

ONGOING STUDIES:

- Blood and Urine Concentrations and Pharmacokinetics* of Flunixin Meglumine Following Transdermal Administrations to Horses (H. Knych, University of California, Davis)

A topical flunixin product (Banamine Transdermal™) has FDA-approval for use in cattle and is commercially available by a veterinarian's prescription. Flunixin is a potent non-steroidal anti-inflammatory drug (NSAID) that has the potential to mask signs of musculoskeletal disease resulting in risk of serious injury if administered to physically compromised horses scheduled to race or train. This project is being conducted to determine whether topical application of flunixin in the horse can be detected by current testing methods in order to ensure control of the use of this medication by this route of administration in proximity to a race.

*Pharmacokinetics is the study of how a substance is absorbed, distributed, metabolized, and eliminated by the body. This knowledge informs among other things if, and how, a substance can be regulated by drug testing. As an example, if a substance is exclusively eliminated in the urine as a metabolite (breakdown product) of the substance administered, testing for the parent (unmetabolized) drug in urine will be ineffective in detecting that the drug was administered.

- Bisphosphonates and Fatal Musculoskeletal Injury (H. Reesink, Cornell University)

Because of bisphosphonates' effects on bone metabolism, concerns have been raised about a potential relationship between their use in young horses and risk of fracture injury. This project includes a retrospective study to estimate the prevalence of bisphosphonate administration in Thoroughbred racehorse in New York, applying liquid chromatography (LC/MS/MS), and sophisticated imaging techniques including NMR, CT, micro-CT, x-ray absorptiometry and spectroscopy to bone fracture fragments. The imaging will evaluate the effects of bisphosphonates on bone remodeling in the young Thoroughbred racehorse. In the second phase of the project, these same methods will be applied to determine and compare the presence of two bisphosphonates** in horse blood, urine, bone, hair, and hoof tissue after single and repeated administrations.

**The two bisphosphonates being studied are clodronate and tiludronate both of which have FDA-approval for use in the horse and are more commonly known by their trade names: OsPhos™ and Tildren™.

- Ketoprofen blood and urine concentrations, pharmacokinetics and effects on biomarkers of inflammation following intravenous administration to exercised horses. (H. Knych, University of California, Davis)

Ketoprofen, commonly known by the trade name Ketofen™, is a non-steroidal anti-inflammatory drug (NSAID) that has FDA-approval for use in the horse. Its use is currently prohibited within 48 hours of a race to prevent its anti-inflammatory effects from impacting the findings of pre-race examinations or horse and jockey/driver safety and welfare during a race. This study is determining the duration of ketoprofen's anti-inflammatory effect by assessing concentrations of inflammatory biomarkers⁺, over time following a single dose of ketoprofen. A controlled laboratory model of inflammation is being used and the suppression of markers of inflammation including beta thromboxane, prostaglandins E₂ and F_{2α}, Leukotriene B₄ is being

measured. Blood and urine concentrations of ketoprofen will be measured at various timepoints after administration, potentially establishing a correlation between concentration and effect.

⁺ Biomarkers are substances produced by the body and can be quantitated for an objective measurement of an inflammatory response to an experimental stimulus or clinical condition. Measuring the suppression of biomarkers over a period of time following the administration of a medication allows investigators to determine the duration and extent of the medication's effect.

- **Transcriptomics[‡] of the Detection of EPO Micro Dosing (H. Knych, University of California, Davis)**

The administration of erythropoietin, often simply referred to as 'EPO', constitutes blood-doping, a prohibited practice for its performance enhancing effects. Currently, the most common approach to detect administration of recombinant human erythropoietin (rHuEPO) in racehorses is immunoassay followed by confirmation using liquid chromatography tandem mass spectrometry (LC-MS/MS). Regulators have raised concerns that this testing method is not sufficiently sensitive as they continue to receive intelligence regarding the use of EPO microdosing—the administration of much smaller doses but at more frequent intervals. The goal of microdosing is to elicit the desired effect while successfully evading detection because the concentration of the EPO present is below the level that can be detected by current test method sensitivity. This project is investigating a combination of transcriptomic analysis and liquid chromatography tandem mass spectrometry as an approach to detect illicit administration of micro-doses of erythropoietin in horses. Investigators will determine the transcriptomic profile of peripheral blood following administration of micro doses of recombinant erythropoietin (rHuEPO) to horses. The pharmacokinetics of rHuEPO following a micro dosing protocol are also being examined.

Transcriptomics[‡] uses RNA analysis to determine genetic activity which can in turn be used to determine which genes are activated or suppressed as a consequence of a specific disease, healing and recovery, or the administration of medications. Rather than demonstrating the presence of a medication, transcriptomics may define a specific 'fingerprint of effect' that applies to an entire class of drugs.

- **Perineural[§] Liposomal Bupivacaine in Exercised Lameness Horses (T. McCarrell, University of Florida)**

Liposomal bupivacaine is an injectable local anesthetic used to control pain in human patients for up to 72 hours. Other local anesthetics have a far shorter duration of effect and would require same day administration for a local anesthetic effect during a horse's race. This study uses a reversible lameness model to examine effective dose and duration of effect of liposomal bupivacaine in the horse. Blood and urine samples will be analyzed at the University of Florida Racing Laboratory to determine testing methods to ensure control on the use of this drug in proximity to a horse's race.

[§]Perineural administration refers to the application of a substance close to a nerve. When a local anesthetic (e.g. Novocaine) is injected perineurally the conduction of impulses through the nerve is temporarily halted and the entire region that is supplied by that nerve loses sensation. If a horse is lame and a veterinarian performs a diagnostic nerve block that eliminates sensation to the foot and the horse no longer shows lameness, the cause of the lameness is isolated to somewhere in the foot, allowing the veterinarian to perform a more targeted and thorough examination. The use of local anesthetics is controlled in horseracing to prevent illicit use in blocking pain that could enable a physically compromised horse to race resulting in risk of severe injury.

COMPLETED STUDIES:

- L- and D-threo ethylphenidate concentrations, pharmacokinetics and pharmacodynamics in horses (funding also provided by AQHA and California Horse Racing Board (H. Knych, University of California, Davis; P. Hartmann, Industrial Laboratories)
Ethylphenidate is a potent stimulant with effects similar to amphetamine; it is considered a Performance Enhancing Drug (PED) and its use is prohibited.
- Improved Detection of Erythropoiesis-Stimulating Agents (ESAs) (B. Moeller, University of California, Davis)
ESAs represent another blood-doping threat and can come in many forms. The identification of their common effect—artificially increasing the horse’s own production of erythropoietin—can be used to identify samples for additional targeted testing to detect the specific blood-doping agent.
- Administration Study of the Selective Androgen Receptor Modulator (SARM) LGD-4033 in the Horse (S. Stanley, University of California, Davis)
SARMs cause a body to increase synthesis of its own anabolic steroids. As the administration of anabolic steroids is prohibited in racing, the use of SARMs represents an illicit effort to induce the desired anabolic effect and evade regulatory consequences.
- Pharmacokinetics and Selected Pharmacodynamic[#] Effects of Phenylbutazone Following Intravenous and Oral Administration to Exercised Horses (H. Knych, University of California, Davis)
Inflammatory biomarker suppression was used to assess the duration and extent of the anti-inflammatory effect of phenylbutazone, commonly referred to as Bute.

[#] Pharmacodynamics examines a substance’s the effect.
- Intravenous Dimethyl Sulfoxide (DMSO) Administration and Withdrawal Guideline Determination – A Clinical Study (J. Blea, S. Hay, F. Northrop, R. Arthur (University of California, Davis), D. Benson (RMTC), A. Kind (Texas A&M), & P. Hartmann (Industrial Laboratories)
DMSO is a non-steroidal anti-inflammatory commonly use in treating racehorses either by topical application to a specific site, or systemic (oral or intravenous) administration for generalized inflammatory conditions.
- Custom Synthesis and Certification of a Dermorphin Reference Standard for Laboratory Use (R. Sams, HFL Sport Science)
Dermorphin, often referred colloquially as Frog Juice, is a potent opioid-like pain reliever and was used illicitly to mask horse injuries.

- **Detection of Extracorporeal Shock Wave Therapy in the Race Horse Using Biomarkers (M. Robinson, University of Pennsylvania)**
Extracorporeal Shock Wave Therapy (ESWT) can promote healing in cases of chronic musculoskeletal disorders, but also induces a limited period of pain relief. If applied in proximity to a race there is the potential for pain and injury to be masked, and for this reason its use is prohibited for 10 days prior to a race. This study investigated whether ESCW use could be controlled through laboratory testing.
- **The Pharmacokinetics of Dexamethasone following Intravenous, Intra-Articular, and Oral Administration (L. Soma, University of Pennsylvania)**
Dexamethasone is a corticosteroid, commonly referred to as Azium. This project facilitated development of threshold and withdrawal guidance to permit therapeutic use while also ensuring the medication did not impact a horse's racing performance.
- **The Pharmacokinetics of Prednisolone following Intravenous Administration (R. Sams, HFL Sport Science)**
Prednisolone is a corticosteroid, that can be administered orally or by injection. The injectable formulation is commonly referred to as SoluCortef. This project facilitated development of threshold and withdrawal guidance to permit therapeutic use while also ensuring the medication did not impact a horse's racing performance.
- **The Pharmacokinetics of Flumethasone following Intravenous Administration (R. Sams, HFL Sport Science)**
Flumethasone is a corticosteroid, commonly referred to as Flucort. This project facilitated development of threshold and withdrawal guidance to permit therapeutic use while also ensuring the medication did not impact a horse's racing performance.
- **The Pharmacokinetics of Triamcinolone Acetonide following Intra-Articular Administrations into Single and Multiple Joints (H. Knych, University of California Davis)**
Triamcinolone acetonide is a corticosteroid, commonly referred to as Vetalog or Kenalog. This project facilitated development of threshold and withdrawal guidance to permit therapeutic use while also ensuring the medication did not impact a horse's racing performance.
- **The Pharmacokinetics of Triamcinolone Acetonide when Co-Administered with Sodium Hyaluronate (H. Knych, University of California, Davis)**
Sodium hyaluronate is also referred to as hyaluronic acid, HA or by trade names including Hylartin-V or Legend.
- **The Pharmacokinetics of Methylprednisolone Acetate following Intra-Articular and Intravenous Administration (H. Knych, University of California, Davis)**
Methylprednisolone is a corticosteroid, commonly referred to as Depo Medrol. This project facilitated development of threshold and withdrawal guidance to permit therapeutic use while also ensuring the medication did not impact a horse's racing performance.

- Disposition of isoflupredone acetate in plasma, urine and synovial fluid following intra-articular administration to exercised Thoroughbred horses. (H. Knych, University of California, Davis)
Isoflupredone is a corticosteroid, commonly referred to as Predef. This project facilitated development of threshold and withdrawal guidance to permit therapeutic use while also ensuring the medication did not impact a horse's racing performance.
- Detection and Pharmacokinetics of AICAR in Horses (M. Robinson, University of Pennsylvania)
AICAR is recognized as a Performance Enhancing Drug (PED) for its effects on energy metabolism and as such is a prohibited substance.
- An Interlaboratory study of the pharmacokinetics of testosterone following intramuscular administration to Thoroughbred horses (B. Moeller and S. Stanley, University of California, Davis and R. Sams, LGC Sport Science)
Testosterone is an endogenous (produced naturally within the body) anabolic steroid. It is necessary to distinguish naturally occurring levels of testosterone from those resulting from an administration in order to control its use.
- Pharmacokinetics of stanozolol in Thoroughbred horses following intramuscular administration (B. Moeller and S. Stanley, University of California, Davis and R. Sams, LGC Sport Science)
Stanozolol is a synthetic anabolic steroid, often referred to as Winstrol.
- Pharmacokinetics of Boldenone and Stanozolol and the Results of Quantification of Anabolic and Androgenic Steroids and in Race Horses and Non-Race Horses (L. Soma, University of Pennsylvania)
Boldenone is a synthetic anabolic steroid, often referred to as Equipoise.
- Method Development and Validation for the Detection of Ziconotide in Equine Serum (K. Zientek, University of Florida)
Ziconotide is derived from the toxin of the Cone Snail; it has the ability to cause paralysis or loss of sensation depending on the type of nerve it is applied to.
- Detection of the cone snail toxin ziconotide in equine serum (C. Kollias-Baker, University of Florida)
- Determination of ethanol in the breath and blood in horses following low doses (S. Stanley, University of California, Davis)
Ethanol or grain alcohol, is a central nervous system depressant. There are anecdotal reports of its use in proximity to a race as a 'calming agent'—an alternative to low doses of tranquilizer that would be readily detected by testing laboratories.

- **Efficacy of furosemide in treatment of EIPH (K. Hinchcliff, The Ohio State University)**
Furosemide is more commonly known by its trade names Lasix or Salix. EIPH is an acronym for Exercise Induced Pulmonary Hemorrhage, a condition where blood vessels in the lungs rupture during maximal exercise.
- **Effects of pre-race administration of Amicar on EIPH (E. Birks, University of Pennsylvania)**
Amicar is a trade name for Aminocaproic acid which is used in human medicine for the control of hemorrhage. It was an approved raceday medication in some states ostensibly for the control of EIPH.
- **Can conjugated estrogens and aminocaproic acid reduce EIPH? (H. Erickson, Kansas State University)**
Conjugated estrogens, often referred to as Estrone, have had demonstrated effect in controlling (not preventing) hemorrhage in human patients.
- **Identification of rHuEPO from horses (R. Sams, Ohio State University)**
rHuEPO is an acronym for recombinant human erythropoietin which is marketed under the trade name Epogen. The use of rHuEPO is recognized as blood doping and is prohibited in horse racing.
- **Determination of a reporting level for procaine (C. Kollias-Baker, University of Florida)**
Procaine is a local anesthetic that is also a component of an effective and commonly used antibiotic procaine penicillin, often referred to as Pen-G, PPG, or Procaine-Pen.
- **Screening of erythropoiesis-stimulating peptides in horse (A. Singh, University of Minnesota)**
- **Identifying poppy seed contamination in the horse by LC/MS (A. Ray, Texas A & M University)**
Poppy seeds are a source of morphine which is a prohibited substance.
- **Pharmacologically based withdrawal time for clenbuterol (E. Birks, University of Pennsylvania)**
Clenbuterol is also known by its trade name, Ventipulmin.