COBALT, LASIX, THRESHOLDS AND WITHDRAWAL TIMES: AN OVERVIEW

BY

Thomas Tobin, Kimberly Brewer and Charlie G. Hughes

Maxwell H. Gluck Equine Research Center

University of Kentucky

for the

HBPA Summer Meeting

Oklahoma City, Oklahoma

Saturday, August 16th, 2014

August 16th, 2014

HISTORY: OKLAHOMA AND REGULATORY THRESHOLDS

The Oklahoma Racing Commission, in about 1986-1987, ***introduced the first plasma threshold for Lasix*** *based on research supported by the Kentucky HBPA. To my knowledge this was the*

FIRST RESEARCH BASED REGULATORY THRESHOLD-WITHDRAWAL TIME GUIDELINE INTRODUCED BY A RACING COMMISSION.

REGULATORY THRESHOLD FOR FUROSEMIDE [1982-3]

The "four hour rule" made possible the approval of Lasix in racing.

Four hour rule at first required detention barns, cumbersome and expensive.

In 1982 the Kentucky HBPA asked us to develop a 4 hour regulatory threshold for furosemide; we dosed 47 horses with 0.5mg/kg furosemide I/V.

The figure to the above right shows the Lasix plasma concentration data points at one hour, clearly a normal distribution.

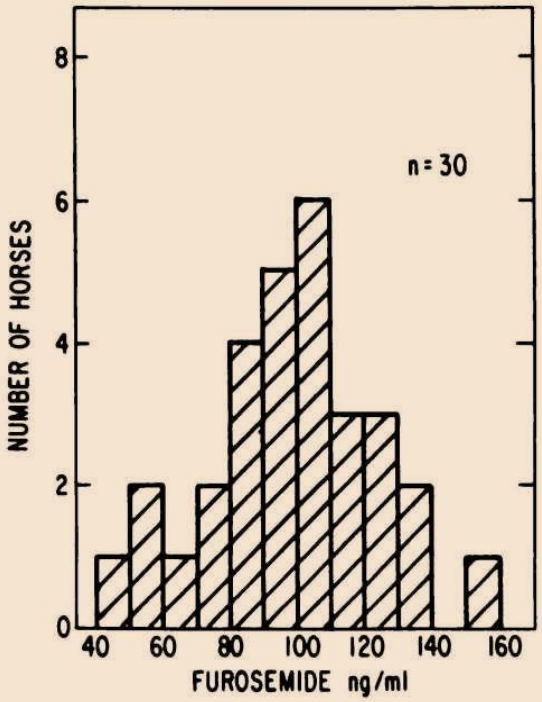


FIG. 5. *Furosemide plasma levels in 30 horses 1 hr after iv administration of 0.5 mg/kg furosemide.*

The vertical bars represent the number of horses found within the indicated ranges of furosemide plasma levels.

REGULATORY THRESHOLD FOR FUROSEMIDE [1983]

Four hour data points show a distribution skewed to the right.

The figure above right shows the raw data, apparently skewed to the right.

The modal concentration is 8.5 ng/ml, the mean is 9.6 ng/ml. There are two "high" samples at about 19 ng/ml. [Outliers??].

A log transformation "normalized" this distribution and about 1/1,000 horses would exceed about 27/30ng/ML.

This became the threshold, adjusted to 60 and then 100 ng/ML in plasma /serum, linked, courtesy of Dr. Sams, to a 1.010 urinary specific gravity "cut-off".

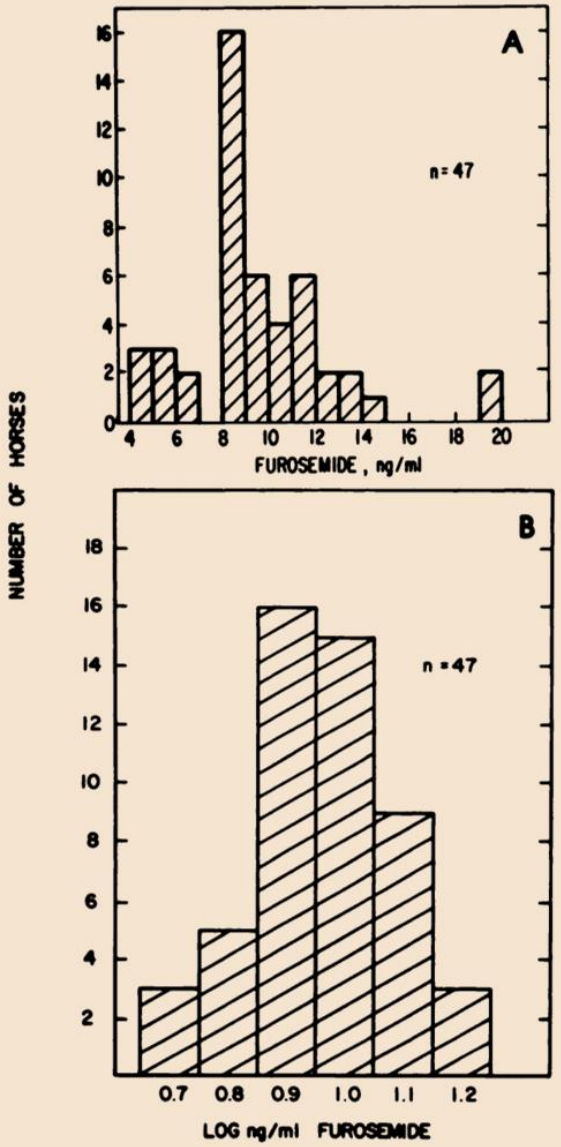


FIG. 6. *Furosemide plasma levels in 47 horses 4 hr after iv administration of 0.5 mg/kg furosemide.*

A, the vertical bars represent the number of horses found within the indicated ranges of furosemide plasma levels; *B*, the vertical bars represent the number of horses found within the indicated ranges of the log of furosemide plasma levels.

HOW DO YOU DEVELOP A REGULATORY THRESHOLD?

1/ Formulation, dose **[mg/kg]** and route of administration, sampling time(s).

2/ Administer to "n" horses in training, the **bigger "N" the better** [EHSLC, 6-8 horses?].

3/ Quantify the samples at the suggested "withdrawal-time" post dosing.

4/ Plot the data at the time of interest as a population distribution: the regulatory threshold is based on mathematical projections from the experimental data.

GROUND RULES FOR THRESHOLDS

1/ The data distribution most commonly, “skewed”, requiring specific mathematical transformations.

2/ “Outliers”: The term “outlier” is undefined. I cannot see how you can have an “outlier” in a medication administration experiment.

3/ So, dose, analyze, define your a mathematical model including all data points and calculate the threshold AND DO NOT HESITATE TO MOVE IT UP.

DISTRIBUTIONS ARE DUE TO BIOLOGICAL VARIABILITY = UNCERTAINTY

P. -L. Toutain, 2010: "*How to extrapolate a withdrawal time from an EHSLC published detection time: A Monte Carlo simulation appraisal*" Equine Vet. J. 42 (3) p 248-254:

FROM SUMMARY

" *In practice, this means that the main sources of* ***uncertainty*** *are of biological origin and* ***cannot be reduced by any managerial options".***

WITHDRAWAL TIME GUIDELINE

A THRESHOLD/WITHDRAWAL GUIDELINE SHOULD INCLUDE A STATISTICAL ESTIMATE OF THE PROBABILITY OF AN INDIVIDUAL WHO RIGOROUSLY FOLLOWS THE WITHDRAWAL GUIDELINES RANDOMLY EXCEEDING THE THRESHOLD, IF YOU HAVE A SCIENTIFICALLY VALID THRESHOLD/ WITHDRAWAL TIME GUIDELINE.

FUROSEMIDE THRESHOLD HISTORY

1/ 30ng/ml threshold was adjusted up to 60 and then to 100 by Regulators.

2/ Adjustment accommodated increases in the furosemide dose to 500 mg/horse by some States.

3/ First scientifically based threshold developed and applied in racing chemistry.

GOOD SCIENCE;

NON-SCIENTIFIC EXPERIENCE BASED ADJUSTMENT

GROUND RULES FOR THRESHOLDS

1/ The more horses the better.

2/ The mathematical nature of the distribution varies, the simplest being the log-normal.

3/ “Outliers” : I cannot see how you can have an “outlier” in a medication administration experiment.

4/ So, dose, analyze, pick a mathematical transformation and calculate the threshold AND THE STATISTICAL UNCERTAINTY for a given withdrawal time guideline.

PHENYLBUTAZONE STUDY, RMTC 2010

1/ 20 horses in training, dose **2g/Horse/IV,** plasma and serum samples taken, late May, 2010.

2/ Prompt analysis in Pennsylvania, much later analysis in Florida.

3/ Sept. 8th, Pennsylvania reports “treat as 36 hour rule or adjust “24hr” dose”.

2UG/ML PHENYLBUTAZONE Raw data

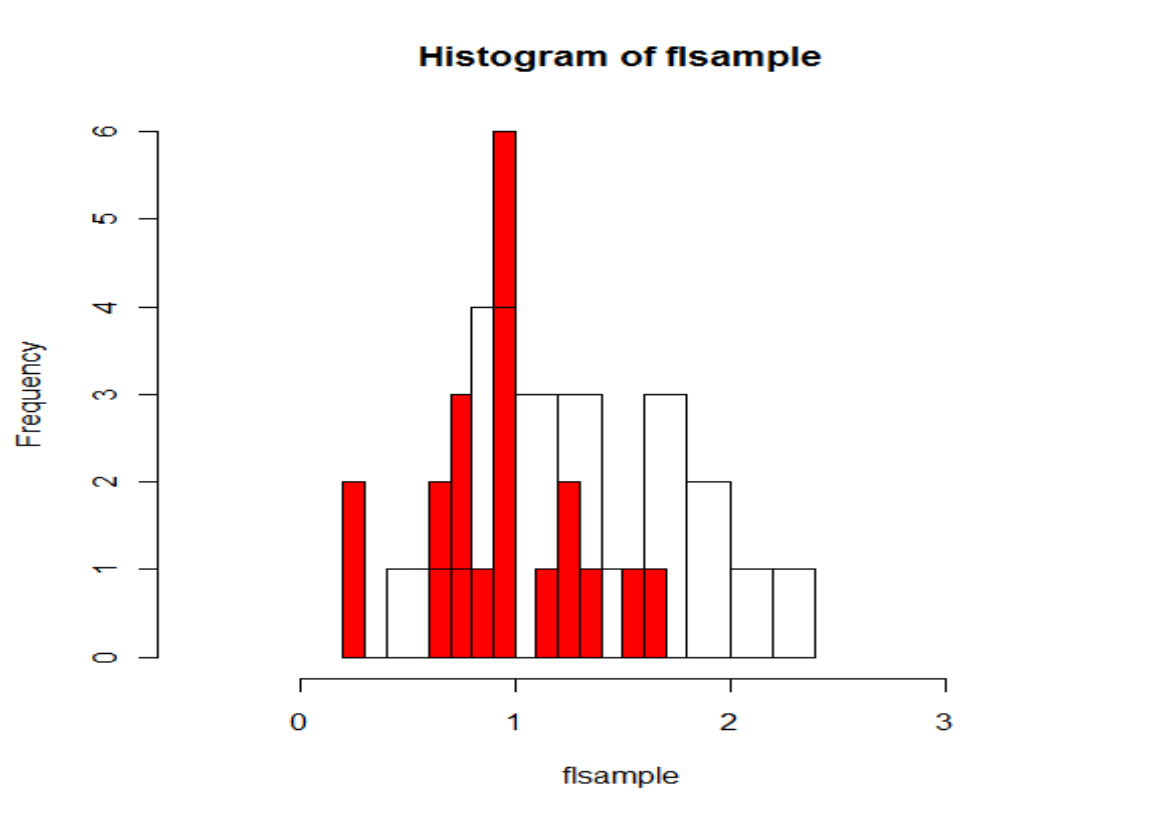
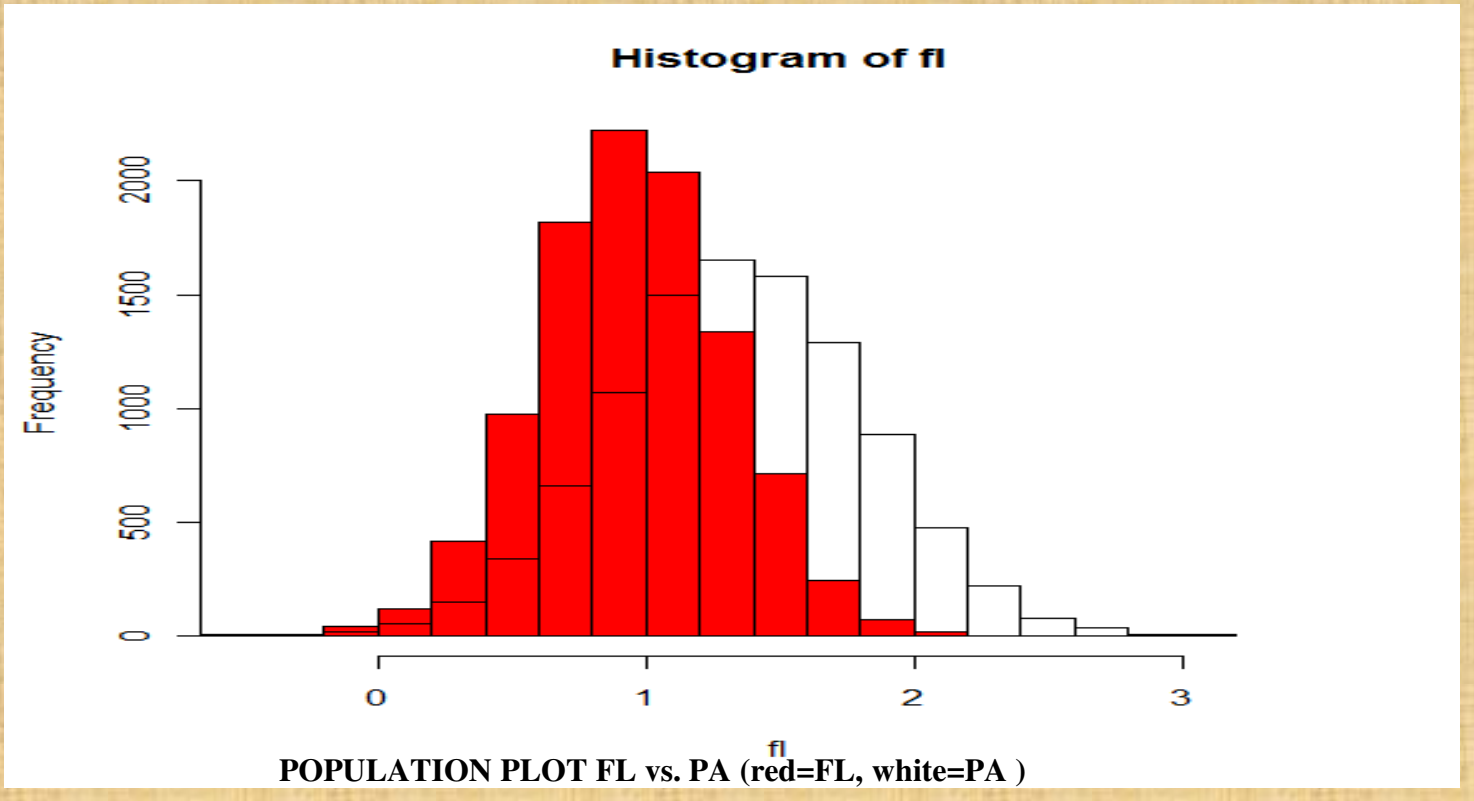


Figure #1: Serum phenylbutazone concentrations sample histograms, Florida samples in red vs Pennsylvania samples in white. (red=fl, white=pa)

2UG/ML PHENYLBUTAZONE Statistical Projections



POPULATION PLOT FL vs. PA (red=FL, white=PA)

# horses out of 17,500	2~3(ug/ml)	3~4(ug/ml)	4~5(ug/ml)	>5(ug/ml)
PA	1,400	3.5	0	0
FL	30.8	0	0	0

RMTC BUTE STUDY, PA ANALYSIS 2010

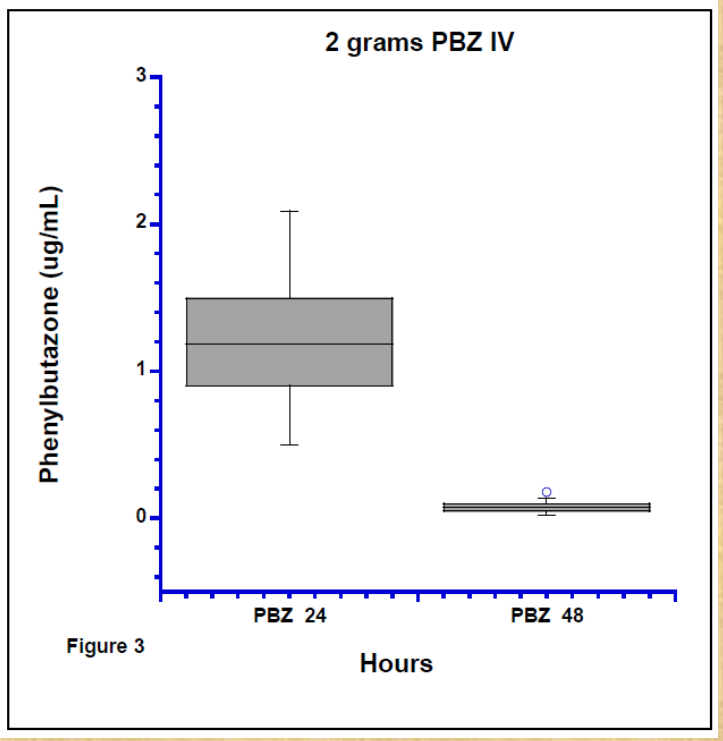


Figure 3

"These data indicate that even with no prior administrations of PBZ a dose of 2 grams 24 hours prior to post-time MAY PRODUCE A VIOLATION of the 2 µg/ml threshold. It is suggested that if a dose of 4.4 mg/kg is to be administered that the weight of the horse be taken into consideration or PBZ be administered at 36 hours prior to race time."

RMTC BUTE PA ANALYSIS

Figure 3 shows the results of a single IV administration of 2 grams (4.4mg/kg) of phenylbutazone (PBZ). This group of 20 horses did not have prior administration of PBZ.

The 24 hour mean post-administration plasma concentration was 1.2 **(range of 0.5 to 2.1) µg/ml** and mean 48 hours plasma concentration was 0.08 (range of 0.02 to 0.18) µg/ml.

These data indicate that even with no prior administrations of PBZ a dose of 2 grams 24 hours prior to post-time may produce a violation of the 2 µg/ml threshold. It is suggested that if a dose of 4.4 mg/kg is to be administered that the weight of the horse be taken into consideration or PBZ be administered at 36 hours prior to race time.

Phenylbutazone IV administration to 20 horses (courtesy of the Drs Sams and Callahan of the University of Florida).

[MATH [& PA] SAYS DANGEROUS THRESHOLD]

TAKE HOME MESSAGE

1/ Dose 2g/horse, but I believe most study horses were above 1,000 lbs., so horses actually/effectively being under dosed?.

2/ Data normally distributed??: a factor might be the above non-scientific dosing schedule.

OUTCOME: Data analysis presented at Spring HBPA meeting, following which RMTC changed the suggested PB dose to 1.8 grams/horse.[Not scientific].

FLUNIXIN

1/ Threshold 20 ng/ml, not a 24 hour withdrawal.

2/ **The RMTC has extended the withdrawal time to 32 hours** based on UC Davis RMTC study. Unclear what the status of the study referred to is.

3/ I suspect that a more scientifically correct approach would be to raise the regulatory threshold based on the scientific data available.

ENGLISH TRANSLATION WITHDRAWAL TIME UNCERTAINTY

"The take-home message is that biological variability is a major source of therapeutic medication overages and is both unpredictable and, as a practical matter, ***ESSENTIALLY UNAVOIDABLE****".*

COBALT: AN OVERVIEW AND REGULATORY THRESHOLD DEVELOPMENT

www.thomastobin.com

FIGURE 2: COBALT INJECTION.

Cobalt Injection Solution (30 mg cobalt/mL) and 'protecting' (Detox) agent of unspecified origin marketed out of Canada. At this time we have been unable to determine the identity, if any, of the 'active' ingredients in this particular 'Detox' substance, but thyroid hormones have been excluded.

Vitamin B-12

1/ Cobalt is a metallic element comprising 0.0029% of the earth's crust. Comes from the German "Kobold"= Goblin.

3/ Cobalt carefully put to work by the very earliest life forms as Vitamin B12.

4/ Required for DNA synthesis and activity of certain enzymes, thus for life. Human requirement, 2-3ug/day.

5/ Most mammals acquire their Vitamin B12 from intestinal bacteria.

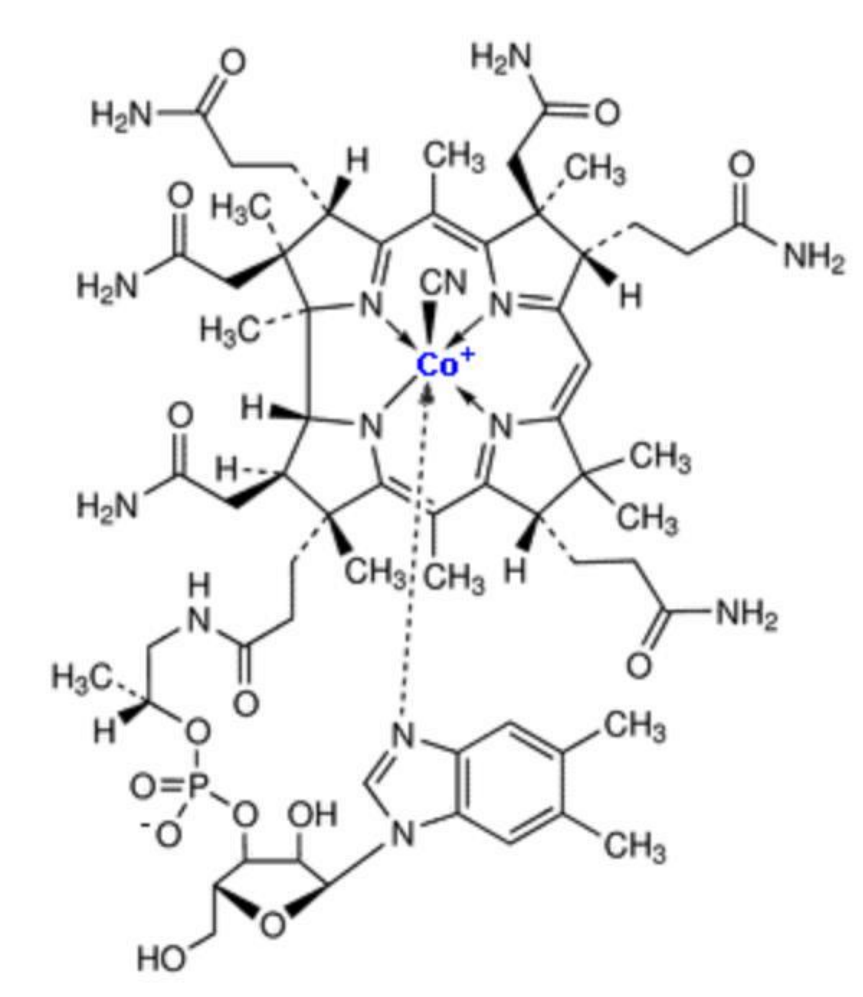


Figure 1. Vitamin B-12, α-(5,6-dimethylbenz-imidazolyl)cobamidcyanide, Mol. Mass 1,355.37 g/mol. Cobalt (inorganic cobalt) is at the center of the above figure surrounded by a four member corrin (tetrapyrrole) ring. Cobalt itself has an atomic weight of 58.9.

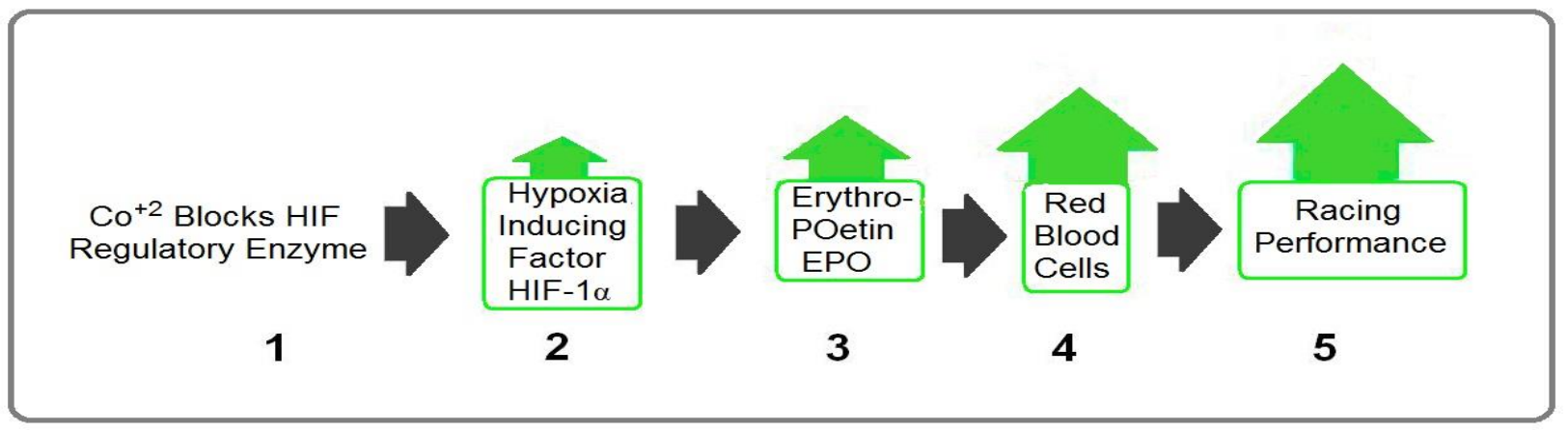
COBALT

1/ Human cobalt requirement 0.1ug/day
Horse presumably 1ug/day or so.

2/ 30 years ago cobalt was noted to stimulate red cell formation→ became standard treatment for anemia→20% improvement in hematocrit.

3/ Later replaced by Testosterone, then by ErythroPOietin [EPO].

EFFECT OF COBALT ON HEMATOCRIT IN HORSES



1/ Cobalt blocks the enzyme that breaks down Hypoxia Inducing Factor (HIF).
2/ Cellular concentration of HIF increases.
3/ Increased HIF promotes synthesis of EPO.
4/ Increased EPO stimulates synthesis of red blood cells.
5/ Increased numbers of red blood cells carry more oxygen to muscles thereby enhancing performance.

COBALT TOXICITY

1/ Cobalt added to beer many years ago caused "**beer drinkers myopathy**".

2/ Cobalt administration to humans for treatment of anemia has been reported to cause thyroid toxicity/goiter.

3/ Cardiomyopathy and heart failure.

COBALT: REGULATORY CONCERNS

1/ First recorded concerns in Canada, 2009.

2/ Later concerns in Australian racing especially Harness racing.

3/ New Jersey racetrack tested for cobalt and excluded two Harness horsemen.

4/ Hong Kong threshold, 100 ng/ml, urine.

5/ Australia, threshold 200 ng/ml urine.

6/ CHRB, Recently, 5ng/ml blood 25 ng/ml urine.

COBALT REGULATORY CONCERNS

Establishing a threshold for cobalt:

1/ Performing a “normal” background study; [Difficult to determine whether or not a high value is a bona fide natural background or due to cobalt administration].

2/ RMTC has proposed a 25 ng/ml urinary threshold, but implementation of this threshold has been delayed.

Acknowledgements

This research has been supported by ongoing research support from The National Horsemen's Benevolent and Protective Association and the Alabama, Arizona, Arkansas, Canada, Charles Town (West Virginia), Florida, Iowa, Indiana, Kentucky, Louisiana, Michigan, Minnesota, Nebraska, Ohio, Oklahoma, Ontario (Canada), Oregon, Pennsylvania, Tampa Bay Downs (Florida), Texas, Washington State, and West Virginia Horsemen's Benevolent and Protective Associations and the Florida Horsemen's Charitable Foundation, the Oklahoma Quarter Horse Racing Association and the Neogen Corporation. The continuing support of the Director, Faculty of the Gluck Equine Research Center, the University of Kentucky Gluck Equine Research Foundation, the administration of the College of Agriculture Food and the Environment and the University of Kentucky

are gratefully acknowledged.