Threshold Value = 20 ppb
- Recommended Withdrawal Time = 24 hr
- Inadvertent Positives - ???

Racehorse Model

- Threshold determined by:
  - Using larger number of TB horses in training (N = ~20 with many post admin. samples)
  - Validated analytical method – exact plasma conc.
  - Calculated measurement uncertainty (>99% confidence interval)
- Threshold Value = 50 ng/mL

Acknowledgements

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- Amel Clifford
- Kris Lomas

Thank You!