Use of Equine Hair as a New Matrix for Anti-Doping Control

Scott D. Stanley, Ph.D.
Professor
University of California – Davis
School of Veterinary Medicine
Contents

• The Maddy Lab
• AQHA zero tolerance policy toward Clenbuterol
• Principles of Hair Analysis
• Hair Segment Analysis
Maddy Equine Analytical Chemistry Lab

**Drug Detection**
Determination, Identification, Confirmation

**Pharmacokinetics**
$T_{1/2}$, AUC, CI, Metabolite ID

**Proteomics**
Erythropoietin, Growth Hormone, Cobratoxin

Clenbuterol $m/z$ 240

![Clenbuterol molecule](image)

![Clenbuterol clearance](image)

![Erythropoietin, Growth Hormone, Cobratoxin](image)
Compounded Clenbuterol

Concentration Issues
Clenbuterol
Compounded Clenbuterol

- Suspension – stated concentration for all products was 100 µg/mL
- 1 Gallon = 1043 doses
Compounded Clenbuterol

- Clear – Regular Strength
- Red – Double Strength
Incorporation of Drugs into Hair

- Each hair follicle has its own blood supply
- After the drug enters the body, it is absorbed into the blood stream
- As hair grows drugs are incorporated into the hair
- Drugs will remain in the hair almost permanently
Incorporation of Drugs into Hair

Diagram showing the incorporation of drugs into hair through the hair follicle and sebaceous gland, with biological fluids involved in the process.
Incorporation of Drugs into Hair

- Epidermis
- Sebaceous Gland
- Hair Follicle
- Biological fluids
- Drugs

One-off Incidence

Drug detection in hair
Incorporation of Drugs into Hair

- Epidermis
- Sebaceous Gland
- Hair Follicle
- Drugs
- Biological Fluids
Growth Rates & History of Use

• Horse hair grows at approx. 2 cm per month (12 cm section = 6 months)
• Consecutive sections provide a retrospective history of drug usage
• Drug use history can be attained as long as the hair allows (i.e., 24 cm of hair = 1 year)
Sample Collection & Preparation

- Collection
  - Mane or tail hair, cut from the proximal end (closest to the horse)
  - Hair is assigned a barcode #, bagged and sealed
- Washing
  - Removes external contaminants
- Extractions
  - Chemical procedures isolates drugs from hair
Hair Collection
Chain of Custody

• Hair sample collection materials are part of the chain of custody

• Important that all relevant fields are completed i.e., hair color, medications, collector info

• The sample is tracked at every stage from collection to the issue of results

• The robustness of the chain of custody procedure is legally defensible in court
Hair Samples

- Lock of mane hair
- Hair is transferred to a glass 1x2½" vial and cut into smaller pieces.
Hair Sample Preparation

Cut hair is transferred to a vial and pulverized.
Hair Analysis

- Unique long window of detection
- May differentiate between regular administration versus single exposure
- Drugs remain locked in hair
- Simple & quick sample collection
- Not appropriate for recent admin. detection, time line in months not days
Segmental Analysis

2 weeks

6 months

1  2  3  4
Drug Groups 1 of 4

- **Anabolic Steroids**
  - **Endogenous** – Androstenedione, DHEA, Dihydrotestosterone, Nandrolone, Testosterone
  - **Exogenous** – Bolasterone, Boldenone, Boldione, Danazol, Drostanolone, Ethylestrenol, Furazbol, Methyltestosterone, Methylandrosterenediol, Methandienone, Norethandrone, Oxandrolone, Stanozolol, Tetrahydrogestrinone (THG), Trenbolone
Drug Groups 2 of 4

- Corticosteroids (steroid hormones)
  - Exogenous – Becomethasone, Betamethasone, Budesonide, Dexamethasone, Flumethasone, Fluticasone, Isofluperedone, Methylprednisolone, Prednisolone, Triamcinolone
  - Endogenous - (Hydrocortisone)
Drug Groups 3 of 4

• β-2 Agonist -
  ▫ Bambuterol, Clenbuterol, Fenoterol, Ractopamine, Xamoterol, Zeranol, Zilpaterol

• Selective Androgen Receptor Modulators (SARMs)
  ▫ Andarine (S-4), Ostarine (S-22)
200 mg of pulverized hair

- UPLC MS/MS
  - Steroid Esters
- LC-HRMS
  - Basic Drugs
- GC-MS/MS
  - Acid Drugs
  - Anabolic Steroid
  - Steroid
Acknowledgements

• Horse Industry Support:
  ▫ CHRB, Los Alamitos Race Track, NMRC, The Jockey Club, Breeder’s Cup, Keeneland Association, AQHA, TOC, CTT, PCQHA, RMTC, and ARCI

• University of California - Davis
  ▪ Dr. Heather Knych
AAS Impact on Performance and Health

**Efficacy:** AAS administration in the horse for therapeutic treatment of conditions is limited.

**Performance:** Used to promote muscle growth

**Health effects:** Effects on reproduction have been studied with a reversible suppression of reproductive function.
AAS Impact on Steroid

1. Steroidogenesis is tightly regulated

2. The impact of AAS usage on altering steroid profiles in the horse is incomplete and inconclusive:
   
   a) Exogenous treatments of decreased testosterone

Roser, J (2008)
Typical Anabolic-Androgenic Steroids Derived from Testosterone
Steroidogenesis
Prohibited Substances

**Anabolics steroids**
- 16β-Hydroxystanozolol
- 1-Androstenedione
- 4-Estrene-3,17-dione
- Altenogest
- Androstadienone
- Bolasterone
- Boldenone
- Boldione
- Calusterone
- Clostebol acetate
- Danazol
- Deoxycortone
- Dimethisterone
- Ethyltestosterone
- Gestrinone
- Hydroxytestosterone
- Medroxyprogesterone acetate
- Methyltestosterone
- Nandrolone
- Norbolethone
- Norethandrolone
- Oxyguno
- Propyltrenbolone
- Stanozolol
- Stenbolone
- Testosterone
- Testosterone propionate
- Trenbolone
- Turinabol
- α- & β-agonists
  - Albuterol
  - Bambuterol
  - Brombuterol
  - Bromchlorbuterol
  - Cimaterol
  - Cimbuterol
  - Clenbuterol
  - Clencyclohexerol
  - Clenhexerol
  - Clenisopenterol
  - Clenpenterol
  - Dobutamine
  - Etafedrine
  - Etilefrine
  - Fenoterol
- Formoterol
- Guanabenz
- Guanfacine
- Hydroxydetomidine
- Mabuterol
- Mapenterol
- Methoxamine
- Methoxyphenamine
- Naphazoline
- Phenylpropanolamine
- Procaterol
- Pseudoephedrine
- Ractopamine
- Romifidine
- Salbutamol
- Terbutaline
- Tuaminoheptane
- Xylazine
- Xylometazoline

**Analgesic/Anesthetic**
- 4-Methylaminophenazone
- Anileridine
- Buprenorphine
- Butorphanol
- Cocaine
- Demorphin
- Dihydrocapsaicin
- Etorphine
- Ketamine
- Lidocaine
- Mepivacaine
- Methadone
- Midazolam
- Nalbuphine
- N-Norprocaine
- Nonivamide
- Noroxymorphone
- O-Desmethyltramadol
- Oxydode
- Oxymorphone
- Paracetamol
- Procaine
- Thebaine
- Tramadol
- Zolazepam
Plasma Concentration Curve

- Distribution
- Removal of drug: Metabolism + Excretion
- Peak Concentration
- Removal of drug: Metabolism + Excretion
- Absorption

- Intensity of drug effect
- Time for drug effect to begin
- Minimum Therapeutic Drug Level

- Concentration in blood
- Time

Plasma or tissue drug level

Time (t)
Metabolism of AAS

- **Phase I**
  - P450 mediated
    - Hydroxylation
    - De-methylation
  - 5α-Reductase and 5β-Reductase mediated
  - Hydroxy Steroid Dehydrogenase mediated
    - 3 and 17 hydroxy positions

- **Phase II**
  - Glucuronidation
  - Sulfation
Urine Analysis

• Good for detecting drugs over 1 - 45 days
• Difficult to collect, time consuming and requires multiple steps for sample preparation
• Requires refrigeration for transportation and storage
• Minimal withdrawal can be employed to avoid most detection
Objectives

- Explain the principles of equine hair testing
- Provide an overview of compounded clenbuterol
- Highlight the benefits of hair analysis over urine and plasma analysis
- Provide an overview of drug groups and their metabolites
- Describe hair testing limitations
Drug Groups 4 of 4

- Peptide Hormones and Growth Factors –
  - Growth Hormone, Growth Hormone Releasing Factor-2, GHRF-6, Insulin-Like Growth Factor-1, Fibroblast Growth Factor, Machano Growth Factor, Vascular Endothelial Growth Factor
Q Exactive MS

- Benchtop Orbitrap MS
  - High resolution (140,000 at m/z 200)
  - High Mass Accuracy (<5 ppm, external)
  - Full scan MS
  - High Sensitivity
  - Advanced LC-MS
    - Proteomics
    - Metabolomics
    - Small Molecule and Structure Elucidation
    - Ultra-trace Level Analysis
      - Sub-fmol Sensitivity
The axial oscillation frequency follows the formula

$$\omega = \sqrt{\frac{k}{m/z}}$$

Where

- $\omega$ = oscillation frequency
- $k$ = instrumental constant
- $m/z$ = mass/charge ratio
MS method: basic compounds

- Data acquisition mode: full scan in m/z range 130-505
- Resolution: 70 K
## Autosampler method: Basic compounds

- **Injection volume**: 40 uL
Stanozolol: LOQ = 10 pg/mL
16-OH-Stanozolol: LOQ = 10 pg/mL
<table>
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<tr>
<th>Name</th>
<th>Formula</th>
<th>Theoretical Mass (M+H)</th>
<th>Experimental Mass</th>
<th>Mass Error (ppm)</th>
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Stanozolol Administration

Plasma sample ~ 18 pg/mL
Blank Plasma sample
Spiked sample 25 pg/mL
Stanozolol - Hair + 4 weeks

Blank Hair

Spiked Hair

Positive Hair
Stanozololol - Urine + 4 weeks
Method Parameters

- **MS:** Thermo TSQ Vantage Triple Quadrupole
- **Ionization:** Heated Electrospray Ionization (HESI)
- **HESI Temp:** 200°C
- **Scan Mode:** SRM
  - Q1 – 0.1 m/z at FWHM
  - Q3 – 0.7 m/z at FWHM
- **Scan Width:** 0.01 m/z
- **Scan Time:** 50 millisecond
Selected Reaction Monitoring

**Fixed m/z**
- 0.7 m/z for SRM
- 0.1 m/z for HSRM

**Pass All**

**Fixed m/z**
- 0.7 m/z for SRM
- 0.7 m/z for HSRM
### Mass Spec Method

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<th>Segment / ESi Mode</th>
<th>Formula</th>
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<th>S-Lens</th>
<th>Precursor / Ion</th>
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<td>291.22</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4 5+</td>
<td>C_{18}H_{28}O_{2}</td>
<td>9.95</td>
<td>78</td>
<td>315.243</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiol 5+/6+</td>
<td>C_{18}H_{28}O_{2}</td>
<td>10.18</td>
<td>74</td>
<td>257.232</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ediol 5+/6+</td>
<td>C_{18}H_{28}O_{2}</td>
<td>10.40</td>
<td>58</td>
<td>257.232</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5 6+/7+</td>
<td>C_{18}H_{28}O_{2}</td>
<td>11.22</td>
<td>75</td>
<td>299.245</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SnDHP 6+/7+</td>
<td>C_{19}H_{30}O_{2}</td>
<td>11.43</td>
<td>65</td>
<td>317.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP 6+/7+</td>
<td>C_{19}H_{30}O_{2}</td>
<td>11.85</td>
<td>87</td>
<td>301.224</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4iol 6+/7+</td>
<td>C_{19}H_{30}O_{2}</td>
<td>11.99</td>
<td>77</td>
<td>285.229</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Mass Spectrometry Diagram](image-url)
Hair Analysis Limitations

- Not currently possible to determine drug purity, dose used, or frequency of administration
- Unable to determine the route of administration
- Cannot pinpoint administration time such as to exactly what day...
Conclusions

• BHA has Zero Tolerance for anabolic steroids for North American horses imported into England
• Anabolic steroid abuse is still a significant concern
• Routine LC-MS/MS has significantly improved ease of detection
• Routine screening in the low part per trillion
• Hair analysis offers potential for long term detection of many historic drugs of abuse
• Segmental analysis may provide a time line for drug administration
Anabolic Androgenic Steroid: Stanozolol

Many modifications possible!
Details on fragmentation of paramount interest
ESI-MS/MS Spectrum of Stanozolol
ESI-MS/MS Spectrum of Stanozolol (LTQ-Orbitrap)

- **Molecular Mass**: 311.25
- **Formula**: C₁₈H₂₉N₂
- **Relative Abundance**: 100
- **Deviation**: -1.6887 ppm

- **Molecular Mass**: 95.0858
- **Formula**: C₇H₁₁
- **Relative Abundance**: 149.11
- **Deviation**: 2.6922 ppm

- **Molecular Mass**: 81.05
- **Formula**: C₅H₇N₂
- **Relative Abundance**: 203.15
- **Deviation**: 2.3914 ppm

- **Molecular Mass**: 329.26
- **Formula**: C₁₂H₁₇N₂
ESI-MS/MS Spectrum of 16-hydroxystanozolol
ESI-MS/MS Spectrum of Stanozolol

- **C₅H₇N₂** (95.0606): 2.6922 ppm
- **C₂H₁₁** (95.0858): 2.3914 ppm
- **C₂₁H₃₁N₂** (311.2477): -1.6887 ppm
**ESI-MS$^3$ Data of Fragments derived from $m/z$ 203**

<table>
<thead>
<tr>
<th>Precursor $m/z$ 329</th>
<th>Precursors $m/z$ 329-203</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fragment</strong> ($m/z$)</td>
<td><strong>measured mass (u)</strong></td>
</tr>
<tr>
<td>81</td>
<td>81.0451</td>
</tr>
<tr>
<td>95</td>
<td>95.0606</td>
</tr>
<tr>
<td>95</td>
<td>95.0858</td>
</tr>
<tr>
<td>133</td>
<td>133.0760</td>
</tr>
<tr>
<td>133</td>
<td>133.1012</td>
</tr>
<tr>
<td>135</td>
<td>135.0917</td>
</tr>
<tr>
<td>135</td>
<td>135.1168</td>
</tr>
<tr>
<td>147</td>
<td>147.0916</td>
</tr>
<tr>
<td>147</td>
<td>147.1167</td>
</tr>
<tr>
<td>203</td>
<td>203.1540</td>
</tr>
</tbody>
</table>
Dexamethasone: LOQ = 5 ng/mL
Dexamethasone: LOQ = 5 pg/mL

Acid_2nd_C4 - m/z= 393.21  SM: 5 RT: 5.92 - 7.92  NL: 8.62E5
F: FTMS + p ESI Full ms [130.00-505.00]
Screening for Abuse

- Sample Load: Hundreds
- Time Frame: 1-2 days
- Requirements:
  - Sensitive
  - Quantitative
  - Selective
  - High Throughput
  - Minimal Sample Preparation
Androgen Production in the Horse

Primary circulating (serum/plasma) androgens are:

![Testosterone](image1.png)

0-1 year old
Colt: 0 – 450 pg/ml
Filly: 0 – 40 pg/ml

0-1 year old
Male: 0 – 450 pg/ml \(^a\)
Filly: 0 – 150 pg/ml \(^a\)

Androstenedione

>3 years old
Male: 550 – 700 pg/ml \(^d\)
Female: 100 – 

References:
\(^a\) – Lemazurier et al (2001)
\(^b\) – Soma et al (2008)
\(^c\) – Gastal et al (2007)
\(^d\) – Silberzahn et al (1989)
Endogenous Production of AAS

**Boldenone**

**Plasma/Serum**

**Stallion:** none reported \(^a\)

**Mare:** none reported

\(^a\) – Soma et al (2008)

**Nandrolone**

**Plasma/Serum**

**Stallion:** 0 – 200 pg/ml \(^a\)

**Mare:** 0 - ?? pregnancy \(^b\)

\(^b\) – Sterk et al (1989)

\(^c\) – Grace et al (2008)
## Steroids Monitored: LOD and LOQ

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOD pg/mL</th>
<th>LOQ pg/mL</th>
<th>Analyte</th>
<th>LOD pg/mL</th>
<th>LOQ pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>6αOH-Androstenedione</td>
<td>500</td>
<td>2,500</td>
<td>Epi-Nandrolone</td>
<td>150</td>
<td>250</td>
</tr>
<tr>
<td>Boldenone Sulfate</td>
<td>250</td>
<td>500</td>
<td>17OH-Progesterone</td>
<td>500</td>
<td>1,250</td>
</tr>
<tr>
<td>Nandrolone Sulfate</td>
<td>250</td>
<td>500</td>
<td>19-Norepiandrosterone</td>
<td>500</td>
<td>1,250</td>
</tr>
<tr>
<td>17β-Estradiol Sulfate</td>
<td>150</td>
<td>250</td>
<td>Dehydroepiandrosterone</td>
<td>1,250</td>
<td>2,500</td>
</tr>
<tr>
<td>Nandrolone Glucuronide</td>
<td>750</td>
<td>2,500</td>
<td>17OH-Pregnenolone</td>
<td>2,500</td>
<td>5,000</td>
</tr>
<tr>
<td>Testosterone Sulfate</td>
<td>250</td>
<td>500</td>
<td>5α-Dihydronandrolone</td>
<td>750</td>
<td>1,250</td>
</tr>
<tr>
<td>1,4-Androstadien-3,17-one</td>
<td>125</td>
<td>125</td>
<td>Epi-Testosterone</td>
<td>25</td>
<td>125</td>
</tr>
<tr>
<td>Testosterone Glucuronide</td>
<td>150</td>
<td>250</td>
<td>5α-Estran-3b,17α-diol</td>
<td>750</td>
<td>1,250</td>
</tr>
<tr>
<td>19-Norandrostendione</td>
<td>250</td>
<td>1,250</td>
<td>5α-Dihydrotestosterone</td>
<td>500</td>
<td>1,250</td>
</tr>
<tr>
<td>Dehydroepiandrosterone Sulfate</td>
<td>150</td>
<td>500</td>
<td>19-Norandrosterone</td>
<td>500</td>
<td>1,250</td>
</tr>
<tr>
<td><strong>Boldenone</strong></td>
<td><strong>100</strong></td>
<td><strong>125</strong></td>
<td>5β-Dihydrotestosterone</td>
<td>250</td>
<td>1,250</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>75</td>
<td>125</td>
<td>Progesterone</td>
<td>50</td>
<td>125</td>
</tr>
<tr>
<td><strong>Nandrolone</strong></td>
<td><strong>150</strong></td>
<td><strong>250</strong></td>
<td>5α-Androstane-3α,17β-diol</td>
<td>2,500</td>
<td>10,000</td>
</tr>
<tr>
<td>Estrone</td>
<td>150</td>
<td>250</td>
<td>Etiocholan-3α,17β-diol</td>
<td>10,000</td>
<td>25,000</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>2,500</td>
<td>5,000</td>
<td>Pregnenolone</td>
<td>1,000</td>
<td>2,500</td>
</tr>
<tr>
<td>17α-Estradiol</td>
<td>1,000</td>
<td>5,000</td>
<td>5α-Dihydroprogesterone</td>
<td>2,500</td>
<td>5,000</td>
</tr>
<tr>
<td><strong>Testosterone</strong></td>
<td><strong>50</strong></td>
<td><strong>125</strong></td>
<td>Allopregnanolone</td>
<td>2,500</td>
<td>5,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pregnanediol</td>
<td>5,000</td>
<td>10,000</td>
</tr>
</tbody>
</table>
Background

Performance horses have the longest established, most elaborate broad-based, and technically accurate systems for drug detection of any competitive sport.
## Blood Flow

### TABLE 4-4

Blood Flow, Perfusion Rate, and Relative Size of Different Organs and Tissues Under Basal Conditions in a Standard 70-kg Young Healthy Adult

<table>
<thead>
<tr>
<th>Organ*</th>
<th>Percent of Body Weight</th>
<th>Blood Flow (ml/min)</th>
<th>Percent of Cardiac Output</th>
<th>Perfusion Rate (ml/min per g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Adrenal glands</td>
<td>0.03</td>
<td>25</td>
<td>0.2</td>
<td>1.2</td>
</tr>
<tr>
<td>2. Blood</td>
<td>7</td>
<td>(5000)*</td>
<td>(100)</td>
<td>-</td>
</tr>
<tr>
<td>3. Bone</td>
<td>16</td>
<td>250</td>
<td>5</td>
<td>0.02</td>
</tr>
<tr>
<td>4. Brain</td>
<td>2.0</td>
<td>700</td>
<td>14</td>
<td>0.5</td>
</tr>
<tr>
<td>5. Adipose</td>
<td>15†</td>
<td>200</td>
<td>4</td>
<td>0.025</td>
</tr>
<tr>
<td>6. Heart</td>
<td>0.4</td>
<td>200</td>
<td>4</td>
<td>0.6</td>
</tr>
<tr>
<td>7. Kidneys</td>
<td>0.5</td>
<td>1100</td>
<td>22</td>
<td>4.0</td>
</tr>
<tr>
<td>8. Liver</td>
<td>2.3</td>
<td>1350</td>
<td>27</td>
<td>0.8</td>
</tr>
<tr>
<td>Portal</td>
<td>1.7 (Gut)</td>
<td>(1050)</td>
<td>(21)</td>
<td>-</td>
</tr>
<tr>
<td>Arterial</td>
<td></td>
<td>(300)</td>
<td>(6)</td>
<td>-</td>
</tr>
<tr>
<td>9. Lungs</td>
<td>1.6</td>
<td>5000</td>
<td>100</td>
<td>10.0</td>
</tr>
<tr>
<td>10. Muscle (inactive)</td>
<td>43</td>
<td>750</td>
<td>15</td>
<td>0.025</td>
</tr>
<tr>
<td>11. Skin (cool weather)</td>
<td>11</td>
<td>300</td>
<td>6</td>
<td>0.04</td>
</tr>
<tr>
<td>12. Spleen</td>
<td>0.3</td>
<td>77</td>
<td>1.5</td>
<td>0.4</td>
</tr>
<tr>
<td>13. Thyroid gland</td>
<td>0.03</td>
<td>50</td>
<td>1</td>
<td>2.4</td>
</tr>
<tr>
<td>Total Body</td>
<td>100</td>
<td>5000</td>
<td>100</td>
<td>0.071</td>
</tr>
</tbody>
</table>

*Some organs (e.g., stomach, intestines, spleen, and pancreas) are not included.
†Includes fat within organs. Because 75–80 kg is more typical of body weight today, a better estimate of this value in an average person is closer to 20%.

THIS CERTIFICATE RECOGNIZES THAT

University of California-Davis Kenneth L. Maddy Laboratory

HAS BEEN AWARDED RMTC LABORATORY ACCREDITATION

AWARDED THIS 11TH DAY OF JUNE, 2013

[Signature]

Dr. Lewis, Chair RMTC Board
Mechanism of Action

CHOLESTEROL

[CYP11A]

Pregnenolone

[17α-HSD] 17-Hydroxypregnenolone

[CYP17] Dehydroepiandrosterone

[17β-HSD] Androstenediol

[3β-HSD]

Progesterone

[17α-HSD] 17-Hydroxyprogesterone

[CYP17] Androstenedione

[17β-HSD] Testosterone

HSP

HSP

HSP

HSP

AR

AR

AR

AR

Down Stream Signaling

GPCR
Implications of Route of Administration

**Routes of Administration**
- A. Intravenous
- B. Oral
- C. Intraperitoneal
- D. Inhalation
- E. Intramuscular
- F. Subcutaneous
- G. Intraarterial

**Arterial Flow**
- Brain
  - Lungs *
    - Liver *
      - Intestines *
        - Gut lumen
        - Kidneys
          - Muscle
            - Fat
              - Skin

**Venous Flow**
- Heart
  - Intestines *
    - Gut lumen
    - Kidneys
  - Muscle
  - Fat
  - Skin

*First-Pass Organs*

**Site of Sampling**

**Elimination (Metabolism Excretion)**
Absorption, Distribution, Excretion of Drugs/Toxicants

- **Ingestion**
  - Gastrointestinal Tract
    - Blood and Lymph
      - Portal Blood
    - Liver
      - Bile
      - Kidney
    - Feces
- **Inhalation**
  - Lung
    - Alveoli
    - Expired Air
    - Secretory Structure
    - Secretions
    - Organs
      - Soft Tissue
      - Bone
- **Intravenous**
  - Target Site
    - Subcutaneous
      - Intramuscular
    - Intraperitoneal
    - Dermal
- **Intraperitoneal**
  - Extracellular Fluid
    - Fat


Ingestion, Inhalation, Intravenous and Intraperitoneal pathways lead to the target site, which in turn influences the distribution and excretion of drugs/toxicants through various organs, tissues and secretions.
Potential of Segmental Analysis

Distal (oldest growth) segment contained highest concentrations

Proximal (newest growth) segment contained lowest concentrations
Program Elements

- Analytical Chemist and a Veterinary Pharmacologist
- Scientific and Industry Advisory Boards to assist in program development and direction
- Able to draw on expertise of faculty in numerous fields at UC-Davis
Gerard Butler charged by British Horseracing Authority after nine horses test positive for steroids

Racing’s ‘Sungate Saga’ has begun with Newmarket trainer Gerard Butler being charged on Friday with seven breaches of the Rules relating to the administering of drugs to racehorses. More Newmarket trainers, among them some of the biggest names in the town, are expected to be charged with similar offences in the coming weeks.
Godolphin rocked by drugs scandal as Mahmood Al Zarooni's horses found with traces of anabolic steroids

Drugs found in 11 horses, including 1,000 Guineas hope Certify at Classic winning trainer's Newmarket stables
Frontpage News

- Testing “In Training” visit
- 45 plasma and hair samples collected
- 4 positive for Stanozolol
- Matched by positives in corresponding hair samples
Frontpage News

- Second inspection 3 weeks after initial visit
- 203 samples, only plasma collected
- 11 positive for Stanozolol
- 3 of 4 original horse still positive
- Concentrations dropped to \(\sim 10\%\) of initial findings
- Hair samples would likely have given more information
BHA announced a new zero tolerance policy regarding anabolic steroids starting in 2015

The headline elements of the policy:

• A horse must not be administered an anabolic steroid at any point in its life

• Any horse administered an anabolic steroid will face a mandatory stand down period from training for 12 months and ineligible to start in any race in Britain for 14 months.

• All horses must be available for testing at any time, regardless of physical location and whose care the horse is under, from the time it is first registered with Weatherbys
BHA announced a new zero tolerance policy regarding anabolic steroids starting in 2015

• All GB bred horses must be registered with Weatherbys within 12 months of birth, phased to six months in two years. Permanently imported horses must be registered with Weatherbys within three months of arrival in Britain accompanied by a sample that shows no evidence of anabolic steroid administration.

• Due to their mirror policies, horses imported from Ireland, France and Germany which have spent 12 months under their equivalent policies will be exempt from this requirement. Likewise, runners from Ireland, France and Germany will be treated as British runners and sampled as per the standard testing policy.

• All other foreign runners must be in Britain (and the BHA notified of their whereabouts) a minimum of 14 days in advance of their intended race to facilitate post-arrival sampling and analysis, the results of which will be received prior to the horse running.
The Sungate® Saga

- Testing “In Training” visit
- 28 plasma samples collected
- 9 positive for stanozolol
- 65-410 picograms per mL
- Trainer reported use of “Sungate®” (Stanozolol, 5 mg/mL) in medication records
- Recommended by Veterinarian
Factors Affecting Drug Incorporation into Hair

• **Melanin content** - The most common form of biological melanin in hair is eumelanin.

• **Lipophilicity** - Refers to the ability of a drug compound to dissolve in fats, oils, and lipids.

• **Alkalinity** - The quantitative capacity of an aqueous solution to neutralize an acid.
Horse Racing Industry Integrity

- Industry Reputation
  - $917.5 million in gross sales
  - 7,161 yearlings ($430 million)

- High Profile Events
  - Challenge Championship
  - Breeder’s Cup Purses = $28 M

- Horse’s Reputation
  - Sept. ’14 - $2.2 million
  - Nov. ’14 - $3.9 million
Clenbuterol 10x

Clenbuterol 756 µg/mL (~10x)
trace acepromazine
Mexican Clenbuterol
- Conc. OK
Syringe
- Clenbuterol ~ 2x
Clenbuterol
33 µg/mL
29 µg/mL
70.5 µg/mL

Clenbuterol
~649 µg/mL
(≈10×)