CALIFORNIA HORSE RACING BOARD
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MEDICATION AND TRACK SAFETY COMMITTEE MEETING

of the **California Horse Racing Board** will be held on **Wednesday, October 22, 2014**, commencing at **2:00 p.m.,** in the **Baldwin Terrace Room** at the **Santa Anita Park Race Track, 285 West Huntington Drive, Arcadia,** California. Non-committee Board members attending the committee meeting may not participate in the public discussion, official committee vote or committee closed session.

AGENDA

Action Items:

1. Discussion and action regarding the **update on the 20th International Conference of Racing Analysts and Veterinarians meeting and other related matters.**

2. Discussion and action regarding an **update on cobalt use in California racing and the proposal to amend and/or add Board rules to regulate its use.**

3. Discussion and action regarding the **report on ketoprofen and the Racing Commissioners International (RCI) recommendation to lower the recommended dosage in order to prevent race day administration.**

4. Discussion and action regarding the **proposed amendment to CHRB Rule 1843.3, Penalties for Medication Violations, to prohibit: 1) suspended trainers from transferring horses to employees; 2) the use of any signage, colors or identifiable tack of a suspended trainer during a suspension and 3) to change from 60 days to 45 days, the requirement that trainers suspended for such time be banned from the enclosure and forfeit all assigned stall space and remove from the inclosure all signage, advertisements, training-related equipment, tack, office equipment and other property.**

5. Discussion and action regarding the **proposed amendment to CHRB Rule 1844, Authorized Medication, to add isofluprodone and its specified authorized level to the list of California's authorized medication**.

6. Discussion and action regarding an update on **the status of regulations previously recommended by the Committee relative to multiple medication violations penalties, fatality review process and third party race day lasix administration.**

7. Discussion and action regarding the **report on the International Federation of Horseracing Authorities (IFHA) recommended policies on medical records and out of competition testing.**

8. Discussion and action regarding the **report on the University of California, Davis's cardiology project, scheduled to commence after the conclusion of the 2014 Breeder's Cup.**

9. **General Business:** Communications, reports, requests for future actions of the Committee.

Additional information regarding this meeting may be obtained from Jacqueline Wagner at the CHRB Administrative Office, 1010 Hurley Way, Suite 300, Sacramento, CA 95825; telephone (916) 263-6000; fax (916) 263-6042. A copy of this notice can be located on the CHRB website at www.chrb.ca.gov. *Information for requesting disability related accommodation for persons with a disability who require aids or services in order to participate in this public meeting, should contact Jacqueline Wagner.

MEDICATION AND
TRACK SAFETY COMMITTEE
1st Vice Chairman Bo Derek, Chairman
Madeline Auerbach, Member
Rick Baedeker, Executive Director
Jacqueline Wagner, Assistant Executive Director

STAFF ANALYSIS
DISCUSSION AND ACTION REGARDING THE UPDATE ON THE 20TH INTERNATIONAL CONFERENCE OF RACING ANALYSTS AND VETERINARIANS MEETING AND OTHER RELATED MATTERS

Medication and Track Safety Committee Meeting
October 22, 2014

BACKGROUND

Business and Professions Code section 19580 provides that the Board shall adopt regulations to establish policies, guidelines, and penalties relating to equine medication in order to preserve and enhance the integrity of horse racing in the state. The International Conference of Racing Analysts and Veterinarians (ICRAV) is a biennial meeting of racing chemists and racing regulatory veterinarians with satellite meetings of the International Federation of Horseracing Authorities dealing with prohibited substances, gene doping and equine welfare.

The 20th ICRAV was held in Mauritius from Saturday, September 20th through Saturday, September 27th. Issues discussed included: emerging threats from proteomics, gene doping, use of cobalt, intra-articular corticosteroids, changing cultural values of horse welfare, use of hair-testing, sampling strategies, medication treatment records in training, fatality reviews, and injury risk factors.

RECOMMENDATION

This item is presented for Committee discussion and action.
The Board's Equine Medical Director is prepared to make a presentation to the Committee.

Report on 20th International Conference of Racing Analysts and Veterinarians

Rick M Arthur, DVM Equine Medical Director

The 20th International Conference of Racing Analysts and Veterinarians (ICRAV) was held in Mauritius from Saturday, September 20th through Saturday, September 27th. ICRAV is a biennial meeting of racing chemists and racing regulatory veterinarians with satellite meetings of the International Federation of Horseracing Authorities dealing with prohibited substances, gene doping and equine welfare. The full program is available at http://www.icrav2014.com/docs/ICRAV_2014_Full_prog_26_08.pdf .

Equine welfare and emerging doping threats from proteomics and gene doping and where current advances in gene therapy, regenerative medicine (stem cell therapy) and therapeutic biologics fit into doping regulation scheme were major topics. There were over 120 presentations plus 19 poster presentations.

Cobalt- There were a number of presentations on cobalt from around the world. There was a major effort to corral the cobalt problem and to set international thresholds. Cobalt is primarily a harness horse issue in North America and Australia/New Zealand.

Intra-articular corticosteroid regulation: There has been a major focus on intra-articular corticosteroid regulation internationally. Most jurisdictions outside the US regulate corticosteroids in urine with lstand down periods as long as 2 weeks. Chris Whitton from University of Melbourne presented data showing IA injections were associated with increased risk of injury for up to 42 days after treatment.

Welfare- Dr. Brian Stewart of Racing Victoria discussed the difficulty in changing the management practice amongst trainers and veterinarians in face of changing cultural value with animal welfare. There were several presentations with the theme that racing is being closely scrutinized everywhere in the worlds on animal welfare. For many international participants, the relative permissive medication policies in the US combined with our 2-4 times higher racing fatality rates than comparable international jurisdictions, is an animal welfare issue.

Other topics relevant to California were the use of hair testing, out-of-competition testing and sampling strategies, medication treatment records in training, fatality reviews with trainers and veterinarians, and risk factors for injury.

Emerging threats from more ever sophisticated doping schemes was a recurring theme of the conference. There were numerous papers on proteomics and peptide identification, gene doping and manipulation of gene expression, and the need to develop "biological passports," similar to WADA human anti-doping strategies, to identify physiologic manipulation that would be otherwise undetectable. Current drug testing strategies do very well identifying typical small molecule drugs. The

consensus of all participants was drug testing is not prepared for peptide drugs and gene doping and racing needs to commit resources to prepare for these emerging doping threats.

The Maddy Lab &/or EMD were author or co-authors on 5 presentations at the 20th ICRAV: Current and Potential Therapies and Gene Doping (Arthur), Gene Expression with Intra-articular Triamcinolone (Arthur, Knych), International Collaboration on Cobalt(Arthur), Selective Androgen Receptors Modulators (Knych, Stanley, Plasma Threshold for Isofluprodone (Arthur)

STAFF ANALYSIS
DISCUSSION AND ACTION REGARDING AN UPDATE ON COBALT USE IN CALIFORNIA RACING AND THE PROPOSAL TO AMEND AND/OR ADD BOARD RULES TO REGULATE ITS USE

Medication and Track Safety Committee Meeting
October 22, 2014

BACKGROUND

Business and Professions Code section 19580 provides that the Board shall adopt regulations to establish policies, guidelines, and penalties relating to equine medication in order to preserve and enhance the integrity of horse racing in the state. Business and Professions Code section 19581 states no substance of any kind shall be administered by any means to a horse after it has been entered to race in a horse race, unless the Board has, by regulation, specifically authorized the use of the substance and the quantity and composition thereof. Board Rule 1843, Medication, Drugs and Other Substances, provides that no horse participating in a race shall carry in its body any drug substance or its metabolites or analogues, foreign to the horse except as hereinafter expressly provided. No drug substance shall be administered to a horse which is entered to compete in a race to be run in this state except for approved and authorized drug substances as provided in these rules.

Cobalt is an endogenous substance as well as a normal dietary substance in mammals, including the horse. Injectable and oral preparations containing cobalt salts are being administered in various racing breeds across the United States. Although cobalt is a naturally existing and necessary dietary mineral for the horse, it is being administered in very high doses. High doses of cobalt containing products are used to increase erythropoiesis. This may allow a horse to oxygenate better than it would in its normal state, a form of "blood doping." Because cobalt in various forms is a normal dietary component as well as an endogenous substance in the form of cobaltoproteins such as cyanocobalamin or Vitamin B12 in the horse, a threshold must be set to differentiate samples collected from a horse that was normally fed and supplemented from a horse administered an extremely high dose of cobalt in enhance performance.

RECOMMENDATION

This item is presented for Committee discussion and action.
The Board's Equine Medical Director is prepared to make a presentation to the Committee.

Cobalt Situation Analysis

Rick M. Arthur, DVM Equine Medical Director

The regulatory blood threshold needs to be 25ng/ml or lower to effectively eliminate cobalt salt administration. The chart below identifies the proportion of horses that would be trigger a violation at the listed levels.

	24 hours	36 hours	48 hours	72 hours	96 hours	120 hours	168 hours
25 ppb	**16/16**	**16/16**	**16/16**	**16/16**	**16/16**	**16/16**	11/16
35 ppb	**16/16**	**16/16**	**16/16**	**16/16**	13/16	9/16	3/16
50 ppb	**16/16**	15/16	11/16	7/16	4/16	2/16	1/16
70 ppb	12/16	6/16	3/16	1/16	1/16	*0/16*	*0/16*

*Based on a dosage of 100mg cobalt chloride intravenously from an administration study conducted by Dr. Knych at the Maddy Lab. The manuscript describing has been accepted by the journal *Drug Testing and Analysis* and will be available on-line shortly.

There does not appear to be a documented instance of cobalt deficiency in the horse. Therefore, cobalt supplementation is unnecessary and cobalt salt administration is medically unjustified. Cobalt does stimulate erythropoiesis in humans and rats and high cobalt dosages are associated with toxicity in humans and rats. Where the horse falls and at what levels relative to blood doping and health risk is unknown. Regardless, the use of cobalt is an issue that needs to be addressed and the administration or cobalt salts, parenterally or orally at unwarranted high dosages, needs to be eliminated.

The international blood threshold proposed by Hong Kong is **2.5ng/ml** using the risk analysis of 1/10,000 false positive rate (3.72SD, the current IFHA standard). Cobalt blood analysis from Dubai, France and a number of European countries appears to support the Hong Kong proposal. The California TB & QH data (n= 125) a false positive risk at 1/10,000 results in a threshold of **13.89 ng/ml** [ng/ml and ppb (parts per billion) can be considered synonymous]. The RMTC nationally collected data supports a blood threshold of 25ng/ml with a risk analysis of 1/33,000 and a **51ng/ml** risk analysis of 1/3,487,966. The press reports of a 1/10,000 risk at 70 ng/ml from the USTA/Maylin project are mathematically incompatible with the reported sample size and reported high of 6.8ng/ml in untreated horses.

There are many cobalt containing supplements and injections. Based on administration studies in Australia, Hong Kong, and Univ of Pennsylvania, cobalt levels can reach 10ng/ml at 24 hours. The long elimination half-life of cobalt could possibly lead to cobalt accumulation. This has not been studied in horses, but in random samples, there is no evidence accumulation is a problem at normal supplement and vitamin B-12 dosages. Indiscriminate cobalt administration could possibly lead to inadvertent elevation of cobalt by negligence rather than an overt doping attempt. The data indicate cobalt greater than 25ng/ml can only be obtained by deliberate cobalt administration. Regardless, negligence warrants a lesser penalty than intentional doping. Therefore I recommend the following approach:

- Cobalt in blood at or above 25ng/ml 25ng/ml but under 50ng/ml should be a Class 4 drug violation with a Category C penalty.
- Cobalt in blood at or above 50ng/ml should be a Class 3 drug violation with a Category B penalty.

Serum cobalt concentration analysis

CHRB Samples

8-19-14

Methods

Serum cobalt concentration (ppb) was evaluated in 204 samples collected from three breeds of racehorses: Quarter Horses, Standardbreds, and Thoroughbreds, with a limit of detection of 1ppb. Samples for which serum cobalt concentration were below the limit of detection were assigned the value of 1ppb for numeric analysis. The assumption of normality was evaluated by breed using Shapiro-Wilk testing. Non-normally distributed data was Box-Cox transformed, then re-evaluated for normality using Shapiro-Wilk testing. For normally-distributed data, data were summarized using mean and standard deviation. Threshold cutoff for normally-distributed data was estimated as the mean + 3.72 standard deviations (which encompasses 99.9900389% of the population). For non-normally-distributed data, data were summarized[1] using percentiles and boxplots. Threshold cutoff for non-normally-distributed data was estimated by identifying the best-fitting[2] distribution for the data using Kolmogorov-Smirnov goodness of fit testing and visual evaluation, then determining the 99.9900389 percentile for that distribution.

Results

Serum cobalt concentration was not normally distributed in any of the breeds ($p<0.0001$), and could not be normally transformed using Box-Cox transformation.

In all breeds, at least 50% of horses sampled had serum cobalt values ≤1 ppb (Table 1). In Quarter Horses, 43 (100%) of 43 samples contained <15ppb cobalt, compared to 81 (98.8%) of 82 Thoroughbred samples and 70 (88.6) of 79 Standardbred samples (Figure 1).

The best-fitting distribution for serum cobalt in 125 Quarter Horses and Thoroughbred Horses was an exponential distribution with standard deviation 1.3992 and mean 2.3992 (Figure 2). The 99.9900389 percentile for this distribution is 13.893, meaning that nearly 99.99% of serum cobalt concentrations would be expected to be less than 13.893 ppb.

Table 1. Descriptive summary of breed-specific serum cobalt concentrations (ppb) in 204 racehorses.

		Serum cobalt concentration (ppb)				
Breed	N	Min	Median	95%	99%	Max
QH	43	1	1	11.1	12	12
STB	79	1	1	290	610	610
TB	82	1	1	9.6	27	27

[1] Stata 13.1, StataCorp LP, College Station, TX
[2] @Risk 5.5, Palisade Corporation

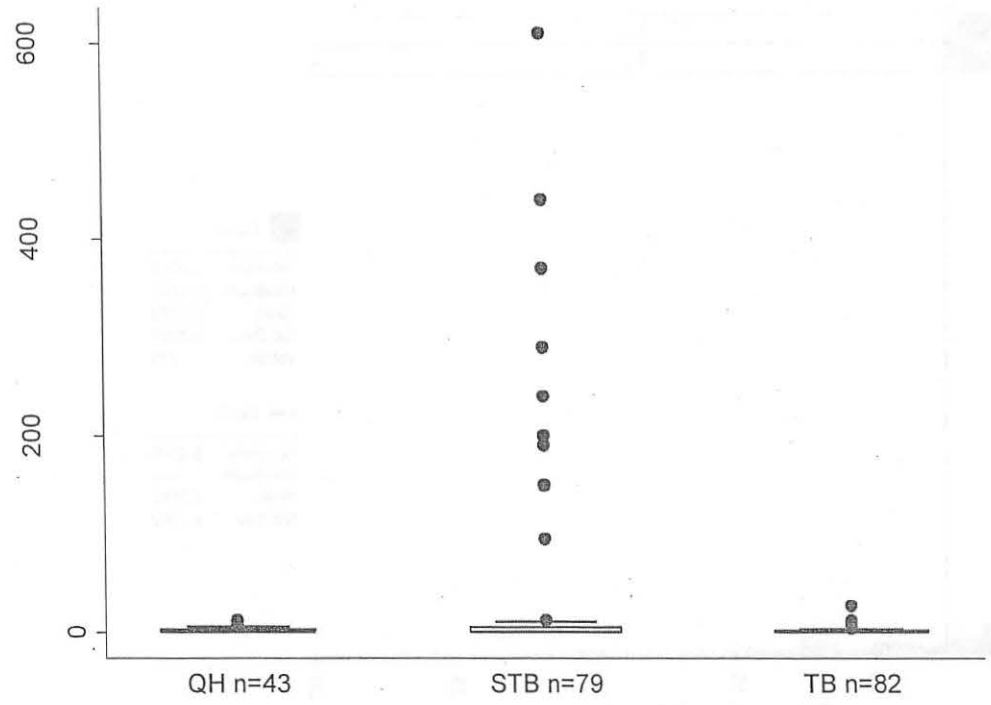


Figure 1. Breed-specific boxplots of serum cobalt concentration in 204 Quarter Horse, Standardbred, and Thoroughbred racehorses.

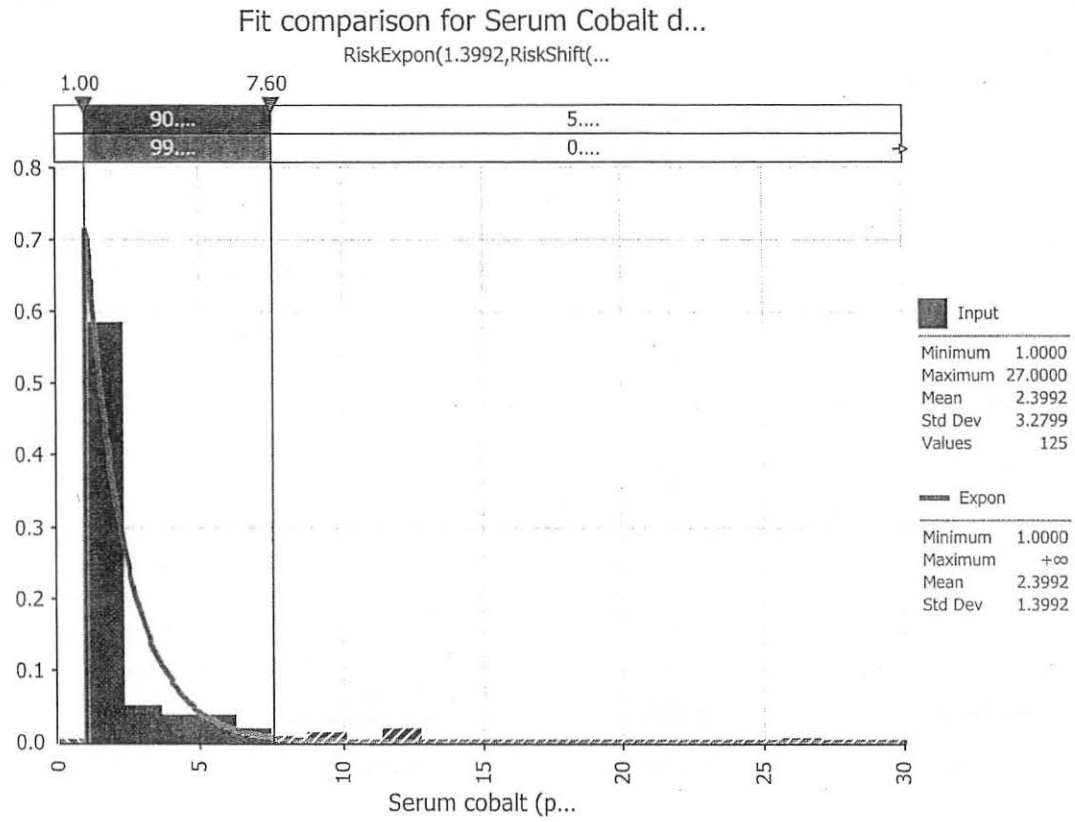


Figure 2. Best-fitting distribution for serum cobalt in 125 samples from 43 Quarter Horse and 82 Thoroughbred racehorses.

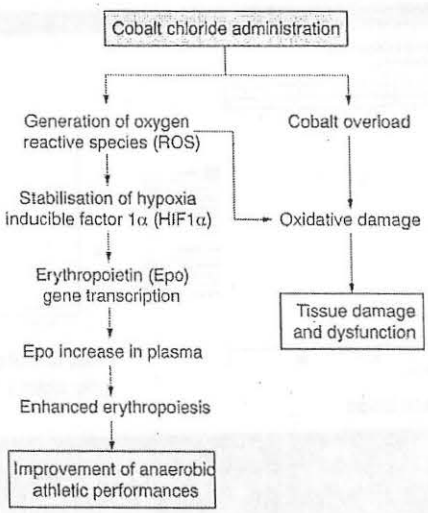
ICRAV conference, Mauritius, September 2014.

An international collaboration on cobalt for setting up a threshold value

Marie-Agnes Popot[1], Emmie N.M. Ho[2], Terence S.M. Wan[2], Rick M Arthur[3], Dionne Benson[4], Charlie Russo[5], Pamela Hincks[6], Clive Pearce[6], Yves Bonnaire[1].

Laboratoire des Courses Hippiques[1], 15 rue de Paradis, 91370 Verrières-le-Buisson, France.
Racing Laboratory[2], The Hong Kong Jockey Club, Sha Tin Racecourse, Sha Tin, NT, Hong Kong.
School of veterinary medicine[3], University of California, Davis, CA 95616, USA.
Racing Medication and Testing Consortium[4], Lexington, KY 40503, USA
Racing Chemistry Laboratory, ChemCentre[5], PO Box 1250, Bentley Delivery Centre, Western Australia, 6983, Australie.
LGC (HFL)[6], UK, Newmarket Road, Fordham, Cambridgeshire, CB7 5WW, UK.

Potential ergogenic effects and complications of cobalt chloride administration in athletes.

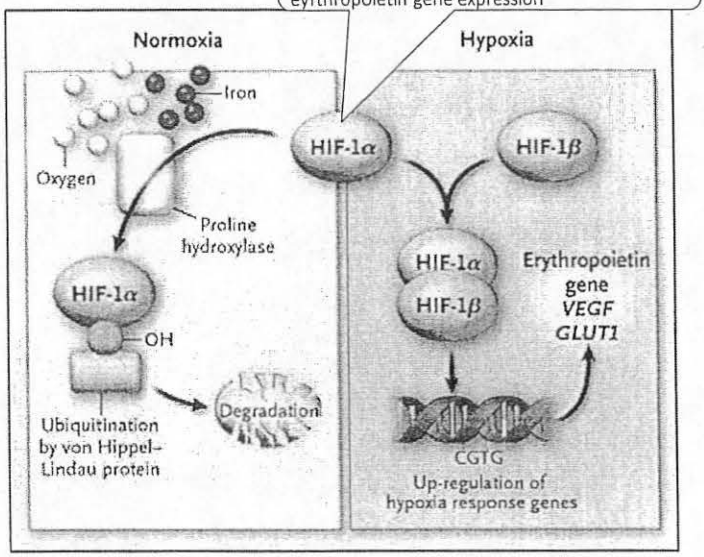


Lippi G et al. Br J Sports Med 2005;39:872-873

Added Slide

- Hypoxia Inducing Factors (HIF)

Cobalt (as does Xenon) stabilizes HIF-1α causing eyrthropoietin gene expression

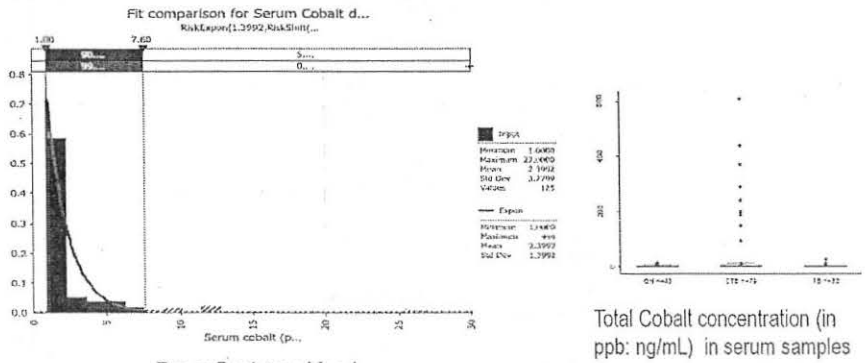


Prchal JT. N Engl J Med 2003;348:1282-1283.

Added Slide

Results from serum samples in California

ICRAV conference, Mauritius, September 2014



Pre or Post-race blood

Total Cobalt concentration (in ppb: ng/mL) in serum samples

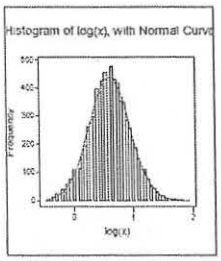
Medium (Serum)	n	Min (ng/mL)	Max (ng/mL)	Mean# (ng/mL)	Transformation To a normal distribution	Possible Threshold (ng/mL) (risk of 1 in 10000)
California	125	1.0*	27	2.39	Risk Analysis	13.89

* Samples ≤ 1ng/ml reported as 1ng/ml

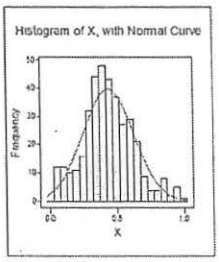
16

Results from raceday samples in Hong Kong and the UAE:

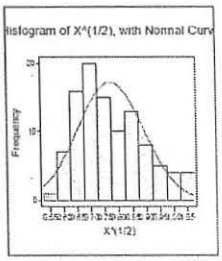
ICRAV conference, Mauritius, September 2014.



HKJC Post-race urine



HKJC Post-race blood



ERA# Raceday blood

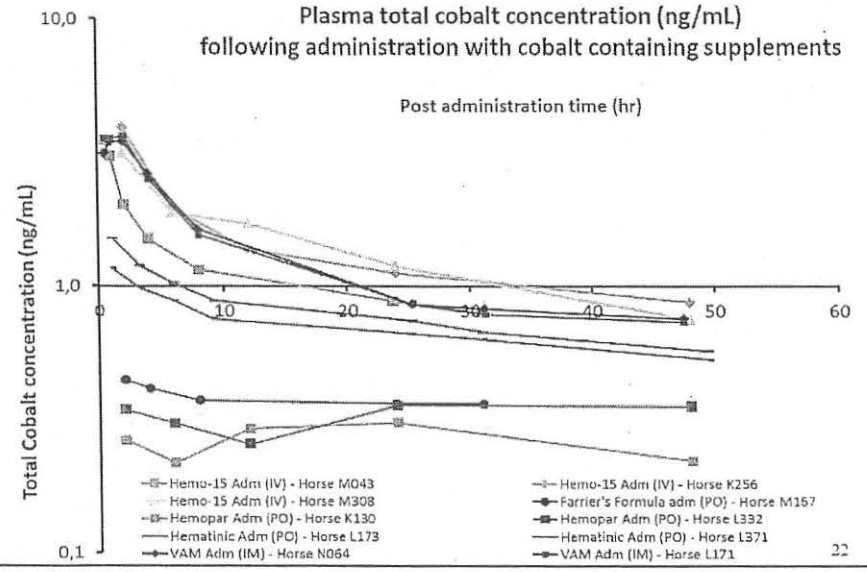
Medium	n	Min (ng/mL)	Max (ng/mL)	Mean (ng/mL)	Transformation To a normal distribution	Possible Threshold (ng/mL) (risk of 1 in 10000)
HKJC Urine	7462	0.38	60.6	5.5	Log	74.5
HKJC Plasma	375	0.03	0.98	0.44	None needed	1.14
ERA# Plasma	103	0.32	1.10	0.61	Square-root	1.47

Emirates Racing Authority

E.N.M. Ho *et al.*, *Drug Testing Anal.*, in press (DOI 10.1002/dta1719)

Method: Administration study data in plasma (Hong Kong)

ICRAV conference, Mauritius, September 2014.



22

RMTC
Racing Medication & Testing Consortium

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RMTC Position Statement on Cobalt

Introduction

Cobalt is an endogenous substance as well as a normal dietary substance in mammals, including the horse. The cobalt dietary requirement for a horse is less than 0.05 ppm.[1] It is used in the incorporation of vitamin B12 in the cecum and colon of the horse.[2] Strictly speaking, if B_{12} vitamins are incorporated into the diet, any administration of cobalt is superfluous.

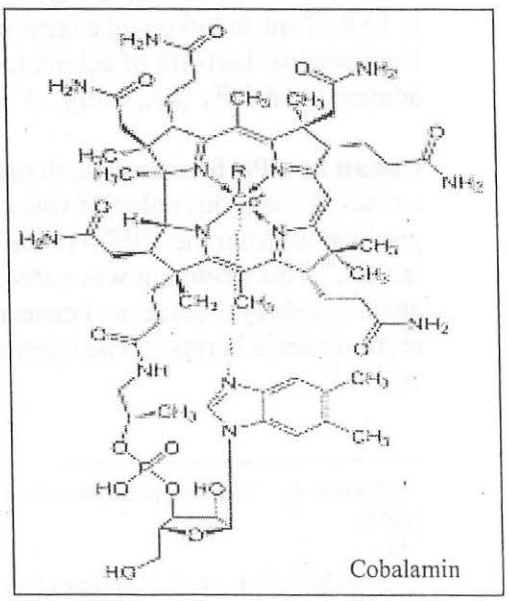
Injectable and oral preparations containing cobalt salts are being administered in various racing breeds across the United States. Although cobalt is a naturally existing and necessary dietary mineral for the horse, it is being administered in very high doses – well in excess of 0.05 ppm. High doses of cobalt containing products are used to increase erythropoiesis. This may allow a horse to oxygenate better than it would in its normal state, a form of "blood doping" similar to treating a horse with human recombinant EPO.

Because cobalt in various forms is a normal dietary component as well as an endogenous substance in the form of cobaltoproteins such as cyanocobalamin or Vitamin B12 in the horse, a threshold must be set to differentiate samples collected from a horse that was normally fed and supplemented from a horse administered an extremely high dose of cobalt in enhance performance. It should be noted, however, that there are no documented cases of cobalt deficiencies in the horse in scientific literature so the use of cobalt containing supplements is unnecessary and medically unjustified.

Chemistry[3]

Cobalt is an element with the atomic number 27 in the periodic table. In the environment, cobalt is typically found in compounds with other elements. Pure cobalt is a hard, gray, metallic substance. The chemical symbol for cobalt is Co.

Cobalt forms the active center of cobalamins – particularly vitamin B_{12}. In the horse, vitamin B_{12} creation occurs in the gastro-intestinal tract (specifically the cecum and the colon). Vitamin B_{12} is used in the production of red blood cells.[4] Cobalt is also a component of a number of other cobaltoproteins such as aminopeptidase 2, a ubiquitous enzyme involved in peptide synthesis.


Cobalamin

[1] Kahn, C.M., *et al.*, The Merck Veterinary Manual, Merck & Co., Inc., (8th Edition) p. 1878 (1998).
[2] *Id.*
[3] See, generally: http://en.wikipedia.org/wiki/Cobalt
[4] Paustenbach, D.J., *et al.*, *A review of the health hazards posed by cobalt*, Critical Reviews in Toxicology, 2013; 43(4): 316-362.

Pharmacokinetics

Absorption

Cobalt is rapidly but incompletely absorbed following ingestion in feeds and supplements. Furthermore, bacteria in the gut utilize cobalt in the biosynthesis of cobalamin. Cobalamin from dietary sources or microbial synthesis binds with intrinsic factor in the digestive tract. The cobalamin-IF complex binds to cubilin, a membrane receptor in the terminal ileum. Upon endocytotic uptake from the ileum, cobalamin dissociates from intrinsic factor and combines with transcobalim II in which form it enters the blood and is carried to various tissues. The serum concentration of Vitamin B12 in 16 mature, partly warm-blooded, partly Finnish race horses was 1.54 ± 0.16 nanograms per milliliter of serum.[5]

Cobalt as cobalt chloride injected intravenously rapidly binds to serum proteins. The majority of cobalt ends up stored in body tissues such as the liver, skeletal muscle, and other tissues. A portion of the injected cobalt is secreted into the gastrointestinal tract where bacteria utilize it to produce cobalamin. This appears in the systemic circulation within 2 hours after administration of intravenous cobalt indicating rapid synthesis and absorption. Thus, the serum contains cobalt ion bound to serum proteins and cobalt as a component of Vitamin B12-transcobalamin II.[6]

Distribution

Cobalt ion (from cobaltous chloride administration) is widely distributed with an apparent steady-state volume of distribution of 0.74 L/kg. Cobalt ion is slowly transported into erythrocytes via a calcium channel where it binds to cytosolic components. Analysis of total cobalt was performed as it is not certain that bound portions of the cobalt are not metabolically active nor is it clear how various sample handling would alter the amount of cobalt that is free in the sample.

Excretion

Cobalt ion (from cobaltous chloride administration) is cleared slowly with a total clearance estimated to be 0.07 mL/min/kg and a terminal phase half-life estimated to be about 156.4 hours. Due to the long terminal half-life of cobalt, there may be a substantial accumulation factor (~7-fold) if it is administered daily (*i.e.*, every 24 hours) for 3-4 half-lives.

Cobalt as a Performance Enhancer?

Cobalt as cobaltous chloride was used clinically in humans to stimulate erythropoiesis (red blood cell production) from the 1950s-1980s.[7] This was accomplished through daily doses of cobalt chloride tablets.[8] This treatment was successful in inducing erythropoiesis in human patients with sickle cell anemia, kidney disease, and cancer.[9] It was also determined to be effective in inducing erythropoiesis in rats.[10] The international unit for EPO was set as the equivalent of 5 mmol of cobalt.[11]

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[5] Salminen, K. *Cobalt metabolism in horse. Serum level and biosynthesis of Vitamin B12*. Acta Vet. Scand. 16: 84-94, (1975)

[6] *Id.*

[7] Paustenbach, D.J., *et al.*, *A review of the health hazards posed by cobalt*, Critical Reviews in Toxicology, (2013); 43(4): 316-362.

[8] *Id.*

[9] Elliott, S., *Erythropoiesis-stimulating agents and other methods to enhance oxygen transport,* British Journal of Pharmacology, (2008) 154(3):529-41.

[10] *Id.*

[11] Sytkowki, A.J., *Erythropoietin*, Germany, Wiley-VCH Verlag GmbH& Ko, (2004) pp. 5, Print.

Cobalt stimulates synthesis of red blood cells by stabilizing hypoxia inducible factor (HIF).[12] In a normally oxygenated mammal, HIF degrades quickly.[13] In hypoxia, HIF is stabilized and induces erythropoiesis by upregulating the gene for erythropoietin and related genes.[14]

The increased erythropoiesis that occurs as a result of this process, in turn, leads to increased oxygen carrying capacity. Increased oxygen carrying capacity leads to improved performance.

Horses, as all mammals, regulate erythropoietin production through HIFs.[15] In fact, in one case, researchers induced the expression of HIF-1α by the addition of cobalt chloride.[16] The researchers found that the addition of cobalt chloride to equine tissue "consistently and rapidly induced the expression of HIF-1 α protein."[17] The increase in expression of the HIFs was 3 fold at 3 and 6 hours, double at 12 hours, and returned to normal at 24 hours.[18]

The World Anti-Doping Agency categorizes HIFs as banned substances on its Prohibited Substances List and prohibits enhancing oxygen transport in any manner.[19]

Toxicity Issues/Potential Adverse Health Effects

In addition to potential performance enhancing attributes of cobalt, there are a variety of adverse health effects of cobalt use that have been documented in humans and other mammals. Both acute and chronic toxicity issues have been reported.

Acute Toxicity

In three reported human case studies of acute cobalt toxicity following oral ingestion, symptom severity varied. Post oral ingestion, the reported symptoms included stomach mucosa necrosis, vomiting, abdominal pain, brain edema, and death.[20] The concentration of cobalt in one of these case studies was as high as 426,000 ppb.

Additionally, research has been done in rats and mice on the effect of a one-time exposure to oral cobalt from a variety of cobalt containing compounds. In those studies, acute oral toxicity symptoms included: diarrhea, ataxia, motor activity reduction, hypothermia, degenerative changes to the liver and heart, and increased blood flow to the kidneys and liver.[21] Dose-dependent acute toxicity was likely related to the bioavailability of cobalt in the different substances.

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[12] *Id.*
[13] *Id.*
[14] *Id.*
[15] De Ceulaer, K., *et al.*, *Morphological Data Indicate a Stress Response at the Oral Border of Strangulated Small Intestine in Horses*, Research in Veterinary Science, (2011); 91:294-300; Deschene, K., *et al., Hypoxia Regulates the Expression of Extracellular Matrix Associated Proteins in Equine Dermal Fibroblasts via HIF1*, Journal of Dermatological Science (2012); 65:12-18.
[16] *Id* at 14.
[17] *Id.*
[18] *Id.*
[19] Knych, H.K., *et al.*, *Detection, Pharmacokinetics and Selected Pharmacodynamics of Cobalt Following a Single Intravenous Administration to Horses*, submitted for publication 8/22/14.
[20] Paustenbach, D.J., *et al.*, *A review of the health hazards posed by cobalt*, Critical Reviews in Toxicology, 2013; 43(4): 316-362.
[21] Paustenbach, D.J., *et al.*, *A review of the health hazards posed by cobalt*, Critical Reviews in Toxicology, 2013; 43(4): 316-362.

Chronic Toxicity

Toxicity associated with chronic administration of cobalt salts to humans includes various neuropathies, thyroid dysfunction, and heart failure. Chronic exposure to cobalt added to beer to stabilize the foam results in cobalt-beer cardiomyopathy that is characterized by abrupt left ventricular failure, cardiogenic shock, and acidosis. [22] The use of cobalt salts as therapeutic agents ceased in the 1980s after toxicity associated with its chronic use was reported in patients and the introduction of human recombinant erythropoietin as a safer and more effective alternative. Specifically, in human studies, a number of effects were associated with chronic cobalt administration. These effects included:

- hematological (polycythemeia/increased hematocrit);
- thyroid (decreased iodine uptake causing goiter/hypothyroidism);
- neurologic effects (reversible hearing and vision impairment – particularly at high plasma concentrations);
- cardiac effects (cardiomyopathy – with very high dose); and
- dermatological effects (skin rashes, pimples, dermatitis, dermal flares).[23]

In animal studies, the following effects were associated with chronic cobalt administration:

- hematological (increased red blood cells, hemoglobin, hematocrit - rats);
- thyroid (histopathological changes to thyroid – mice, changes in thyroid hormones – rats);
- neurological effects (optic toxicity and auditory system toxicity – rabbits);
- cardiac effects (general – guinea pigs, myocardial degeneration/high mortality – rats, cardiomyopathy – dogs with thiamine deficiency);
- reproductive (decreased sperm concentration in mice, testicular atrophy – rats); and
- kidney and liver (organ damage at high doses – rats and mice).[24]

Cobalt Research

A number of research projects were performed in order to determine an appropriate regulatory threshold for cobalt in race horses.

The RMTC and the Kentucky EDRC co-sponsored an administration a study of cobalt salts at the University of California Davis under the direction of Dr. Heather Knych and the University of Kentucky's Dr. Cynthia Gaskill. In this study, Dr. Knych administered 100 mg of cobalt chloride salts intravenously into 16 research horses at UC Davis. Blood and urine samples were collected at specific time points over 10 days post administration. Dr. Gaskill analyzed the samples blood and urine samples at the University of Kentucky. The resulting scientific paper describing the pharmacokinetics and pharmacodynamics of cobalt chloride administration has been peer-reviewed and accepted for publication in Drug Testing and Analysis and will be available soon. An important finding of this study was a prolonged gamma (elimination) half-life (156.4 hrs) of cobalt in serum, which will be useful in regulating its use.

Additionally, laboratories from University of Kentucky (using Kentucky Equine Drug Research Council funds), Pennsylvania, UC Davis and Truesdail Laboratories (multiple jurisdictions) determined serum cobalt concentrations in samples collected from racing horses tested for cobalt as a

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[22] Alexander CS. *Cobalt-beer cardiomyopathy. A clinical and pathologic study of twenty-eight cases.* American Journal of Medicine, 53: 395-417, 1972).

[23] *Id.*

[24] *Id.*

part of research and/or screening. The inter-laboratory data agreement results showed that the method was transferrable.

The RMTC combined the cobalt serum data from these sources into one population and asked Dr. Ashley Hill to analyze the data. Dr. Hill is an epidemiologist who works with testing laboratory probability calculations for the CAHFS laboratory at University of California Davis. The objective was a threshold for cobalt equivalent of the mean plus 5 standard deviations as was done for arsenic threshold in the late 80's. Five standard deviations is the 99.99997133 percentile and approximately a probability of 1 in 3,487,966 of a false positive.

Dr. Hill performed analyses of the data using all horses (except CA STB's where veterinarians and trainers admitted administering cobalt salts) and with just TBs and QHs (all STBs excluded). Including all horses, the mean plus 5SD (99.99997133 percentile) yielded a threshold of 51 ppb. If two outliers (153 and 338 ppb) suspected of cobalt salt administration were statistically excluded and omitted, the threshold was 36.3 ppb. Similar to the CA data where trainers and veterinarians acknowledged cobalt salt administration, the STB data include some very high values that greatly influence the results. Eliminating all STBs the mean plus 5SD (99.99997133 percentile) was 35.9 ppb.

Because we suspect that a number of horses in the TB population used to develop this threshold have been treated with cobalt, Dr. Hill was tasked to determine the relative risk of a threshold of 25 ppb. A 25ppb threshold using all the QH & TB data is equivalent to 4 standard deviations and carries an estimated risk of a false positive of 1 in 33,000 horses. Note that these calculations using mean plus 4 or 5 times the SD result in higher thresholds than the mean plus 3.72 SD used in IFHA threshold calculations. Dr. Hill had previously determined a cobalt threshold from Quarter Horses in CA which was the only population in that jurisdiction with data that could be normalized and analyzed with standard statistics. Using the CA Quarter Horse sample population (n=43) and otherwise familiar statistical analysis, the equivalent mean + 5SD resulted in a cobalt threshold in serum of 24.57 ppb.

In addition to these studies, Dr. Mary Robinson of the Pennsylvania Equine Toxicology and Research Laboratory and University of Pennsylvania has completed several administrations of cobalt containing supplements. This research consisted of single-dose administration studies of cobalt containing supplements which are routinely administered to racing horses. Based upon the results from those administration studies in research horses as well as other international information, we have determined that it is extremely unlikely that typical cobalt containing substances such as these when used in a normal fashion would cause cobalt concentrations in serum or plasma to exceed a 25 ppb threshold.

The three substances that were investigated were: vitamin B_{12} (0.07 mg IM), Red Cell (3.9 mg PO), and Vita 15 (2.4 mg IM).[25] At no time did the plasma concentration exceed: 1 ppb for vitamin B12, 6 ppb for Red Cell, or 13 ppb for Vita 15.[26] Additionally, Dr. Robinson will be investigating a 10 mL dose of vitamin B_{12} IV. Dr. Robinson's presented the results of this research at the ICRAV conference in September and will be publishing these results in the full proceedings.

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[25] Robinson, M.A., *et al.*, *Cobalt-Containing Supplements and Sweet Feed Increase Equine Plasma and/or Urine Cobalt Concentrations*, Abstract 20th ICRAV Convention Proceedings, pp. 108.
[26] *Id.*

International Research and Collaborations

International cobalt thresholds in urine and blood were discussed extensively at the International Conference of Racing Analysts and Veterinarians (ICRAV) in September 2014. There were a number of presentations on efforts to determine a global threshold for cobalt as well as discussions of existing cobalt thresholds.

New South Wales in Australia is currently using a cobalt threshold of 200 ppb in urine. This threshold is based upon race day sampling where the concentrations of cobalt ranged from 1 to 3,460 ppb.[27] The 200 ppb urine threshold was considered as having a 1:50,160 chance of a false positive.[28] Australia is amenable to lowering that threshold based upon the international recommendation.[29]

In addition to the race-day survey completed in Australia, the researchers there also performed several administrations of cobalt containing substances. Five horses were administered Hemo-15 (10 mL containing7 mg total cobalt) and 10 mls of 150 mg/mL vitamin B-12 IV for three consecutive days.[30] In those horses, the blood cobalt concentration peaked at 15 minutes post administration. None of the blood samples exceeded 10 ppb in cobalt.[31]

Additionally, a presentation was made reflecting the ongoing international collaboration on cobalt.[32] In this presentation, a review of various survey data was completed from several jurisdictions around the globe.[33] Below is a chart of the respective jurisdictions, number of horses, and resulting thresholds when using the international standard of a 1:10,000 chance of a false positive (*i.e.*, mean plus 3.72 times the SD).[34]

Jurisdiction	Number of Horses	Statistical Threshold in Plasma
Hong Kong	375	1.14 ppb
United Arab Emirates	103	1.47 ppb
United States (California Data)	125	13.89 ppb

Hong Kong Jockey Club researchers have just published a peer-reviewed scientific paper that recommends a threshold of 2 ppb in serum at mean + 3.72SD (1/10,000 risk).[35] The Hong Kong paper includes data on plasma cobalt concentrations found in horses after administration of cobalt containing supplements (Hemo-15™, Hemopar™, Hematinic™, Farrier's Formula, VAM®).[36] At all times, after administration of these substances, the total plasma concentrations of cobalt were below 10 ppb.[37]

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[27] Wainscott, M., Hibbert, D.B.; *Study of Cobalt in Racing Standardbred Horses*, Abstract 20th ICRAV Convention Proceedings, pp. 46.
[28] *Id.*
[29] *Id.*
[30] *Id.*
[31] *Id.*
[32] Popot, M.A., *et al.*, *An International Collaboration on Cobalt for Setting up a Threshold Value*, Abstract 20th ICRAV Convention Proceedings, pp. 48.
[33] *Id.*
[34] *Id.*
[35] Ho, E.N.M, *et al.*, *Controlling the Misuse of Cobalt in Horses*, Drug Testing and Analysis, 2014 August 17, doi: 10.1002/dta.1719 [epub ahead of print].
[36] *Id.*
[37] *Id.*

Several thresholds are being considered across the United States and within the RMTC – based upon serum data from the pharmacokinetic study of cobalt salts in horses, the following will show the length of time that each considered threshold would likely regulate the use of cobalt salts at typical doses reported to be used in race horses. There were 16 horses in the study. The following table reflects the number of horses whose plasma concentration exceeds the suggested threshold at each time compared to the total number of horses in the study (n=16).

	24 hours	**36 hours**	**48 hours**	**72 hours**	**96 hours**	**120 hours**	**168 hours**
25 ppb	16/16	16/16	16/16	16/16	16/16	16/16	11/16
35 ppb	16/16	16/16	16/16	16/16	13/16	9/16	3/16
50 ppb	16/16	15/16	11/16	7/16	4/16	2/16	1/16
70 ppb*	12/16	6/16	3/16	1/16	1/16	0/16	0/16

* This number reflects the proposed threshold from the September 30, 2014 USTA press release.

The 25ppb threshold is the only threshold that regulates the administration of as much as 100mg of cobalt chloride at 5 days and the majority of horses at 1 week.

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Controlling the misuse of cobalt in horses

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Cobalt is a well-established inducer of hypoxia-like responses, which can cause gene modulation at the hypoxia inducible factor pathway to induce erythropoietin transcription. Cobalt salts are orally active, inexpensive, and easily accessible. It is an attractive blood doping agent for enhancing aerobic performance. Indeed, recent intelligence and investigations have confirmed cobalt was being abused in equine sports. In this paper, population surveys of total cobalt in raceday samples were conducted using inductively coupled plasma mass spectrometry (ICP-MS). Urinary threshold of 75 ng/mL and plasma threshold of 2 ng/mL could be proposed for the control of cobalt misuse in raceday or in-competition samples. Results from administration trials with cobalt-containing supplements showed that common supplements could elevate urinary and plasma cobalt levels above the proposed thresholds within 24 h of administration. It would therefore be necessary to ban the use of cobalt-containing supplements on raceday as well as on the day before racing in order to implement and enforce the proposed thresholds. Since the abuse with huge quantities of cobalt salts can be done during training while the use of legitimate cobalt-containing supplements are also allowed, different urinary and plasma cobalt thresholds would be required to control cobalt abuse in non-raceday or out-of-competition samples. This could be achieved by setting the thresholds above the maximum urinary and plasma cobalt concentrations observed or anticipated from the normal use of legitimate cobalt-containing supplements. Urinary threshold of 2000 ng/mL and plasma threshold of 10 ng/mL were thus proposed for the control of cobalt abuse in non-raceday or out-of-competition samples. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: cobalt; inductively coupled plasma-mass spectrometry; urine; plasma; horse; threshold

Introduction

Cobalt is a well-established chemical inducer of hypoxia-like responses and had been used to treat anaemia in pregnant women, infants, and patients with chronic anaemia.[1] Hypoxia causes gene modulation at the hypoxia inducible factor (HIF) pathway, leading to cell and tissue adaptation to the low oxygen conditions. The main mediator hypoxia inducible factor 1α (HIF1α) activates genetic sequences, including those of the erythropoietin (EPO) gene, which promotes efficient adaptation to hypoxia.[2] Apart from the haematopoietic effects, cobalt also induces the pleiotropic and non-haematopoietic effects of erythropoietin, including modification of several parameters of lipid and glucose metabolism.[3] The seminal studies of the effects of inorganic cobalt administration in healthy men revealed that a daily intake of 150 mg of cobalt chloride would produce an increase in red blood cell (RBC) counts by about 1 million cells per microlitre of blood within 7 to 22 days. The high RBC counts would return to normal 9 to 15 days after cobalt administration.[4] Nevertheless, cobalt salt is no longer used for anti-anaemia treatment due to its adverse effects.[3,5] The role of cobalt in erythropoiesis is disparate. Cobalamin deficiency can result in anemia. However, supplementing with cobalamin does not benefit performance unless there is a nutritional deficit.[6] Inorganic cobalt ion (Co^{2+}) stimulates erythropoiesis through the stabilization of HIF as discussed, with increased expression of the EPO gene even in non-anemic subjects. Indeed, the activity of an International EPO Unit (IU) was originally referenced against the biological effect of 5 μM of cobalt chloride.[7]

Cobalt is an essential micronutrient in the form of vitamin B12 (cobalamin), but inorganic cobalt as such is not required in the human diet. Cyanocobalamin is the synthetic form of vitamin B12 and the form commonly available in vitamin B12 supplements. The daily nutritional requirement of an adult amounts to 2 to 3 mg of cobalamin. Inorganic cobalt is also obtained from the diet. The normal daily intake is on average about 7.5 μg.[8] Cobalt is acutely toxic in larger doses; cobalt ions and cobalt metal (nanoparticles) are cytotoxic and induce apoptosis and at higher concentrations necrosis with inflammatory response. There is evidence suggesting that cobalt salt may cause severe gastrointestinal, endocrine, cardiovascular, haematological, reproductive, neurological, and immunological responses.[9] Cobalt metal and salts are also genotoxic, mainly resulting from oxidative DNA damage by reactive oxygen species. Cobalt salt was further shown to inhibit thyroidal iodide uptake,[10] and chronic cobalt chloride ingestion can cause hypothyroidism and goiter.[11] This may be the reason why the administration of cobalt chloride for performance enhancement is suspected to be supplemented with thyroid hormone. More impor-

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tantly, the cobalt-induced activation of HIF, present in almost all animal cells, with transcription of a range of hypoxia responsive HIF-target genes, probably promotes tumor development and growth.[12,13]

Considering that cobalt salts are low cost, readily available, orally active, and effective in boosting endogenous erythropoietin production, they are attractive blood doping agents to enhance aerobic performances. Indeed, gene therapy targeting the HIF pathway has been reported as an attractive alternative to traditional techniques of blood doping since the last decade.[14–16] The stimulated erythropoietin production and increased erythropoiesis increase the oxygen-carrying capacity of blood. Moreover, preconditioning with cobalt salts promotes tissue adaptation to hypoxia, improves hypoxic/ischemic tolerance, protects skeletal muscles from exercise-induced oxidative damage and enhances physical endurance performance.[3,17] It has also been proposed that cobalt preconditioning could possibly avoid high altitude-induced oxidative stress and ameliorate mountain sickness. While there has been no reported study confirming the effect of cobalt on the performance of racehorses, recent intelligence from the USA and investigations of overseas out-of-competition and post-competition samples in the authors' laboratory, as well as a number of reported cases in Australia, have confirmed that cobalt is being abused in equine sports.

Due to the ability of cobalt to act as an erythropoietic agent in equine sports, a method to control cobalt misuse is needed. Inductively coupled plasma mass spectrometry (ICP-MS) is by far the best technique to quantify elements other than C, H, O, F, and the inert gases in biological samples. Besides its high sensitivity and fast turnaround time, another major advantage of using ICP-MS to quantify total cobalt in biological samples is the simple sample preparation required. Blood and urine can often be analyzed directly after dilution with acid.[18,19] This paper describes ICP-MS methods for the quantification of total cobalt in equine plasma and urine. Equine plasma was first protein-precipitated. An aliquot of the deproteinated plasma or a portion of urine was then diluted with nitric acid and submitted directly to ICP-MS analysis. The total cobalt concentration was determined from a multi-point linear regression calibration curve using Germanium (Ge) as the internal standard.

As cobalt is naturally occurring in equine biological samples, a threshold is necessary to control its misuse in horses. With a threshold established, any equine sample is deemed to be positive for a prohibited substance if its cobalt concentration exceeds the respective threshold and if its presence in the sample can be independently confirmed using an unequivocal identification method. A few papers have reported the qualitative identification of the presence of cobalt by the formation of metal-complexes that can be analyzed by gas chromatography–mass spectrometry (GC-MS)[20] or electrospray ionization mass spectrometry (ESI-MS).[21,22] Based on the study reported by Minakata *et al.*,[21] definitive liquid chromatography-mass spectrometry (LC-MS) methods for confirming the presence of cobalt in equine plasma and urine were developed by monitoring its diethyldithiocarbamate complex.

Materials and methods

Materials

Reference standard solutions of cobalt (Co) and germanium (Ge), with certified values traceable to the respective Standard Reference Material (SRM) 3113 and 3120a of the National Institute of Standards and Technology (NIST), were obtained from High Purity Standards (Charleston, SC, USA). Triton-X (Ultra), diethyldithiocarbamate (DDC) and isoamyl alcohol (IAA) were purchased from Sigma-Aldrich (St Louis, MO, USA). Nitric acid (Suprapur grade; 65%), trichloroacetic acid (pro analysi grade), citric acid (pro-analysi grade) and sodium chloride (pro analysi grade) were obtained from Merck (Darmstadt, Germany). High purity deionised water was obtained from a Milli-Q Element A10 water purification system (Milli-Q, Molsheim, France). Blank plasma and urine samples were taken from post-race samples collected from horses after their races in Hong Kong.

Working standard solutions

The working standard solutions of Co and Ge were prepared from the respective reference standard solutions by dilution with 3.25 % (*v/v*) nitric acid. Only plastic containers and labware were used for all ICP-MS analyses.

Sample preparation for ICP-MS analyses

Blood

Blood samples (collected in lithium heparin tubes) were centrifuged at 3000 rpm (~1650 g) for 10 min and the plasma fraction was isolated. Germanium standard solution (40 ng) was added as an internal standard to plasma (80 μL). The concentration of the internal standard in the plasma sample was equivalent to 500 ng/mL. The mixture was deproteinated by the addition of trichloroacetic acid (300 μL; 10 g trichloroacetic acid and 120 mg NaCl in 100 mL deionized water) and nitric acid (3.25 %) to give a total volume of 4 mL. The mixture was vortexed briefly and left standing at room temperature for 10 min and then centrifuged at 2000 rpm (~750 g) for 10 min. The supernatant (3.6 mL) was transferred to an ICP-MS autosampler tube (a 4-mL polypropylene tube) and then infused *via* an autosampler to the ICP-MS.

Urine

Urine samples were centrifuged at 3000 rpm (~1650 g) for 10 min. Germanium standard solution (40 ng) was added as an internal standard to urine (80 μL). The sample was then diluted with nitric acid (3.25 %) to give a total volume of 4.0 mL. The diluted sample was then infused *via* an autosampler to the ICP-MS.

Protein precipitation for plasma sample for confirmation by LC-MS

Blood samples were centrifuged at 3000 rpm (~1650 g) for 10 min and the plasma fraction was isolated. Trichloroacetic acid (50 μL, 10 %, *w/v*) was added to an aliquot of plasma (300 μL). The mixture was then vortexed for 1 min. After standing for 10 min, the mixture was centrifuged at 14 000 rpm (~13 000 g) for 1 min. Two hundred microlitres of the supernatant was then subjected to complex formation.

Sample preparation for confirmation by LC-MS

Diethyldithiocarbamate (DDC; $(C_2H_5)_2NCSS^-$, 20 μL, 1 M) was added into either deproteinated plasma (200 μL) or urine (200 μL). Internal standard was not used. The mixture was then vortexed for 1 min and shaken at 1400 rpm at 25 °C (in a thermo-mixer) for 10 min. Citric acid (20 μL, 0.2 M) and isoamyl alcohol (IAA, 500 μL) were added to the mixture. After further mixing in the thermo-mixer at 1400 rpm at 25 °C for 10 min, the mixture was centrifuged at 14

000 rpm (~13 000 g) for 1 min. The supernatant was taken out and mixed with 100 μL of methanol to facilitate evaporation. The mixture was blown down to dryness at 60 °C with nitrogen. The dry residue was then reconstituted with methanol (40 μL) and subjected to LC-MS analysis.

Instrumentation

ICP-MS analyses were performed on an Agilent 7500ce inductively coupled plasma mass spectrometer equipped with a G3160A integrated autosampler and a MicroMist nebulizer (Agilent Technologies, Santa Clara, CA, USA). LC-MS analyses were performed on a Thermo Finnigan TSQ Quantum Classic mass spectrometer (Thermo Finnigan, San José, CA, USA) equipped with a Waters Acquity UPLC system (Waters Corporation, Milford, MA, USA).

ICP-MS conditions

An RF power of 1400 W was employed. The argon carrier gas flow rate was set at 1.05 L/min. The spray chamber temperature was set at 2 °C. Helium (4.0 mL/min) was used as the collision gas. The peristaltic pump speed was set at 0.2 revolutions per sec (rps) during analysis. The sample uptake rate was about 0.8 mL/min, and the sample uptake time was set at 30 s. The isotopes to be monitored for Co and Ge were *m/z* 59 and *m/z* 72, respectively. All data acquisitions were performed in Spectrum Analysis mode with triplicate measurements. Peak Area Integration mode was used, and the integration time per mass was 2 s for Co, and 1 s for Ge. The total acquisition time per sample was about 3 min. After each injection, the autosampler probe was rinsed with deionized water for 5 s in the rinse port and 5 s in the rinse vial, followed by intelligent rinse with 0.07 % Triton-X for a maximum of 100 s to minimize carry over. The autosampler probe was finally rinsed with deionized water for 20 s before the next infusion.

LC-MS conditions for confirmation of cobalt

A reversed-phase UPLC column (Waters; Acquity BEH C18; 10 cm L x 2.1 mm ID; 1.7 μm particle size) was used for the analysis of cobalt diethyldithiocarbamate (Co-DDC) complex. The mobile phase was composed of 5 mM ammonium formate (pH 3) in deionized water as solvent A and 0.1% formic acid in acetonitrile as solvent B. A linear gradient was run at a constant flow rate of 350 μL/min, with 100 % solvent A at the initial condition (t = 0 min), decreasing to 0 % solvent A from t = 1 min to t = 6 min, and held for 0.1 min (until t = 6.1 min). The gradient was then returned to 100 % solvent A from t = 6.1 min and equilibrated until t = 10 min before the next injection. The injection volume was 5 μL.

Detection of the Co-DDC complex was performed in positive electrospray ionisation mode in a single time segment using selected reaction monitoring (SRM). Spray voltage of 3800 V and capillary temperature of 320 °C were employed. The nitrogen sheath and auxiliary gas flow rates were set at 60 and 10 arbitrary TSQ Quantum units respectively. The selected precursor ion of the Co-DDC complex was *m/z* 355, while the product ions monitored were *m/z* 116, 174, 208, and 291. The collision offset voltages were set from 25 V (for *m/z* 116, 174) to 35 V (for *m/z* 208, 291). Argon was used as the collision gas and set at 1.2 mTorr. The peak widths for the precursor and product ions in respectively Q1 and Q3 were set at 0.7 amu (FWHM). The scan width for the product ions was set at 0.01 amu and the scan time for each transition was 50 msec. Data processing was performed using the Thermo Finnigan Xcalibur software (Version 2.0.6).

Calibrators and quality control samples for ICP-MS analyses

Calibration curves were established by analyzing a set of cobalt calibrators at concentrations of 0, 2, 4, 6, 8, and 10 ng/mL and 0, 30, 60, 90, 120, and 150 ng/mL in deionized water for equine plasma and urine, respectively. Quality control (QC) samples at 1 and 4 ng/mL (for plasma) and 60 ng/mL (for urine) were prepared in duplicate by spiking cobalt standard to blank plasma and blank urine, respectively. The calibrators, QC samples, and their corresponding blank matrices were analyzed alongside each batch of test samples using identical procedures. As a QC measure, the calibrators and QC samples were made up from Co working standard solutions that had been prepared separately. The peak area count ratios of cobalt to the internal standard (Ge) versus the spiked Co concentrations were fitted using linear regression to obtain the calibration curve. Concentrations of total cobalt in the test samples were interpolated from the calibration curve using standard ChemStation quantification software. For the QC samples, the actual recovered concentration of cobalt was derived by subtracting the concentration of the corresponding blank matrix from the total concentration determined.

Statistical analysis

Statistical analysis was performed with Minitab computer software version 13.32 (2000) (Minitab Inc., State College, PA, USA). The Kolmogorov-Smirnov normality test was used to compare the observed frequencies with the calculated distribution. Outliers in a data set were identified using the standard function from Microsoft Excel. Any number in a data set with the absolute value of Z-score exceeding 3.5 is considered an outlier.

Drug administration experiments (at manufacturers' recommended daily dosages)

Hemo-15

Hemo-15® (10 mL each, Virbac, Milperra, NSW, Australia) was administered daily by intravenous injection to 3 thoroughbred geldings for 3 consecutive days.

VAM® Injection

VAM® Injection (11 mL each, Nature Vet, NSW, Australia) was administered twice on alternate days by intramuscular injection to 2 thoroughbred geldings.

Farrier's Formula®

Farrier's Formula® (1.5 cups, ~255 g, Life Data Labs, Inc., Cherokee, Alabama, USA) was administered daily by stomach tubing to 1 thoroughbred gelding for 3 consecutive days.

Twydil® Hemopar

Twydil® Hemopar (60 mL each, PAVESCO AG, Basel, Switzerland) was administered daily by mixing with the daily feed to 2 thoroughbred geldings for 3 consecutive days.

Twydil® Hematinic

Twydil® Hematinic (40 mL each, PAVESCO AG, Basel, Switzerland) was orally administered twice daily to 2 thoroughbred geldings for 3.5 consecutive days (totally 7 times per horse).

Blood and urine samples were collected before and after administration. Blood samples were collected in lithium heparin tubes and centrifuged upon receipt and the corresponding plasma samples were kept at below −60 °C until analysis. Approval of the drug administration experiments in this study has been obtained from the Animal Ethics Committee of the Hong Kong Jockey Club (reference HKJC-ERC004).

Method validation

Quantification method by ICP-MS

The inter-day accuracy and precision were assessed by analyzing the QC samples with cobalt spiked at various concentrations (1 and 4 ng/mL in plasma and 60 ng/mL in urine and the corresponding blank samples). The limit of detection (LoD) and limit of quantification (LoQ) of cobalt in equine urine and plasma were estimated by replicate analyses of blank sample matrices (n = 6 each). The impact on method recovery of the different common forms of cobalt (inorganic cobalt, cyanocobalamin and cobalt gluconate) found in some cobalt-containing supplements was evaluated by analyzing different untreated plasma samples spiked with different forms of cobalt (equivalent to 1 ng/mL cobalt) and their corresponding sample blanks. Recoveries were corrected by subtracting the cobalt concentration in the corresponding blank matrix from the total cobalt concentration determined for the spiked sample. Pairs of plasma and serum samples isolated from blood collected (with and without anticoagulant respectively) from the same horses (n = 6) were analyzed in quadruplicate for total cobalt to assess the method applicability to serum samples.

Confirmation method by LC-MS

The inter-day precisions of area count and retention time were assessed by analyzing cobalt spiked urine sample at 100 ng/mL and spiked plasma sample at 2 ng/mL. The detection sensitivity was evaluated by analyzing post-administration samples with their concentrations pre-determined by the ICP-MS method. Matrix suppression was studied by comparing the area counts obtained from matrix-spiked samples (corrected for contribution from their corresponding blank matrices) with those from the water spiked samples at the same cobalt concentrations. Method applicability to other forms of cobalt was studied by analyzing spiked plasma with cyanocobalamin and cobalt gluconate at cobalt equivalent concentrations of 1 and 4 ng/mL.

Results and discussion

Validation of ICP-MS quantification method for total cobalt in equine urine and plasma

It is well known that polyatomic isobars ($^{36}Ar^{23}Na$, $^{43}Ca^{16}O$ and $^{40}Ar^{18}OH$) could interfere with ICP-MS analysis of cobalt. The contribution of various polyatomic interferences was reported to be equivalent to about 0.4 ng/mL of cobalt in human serum.[23] Collision/reaction cell (CRC) technology has been shown to be a very effective tool to remove isobaric interferences from polyatomic species.[24] This technique was employed in the present study using helium as the collision gas. The inter-day accuracy and the precision of the method were determined to be within ±5 % and ≤9 % RSD, respectively, at all QC levels in both matrices (Table 1). The LoD and LoQ were found to be 0.16 ng/mL (equivalent to 3 × SD) and 0.52 ng/mL (equivalent to 10 × SD) in equine urine, and 0.06 ng/mL and 0.21 ng/mL in equine plasma. Calibration curves were linear within the concentration range, with correlation coefficients (r) greater than 0.99 in all cases.

Method recovery based on untreated plasma samples spiked with inorganic cobalt (equivalent to 1 ng/mL cobalt) was found to be 101 %, while slightly lower method recoveries of about 90 % were observed for untreated plasma spiked with other forms of cobalt (cyanocobalamin and cobalt gluconate) at equivalent cobalt concentration. As the magnitude of the negative recovery (about −10 %) for the non-salt forms is not significantly different from the method precision (about 9 % RSD at 1 ng/mL), the impact was not considered significant for the purpose of population surveys, particularly when a high confidence limit would be used to establish the thresholds.

There was no significant difference between the total cobalt concentrations determined in plasma and serum collected from the same horse (n = 6), as shown in Table 2. Therefore, population data of total cobalt in either equine plasma or equine serum can be considered to be equivalent.

Total cobalt population survey in post-race equine urine

In Hong Kong, the screening of total cobalt in equine urine samples has started since 2006. Equine urine was analyzed by ICP-MS after a simple dilution with nitric acid. Germanium (Ge) was used as the internal standard because there was essentially no interference with cobalt determination. In addition, Ge is not normally present in horse urine and blood, and has a mass (72 Da) close to that of Co (59 Da), minimizing possible mass-dependent difference in detector sensitivity. The total cobalt concentration in a urine sample was interpolated directly from a multi-level linear regression calibration curve constructed using calibrators prepared from water spiked with various

Table 1. Precision and accuracy of spiked quality control (QC) samples

QC sample (ng/mL)	Number of QC samples analyzed	Precision (%RSD)	Accuracy (%)
Equine urine			
60	550	4.7	95
Equine plasma			
1	21	9.0	101
4	19	3.2	98

Table 2. Total cobalt concentrations in plasma and serum samples collected from the same horse (n = 6)

Horse	Total cobalt in plasma (ng/mL)	Total cobalt in serum (ng/mL)
G118	0.24	0.26
G312	0.30	0.32
L098	0.34	0.35
M113	0.35	0.34
M266	0.28	0.26
N093	0.30	0.28

concentrations of cobalt. A cobalt threshold in equine urine could be established based on a population survey of 7462 post-race urine samples collected from horses after their races in Hong Kong. The population mean ± standard deviation (SD) was 5.5 ± 5.0 ng/mL. This set of data showed a skewed distribution and could not be used directly to establish a threshold. A normal distribution could be obtained for the whole set of data after a logarithm transformation (Figure 1a). The transformed data were then subjected to the Kolmogorov-Smirnov normality test, resulting in an acceptable significance level of 0.05. A possible threshold could then be set at a level equal to the untransformed 'mean + 3.72 SD' value of 74.5 ng/mL, representing a risk of 1 in 10 000 (assuming the degree of freedom to be infinity) for a normal sample to exceed this threshold. Based on this approach which is often used to establish internationally-accepted thresholds in the horseracing industry,[18,19,25–27] a 'rounded-up' threshold of 75 ng/mL of total cobalt in raceday equine urine samples was proposed. The risk associated with this threshold was about 1 in 10 315 (with a degree of freedom of 7461). This proposed threshold could be used to control total cobalt concentration in a raceday urine sample.

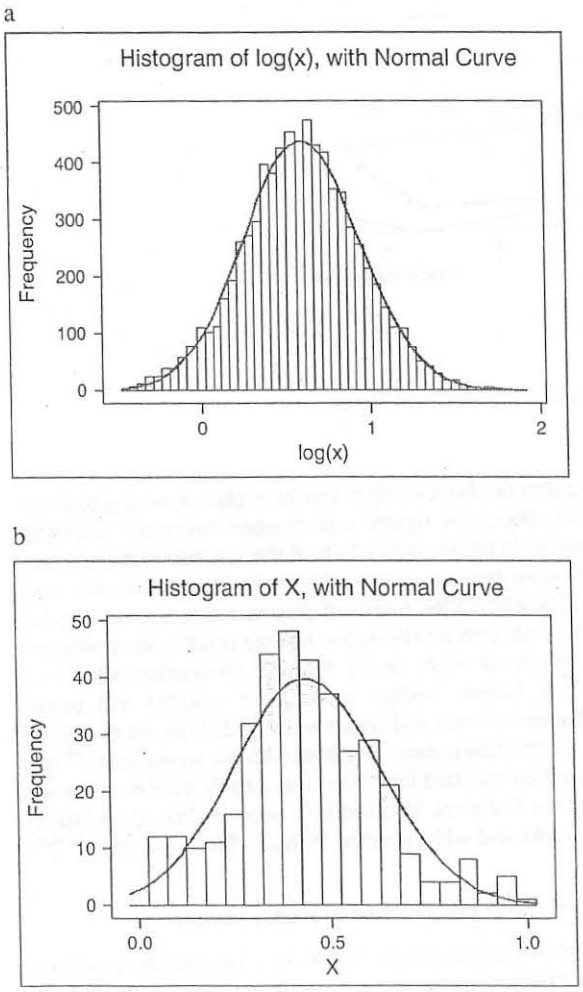

Figure 1. (a) Total cobalt concentration in equine urine samples after logarithm transformation (b) Total cobalt concentration in equine plasma samples (without transformation).

Total cobalt population survey in post-race equine plasma

Owing to the increasing popularity of using blood samples for doping control testing, the authors have also started monitoring total cobalt in blood samples since April 2013. The sample preparation procedures for blood samples were similar to those for urine samples, except that an additional protein precipitation step was included. Total non-protein-bound cobalt in plasma was measured. A proposed cobalt threshold in equine plasma could be established based on a population of 375 post-race blood samples using the same approach described above for equine urine. The population mean ± SD was 0.44 ± 0.19 ng/mL. This whole set of data fits a normal distribution as shown Figure 1b, resulting in an acceptable significance level of 0.068 in the Kolmogorov-Smirnov normality test. A possible threshold could then be set at the 'mean + 3.72 SD' value of 1.14 ng/mL, representing a risk of 1 in 10 000 (assuming the degree of freedom to be infinity) for a normal sample to exceed this threshold. As a relative small population was used to derive the threshold, a 'rounded-up' threshold of 2 ng/mL of total cobalt in raceday equine plasma samples was proposed. The risk associated with this threshold was about one in 1000 trillion (with a degree of freedom of 374).

The proposed total cobalt threshold in equine plasma was verified using an independent population of 109 raceday blood samples from the Emirates Racing Authority (ERA) analyzed in Hong Kong using the same quantification method. Blood samples from ERA are a good choice for cobalt population survey because, like in Hong Kong, injections are not allowed to be given on raceday, thus minimizing the risk of samples being affected by injection with cobalt-containing supplements. The mean ± SD for the plasma total cobalt in the ERA population was determined to be 0.70 ± 0.44 ng/mL. Among these 109 samples, 6 (with plasma total Co levels at 1.5–2.8 ng/mL) were considered outliers. These 6 samples were reportedly collected from horses belonging to two trainers and the elevated cobalt levels in these 6 samples were probably remnants of earlier treatments with cobalt-containing supplements. This set of data gave, after removal of the outliers, a mean ± SD value of 0.61 ± 0.19 ng/mL and a normal distribution after a square-root transformation with an acceptable significance level of 0.05. The untransformed 'mean + 3.72 SD' value was 1.47 ng/mL, which was below the proposed threshold of 2 ng/mL, suggesting that the proposed plasma total cobalt threshold may also be applied to raceday blood samples from other countries.

Administration trials with cobalt-containing supplements

In order to evaluate the impact of legitimate cobalt-containing supplements on the proposed thresholds, administration trials were conducted with 2 cobalt-containing injectables (Hemo-15 and VAM® Injection) and 3 cobalt-containing oral supplements (Farrier's Formula®, Hemopar, and Hematinic). The dose regimens used in the trials were based on those recommended by the manufacturers. Details of the listed cobalt ingredients and the cobalt equivalent in a daily dose for each supplement are summarized in Table 3, and the elimination profiles of total cobalt in urine and plasma are shown in Figures 2 and 3, respectively.

Elevated total cobalt levels in urine and plasma above the respective proposed thresholds were observed for the two injectables (Hemo-15 and VAM® Injection) and observed only in urine for the oral supplement with the highest daily dose of cobalt (Hematinic). Peak urinary and plasma total cobalt levels for these three products were all observed within 2 h of the last administration. Despite a

Table 3. Listed cobalt ingredients and comparison of Co equivalent per daily dose

Cobalt-containing supplement	Cobalt ingredient as listed	Recommended daily dose	Cobalt equivalent per daily dose (mg)
Injections			
Hemo-15	Cyanocobalamin 150 μg/mL Cobalt gluconate 0.7 mg/mL	10 mL	0.99
VAM® Injection	Cyanocobalamin 150 μg/mL Cobalt sulphate 240 μg/mL	11 mL	1.08
Oral supplements			
Farrier's Formula	Cobalt carbonate 1.9 mg/cup (cup = 170 gram of product)	1.5 cup (=255 gram)	1.41
Hemopar	Cyanocobalamin 800 μg/L Cobalt sulphate monohydrate 9 mg/L	60 mL	0.19
Hematinic	Cyanocobalamin 180 mg/L Cobalt carbonate 110 mg/L	80 mL (40 mL twice)	4.99 (2.5 twice)

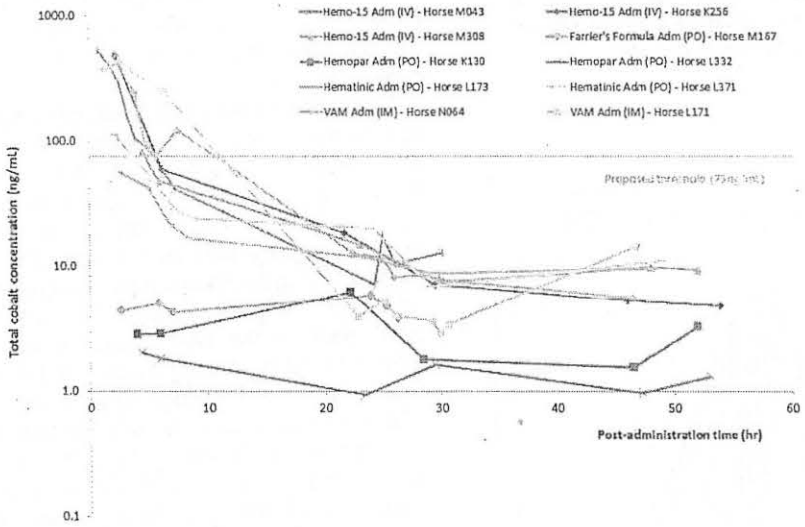

Figure 2. Urinary total cobalt following administration of various forms of cobalt-containing supplements to horses.

much higher last dose of the oral supplement Hematinic than that of the two injectables, its peak total cobalt concentrations in urine and plasma was lower than those for the injectables, indicating that absorption of cobalt by the oral route is far less efficient than by way of injection. For the other two lower-dose oral supplements (Farrier's Formula® and Hemopar), no significant change in urinary and plasma total cobalt levels was observed in post-administration samples, with levels below the respective proposed thresholds at all times. Based on the proposed thresholds in urine (75 ng/mL) and plasma (2 ng/mL), VAM® Injection showed the longest detection time in urine of about 12 h, while both VAM® Injection and Hemo-15 had the longest detection time in plasma of about 6 h (Table 4). The results from these administration trials would suggest that legitimate cobalt-containing injectables should be banned not just on raceday but preferably on the day before racing in order to ensure that the proposed thresholds are not inadvertently breached in raceday samples. The use of oral supplements containing relatively high cobalt content should also be restricted to non-racedays.

The initial elimination half-life for plasma total cobalt was observed to be about 2–6.4 h, and the terminal elimination half-life was found to be about 42–68 h. Similar to plasma, urinary total cobalt levels decreased rapidly and dropped below the proposed threshold of 75 ng/mL within 12 h of the last administration. Our findings were broadly in line with those observed in rats and human. The elimination profile of plasma cobalt appeared to be triphasic in rats with an absorption half-life of 0.9 h, an elimination phase half-life of 3.9 h, and a terminal elimination half-life of 22.9 h.[28] In human studies, it has been reported that cobalt concentration in blood and serum was initially high but decreased rapidly due to tissue uptake combined with urinary excretion.[8] The renal excretion reported for human was initially rapid but decreasing over the first days, followed by a second slow phase lasting several weeks, and with retention in tissues for several years.[29,30]

Control of cobalt misuse in non-raceday samples

The control of cobalt misuse in non-raceday samples would require a different approach since numerous legitimate cobalt-containing supplements are allowed to be used during training. Indeed, cobalt levels in excess of 30 ng/mL (with one exceeding 1000 ng/mL!) have been observed by the authors' laboratory in

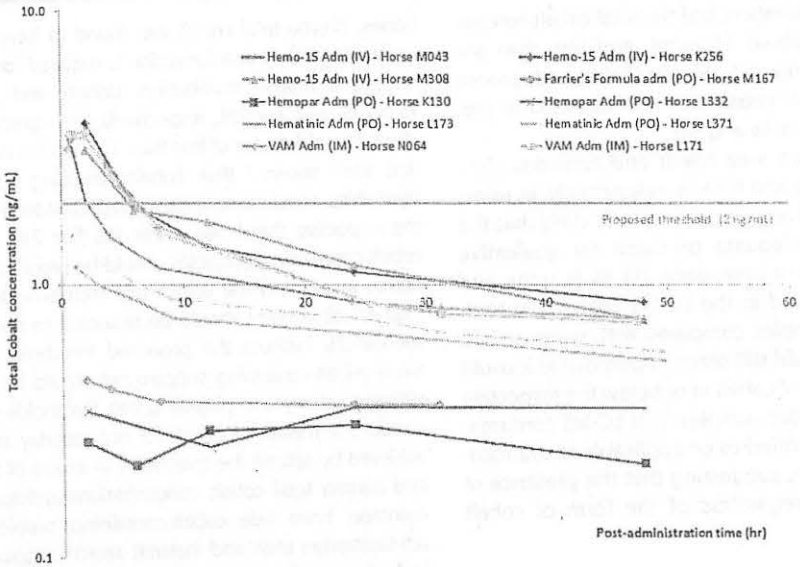

Figure 3. Plasma total cobalt following administration of various forms of cobalt-containing supplements to horses.

several non-raceday blood samples from overseas. A pragmatic approach would be to set a threshold for non-raceday samples in excess of the maximum urinary or plasma total cobalt concentrations expected to be attainable by the use of common *bona fide* cobalt-containing supplements. Based on our administration trials with various products, it would appear that administering a supplement with the largest recommended dose intravenously would provide the highest possible maximum concentration (Cmax) in both urine and plasma to be considered as thresholds for non-raceday samples. A search on the Internet revealed that Hemo-15 was the *bona fide* cobalt-containing equine supplement with the highest recommended IV dose. Based on our trials with Hemo-15, the highest Cmax (by extrapolation to time = 0) would be from Horse K256 at respectively 6 ng/mL in plasma and 1600 ng/mL in urine. Total cobalt thresholds for non-raceday samples could thus be proposed at 10 ng/mL in plasma and 2000 ng/mL in urine. The suitability of these thresholds might warrant further verification by conducting administration trials with other legitimate cobalt-containing supplements not included in the present study.

Confirmation of cobalt in equine urine and plasma by LC-MS

When a regulatory sample is shown to have a total cobalt concentration exceeding the relevant threshold, the presence of cobalt in the sample should ideally be established unequivocally and independently using a confirmation method. The confirmation method adopted was based on the mass-spectrometric method reported by Minakata *et al.*,[21] with additional liquid-chromatographic separation to enhance the degree of proof. Cobalt in either urine or deproteinated plasma was complexed with diethyldithiocarbamate (DDC) and extracted with isoamyl alcohol (IAA) in the presence of critic acid. The resulting Co-DDC complex was analyzed by LC-MS in ESI mode. The precursor ion at *m/z* 355 corresponds to the Co-DCC complex $[Co(DDC)_2^+]$, while the characteristic product-ions monitored were *m/z* 291 $[Co(C_4H_{10}NCS)_2^+]$, *m/z* 280 $[Co(DDCH)^+]$, *m/z* 174 $[CoC_4H_9NCS^+]$, and *m/z* 116 $[C_4H_{10}NCS^+]$. Cobalt could be easily confirmed in a blood sample collected 8.1 h after the last Hemo-15 administration (Figure 4). Both the retention time and mass spectrum of Co-DDC complex obtained from the post-administration sample matched well with those from the cobalt standard. These LC-MS data met the criteria stipulated in the *AORC Guidelines for the Minimum Criteria for Identification by Chromatography and Mass Spectrometry*.[31] The total cobalt concentration in this sample had been determined to be about 1.2 ng/mL by the ICP-MS quantification method, suggesting that this LC-MS method has adequate sensitivity to confirm the presence of cobalt in horse plasma exceeding the threshold of 2 ng/mL. Similarly, cobalt was confirmed in a post-administration urine sample collected 6.4 h

Table 4. Comparison of peak total cobalt concentrations observed and maximum detection times based on the respective proposed thresholds in urine and plasma

Administration with cobalt-containing supplement	Urine		Plasma	
	Peak total cobalt level observed (ng/mL)	Maximum detection times (hrs)	Peak total cobalt level observed (ng/mL)	Maximum detection times (hrs)
Injectables				
Hemo-15	81–530	6.1	3.1–3.9	5.9
VAM® Injection	374–424	11.6	3.5–3.6	6.3
Oral supplements				
Farrier's Formula	Elevated total cobalt level not observed			
Hemopar	Elevated total cobalt level not observed			
Hematinic	56–113	3.8	1.2–1.5	—

after the last Hemo-15 administration, and the total cobalt concentration in the sample was about 55 ng/mL and less than the proposed threshold in urine (Figure 5). Since cobalt is endogenous in the horse, small amount of cobalt was also detected in pre-administration samples (Figures 4a and 5a).

The inter-day precisions on area count and retention time were determined to be 21 % and 0.13 %, respectively, in urine, and 25 % and 0.1 %, respectively, in plasma, indicating that the confirmation method has adequate precision for qualitative identification. Significant ion-suppressions (31 % in urine and 75 % in plasma) were observed in the LC-MS analyses of both urine and plasma spiked samples compared with water spikes. Nevertheless, the method could still serve its purpose as it could reliably confirm the presence of cobalt at or below the respective proposed thresholds for raceday samples. This LC-MS confirmation method has also been verified to be applicable to cyanocobalamin and cobalt gluconate, suggesting that the presence of cobalt could be confirmed regardless of the form of cobalt present in the samples.

Conclusion

ICP-MS quantification methods for total cobalt in equine urine and plasma samples were developed and validated. Urine and deproteinated plasma samples were analyzed directly by ICP-MS after simple dilution with nitric acid. Endogenous total cobalt levels in post-race urine (n = 7462) and plasma (n = 375) samples were determined with the aim to establish thresholds to control the misuse of cobalt in horses. Plasma total cobalt was found to have a normal distribution, while a logarithm transformation is required for urinary total cobalt to achieve a normal distribution. Urinary and plasma thresholds of 75 ng/mL and 2 ng/mL, respectively, were proposed for raceday samples with a risk factor of less than 1 in 10 000. Results from administration trials showed that cobalt-containing supplements, especially injectables, could cause urinary and plasma total cobalt levels to exceed the respective thresholds within the first 24 h. Therefore, the use of cobalt-containing injectables should be prohibited starting on the day before racing and the use of oral supplements containing relatively high cobalt content should be restricted to non-racedays in order to successfully institute the proposed thresholds for raceday samples. Since cobalt-containing supplements could be used during training, different urinary and plasma cobalt thresholds would be required to control the misuse of cobalt for non-raceday samples. This could be achieved by setting the thresholds in excess of the maximum urinary and plasma total cobalt concentrations anticipated from the use of common bona fide cobalt-containing supplements. Results from administration trials and Internet search suggested that Hemo-15, a high-dose supplement administered intravenously, was a good model for establishing cobalt thresholds in non-raceday samples. Based on results from the Hemo-15 administration trials, thresholds of 2000 ng/mL in urine and 10 ng/mL in plasma for total cobalt in non-raceday samples were proposed. The presence of cobalt in the test samples could be confirmed unequivocally and independently by forming a cobalt-diethyldithiocarbamate complex followed by LC-MS analysis. While the diet seems to be a major factor that can influence the observed levels of cobalt in horses, there is still not much known regarding other factors, such as

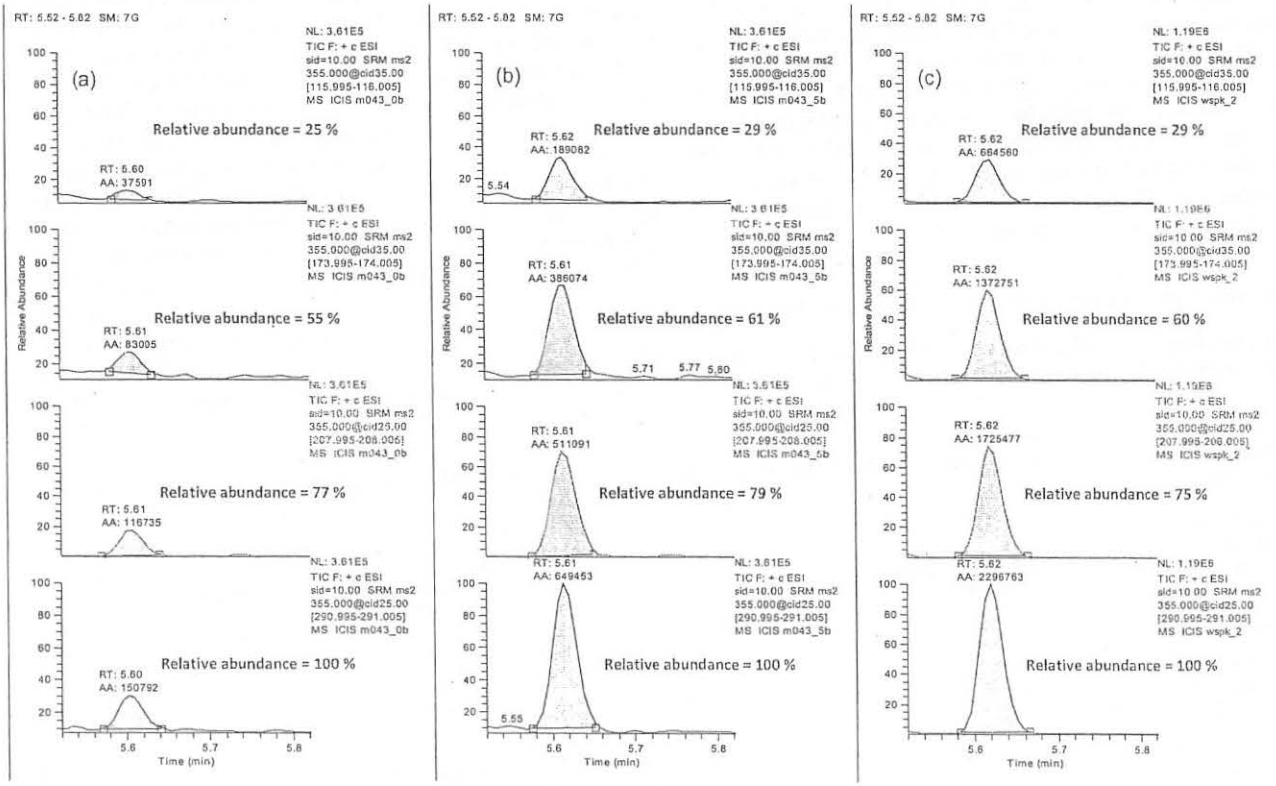

Figure 4. LC-MS product-ion chromatograms of Co-DDC obtained from (a) a pre-administration plasma sample, (b) a post-administration blood sample collected 8.1 h after IV administration of 10 mL of Hemo-15 daily for three days to a horse, and (c) a standard solution of cobalt in water.

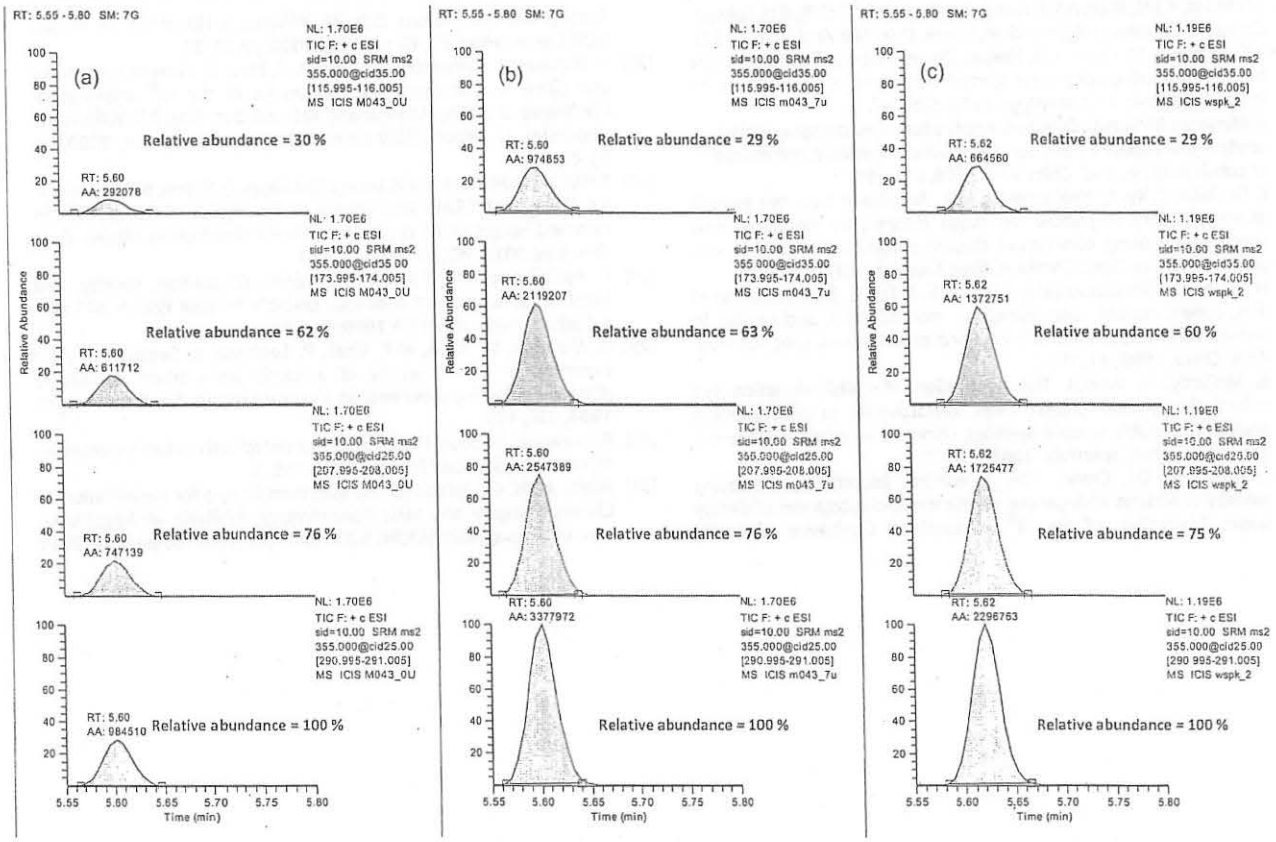

Figure 5. LC-MS product-ion chromatograms of Co-DDC obtained from (a) a pre-administration urine sample, (b) a post-administration urine sample collected 6.4 h after IV administration of 10 mL of Hemo-15 daily for three days to a horse, and (c) a standard solution of cobalt in water.

clinical or pathological conditions, that can influence the pharmacokinetics, and hence the observed levels, of cobalt in horses. In order to further improve the control of the misuse of cobalt in equine sports, a database of basal values of total cobalt in samples from a significant number of untreated horses in different regions should be established. In addition, more administration trials should be conducted with legitimate cobalt-containing equine supplements commonly used in different countries. This objective would require further international collaboration.

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References

[1] R.G. Holly. Studies on iron and cobalt metabolism. *JAMA* **1955**, *158*, 1349.
[2] M.-A.C. Dery, M.D. Michaud, D.E. Richard. Hypoxia-inducible factor 1: regulation by hypoxic and non-hypoxic activators. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 535.
[3] L.O. Simonsen, H. Harbak, P. Bennekou. Cobalt metabolism and toxicology-a brief update. *Sci. Total Environ.* **2012**, *432*, 210.
[4] J.E. Davis, J.P. Fields. Experimental production of polycythemia in humans by administration of cobalt chloride. *Exp. Biol. Med.* **1958**, *99*, 493.
[5] K.M. Unice, A.D. Monnot, S.H. Gaffney, B.E. Tvermoes, K.A. Thuett, D. J. Paustenbach, B.L. Finley. Inorganic cobalt supplementation: prediction of cobalt levels in whole blood and urine using a biokinetic model. *Food Chem. Toxicol.* **2012**, *50*, 2456.
[6] W. Jelkmann. The disparate roles of cobalt in erythropoiesis, and doping relevance. *Open J. Hematol.* **2012**, 3.
[7] P.M. Cotes, D.R. Bangham. The International Reference Preparation of Erythropoietin. *B. World Health Organ.* **1966**, *35*, 751.
[8] T. Smith, C.J. Edmonds, C.F. Barnaby. Absorption and retention of cobalt in man by whole-body counting. *Health Phys.* **1972**, *22*, 359.
[9] B.L. Finley, A.D. Monnot, D.J. Paustenbach, S.H. Gaffney. Derivation of a chronic oral reference dose for cobalt. *Regul. Toxicol. Pharm.* **2012**, *64*, 491.
[10] K.R. Paley, E.S. Sobel, R.S. Yalow. Effect of oral and intravenous cobaltous chloride on thyroid function. *J. Clin. Endocrinol. Metab.* **1958**, *18*, 850.
[11] J.P. Kriss, W.H. Carnes, R.T. Gross. Hypothyroidism and thyroid hyperplasia in patients treated with cobalt. *J. Am. Med. Assoc.* **1955**, *157*, 117.
[12] P. Maxwell, K. Salnikow. HIF-1: an oxygen and metal responsive transcription factor. *Cancer Biol. Ther.* **2004**, *3*, 29.
[13] Q. Ke, M. Costa. Hypoxia-inducible factor-1 (HIF-1). *Mol. Pharmacol.* **2006**, *70*, 1469.
[14] G. Lippi, G.C. Guidi. Gene manipulation and improvement of athletic performances: new strategies in blood doping. *Brit. J. Sports Med.* **2004**, *38*, 641.
[15] G. Lippi, M. Franchini, G.C. Guidi. Cobalt chloride administration in athletes: a new perspective in blood doping. *Brit. J. Sports Med.* **2005**, *39*, 872.
[16] G. Lippi, M. Franchini, G.C. Guidi. Blood doping by cobalt. Should we measure cobalt in athletes? *J. Occup. Med. Toxicol.* **2006**, *1*, 18.
[17] S. Saxena, D. Shukla, S. Saxena, Y.A. Khan, M. Singh, A. Bansal, M. Sairam, S.K. Jain. Hypoxia preconditioning by cobalt chloride enhances endurance performance and protects skeletal muscles from exercise-induced oxidative damage in rats. *Acta Physiol.* **2010**, *200*, 249.
[18] E.N.M. Ho, D.K.K. Leung, T.S.M. Wan, A.S.Y. Wong. ICP-MS detection of metallic poisons in equine urine. *Proceedings of the 16th International Conference of Racing Analysts and Veterinarians.* (Eds: E. Houghton, I. Kijima-Suda, R. Wade, J.F. Wade), R&W Communications, Newmarket, **2006**, pp 469–476.

[19] E.N.M. Ho, T.S.M. Wan, A.S.Y. Wong, K.K.H. Lam, P.J. Schiff, B.D. Stewart. Control of the misuse of bromide in horses. *Drug Test. Anal.* **2010**, *2*, 323.
[20] S.K. Aggarwal, M. Kinter, D.A. Herold. Determination of cobalt in urine by gas chromatography-mass spectrometry employing nickel as an internal standard. *J. Chromatogr.* **1992**, *576*, 297.
[21] K. Minakata, M. Suzuki, O. Suzuki. Application of electrospray ionization tandem mass spectrometry for the rapid and sensitive determination of cobalt in urine. *Anal. Chim. Acta* **2008**, *614*, 161.
[22] E. Dodbiba, C. Xu, E. Wanigasekara, D.W. Armstrong. Sensitive analysis of metal cations in positive ion mode electrospray ionization mass spectrometry using commercial chelating agents and cationic ion-pairing reagents. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 1005.
[23] H. Vanhoe, C. Vandecasteele, J. Versieck, R. Dams. Determination of iron, cobalt, copper, zinc, rubidium, molybdenum, and cesium in human serum by inductively coupled plasma mass spectrometry. *Anal. Chem.* **1989**, *61*, 1851.
[24] E. McCurdy, G. Woods. The application of collision/reaction cell inductively coupled plasma mass spectrometry to multi-element analysis in variable sample matrices, using He as a non-reactive cell gas. *J. Anal. Atom. Spectrom.* **2004**, *19*, 607.
[25] E. Houghton, D.L. Crone. The approaches adopted by the racing industry to address endogenous substances and substances of dietary origin. *Proceedings of the 13th International Conference of Racing Analysts and Veterinarians.* (Eds: R.B. Williams, E. Houghton, J.F. Wade), R&W Communications, Newmarket, **2000**, pp 23–27.
[26] Y. Bonnaire, L. Dehennin, M.A. Popot, P. Plou. Testosterone in mares and fillies: A new threshold. *Proceedings of the 13th International Conference of Racing Analysts and Veterinarians.* (Eds: R.B. Williams, E. Houghton, J.F. Wade), R&W Communications, Newmarket, **2000**, pp 60–63.
[27] E.N.M. Ho, W.H. Kwok, D.K.K. Leung, C.M. Riggs, G. Sidlow, B.D. Stewart, A. S.Y. Wong and T.S.M. Wan. Control of the misuse of testosterone in castrated horses based on an international threshold in plasma. *Drug Test. Anal.* **2014**, DOI 10.1002/dta.1681
[28] F. Ayala-Fierro, J.M. Firriolo, D.E. Carter. Disposition, toxicity, and intestinal absorption of cobaltous chloride in male Fischer 344 rats. *J. Toxicol. Environ. Health A* **1999**, *56*, 571.
[29] G. Mosconi, M. Bacis, M.T. Vitali, P. Leghissa, E. Sabbioni. Cobalt excretion in urine: results of a study on workers producing diamond grinding tools and on a control group. *Sci. Total Environ.* **1994**, *150*, 133.
[30] R. Lauwerys, D. Lison. Health risks associated with cobalt exposure — an overview. *Sci. Total Environ.* **1994**, *150*, 1.
[31] AORC. AORC Guidelines for the Minimum Criteria for Identification by Chromatography and Mass Spectrometry. Available at: http://www.aorc-online.org/AORC%20MS%20Criteria.pdf [accessed on 5 Sep 2014]

Review

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Intolerability of cobalt salt as erythropoietic agent

Bastian Ebert and Wolfgang Jelkmann*

Unfair athletes seek ways to stimulate erythropoiesis, because the mass of haemoglobin is a critical factor in aerobic sports. Here, the potential misuse of cobalt deserves special attention. Cobalt ions (Co^{2+}) stabilize the hypoxia-inducible transcription factors (HIFs) that increase the expression of the erythropoietin (Epo) gene. Co^{2+} is orally active, easy to obtain, and inexpensive. However, its intake can bear risks to health. To elaborate this issue, a review of the pertinent literature was retrieved by a search with the keywords 'anaemia', 'cobalt', 'cobalt chloride', 'erythropoiesis', 'erythropoietin', 'Epo', 'side-effects' and 'treatment', amongst others. In earlier years, cobalt chloride was administered at daily doses of 25 to 300 mg for use as an anti-anaemic agent. Co^{2+} therapy proved effective in stimulating erythropoiesis in both non-renal and renal anaemia, yet there were also serious medical adverse effects. The intake of inorganic cobalt can cause severe organ damage, concerning primarily the gastrointestinal tract, the thyroid, the heart and the sensory systems. These insights should keep athletes off taking Co^{2+} to stimulate erythropoiesis. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: anaemia therapy; cobalt; doping; erythropoiesis; erythropoietin

Introduction

Cheating athletes seek ways to stimulate erythropoiesis, because the aerobic capacity correlates with the total mass of haemoglobin (Hb). Erythropoiesis requires the glycoprotein hormone erythropoietin (Epo), which is produced in the kidneys and – to a minor degree – in a few other organs such as the liver. Recombinant human Epo (rhEpo) and its analogues have been misused for doping purposes,[1,2] but their intake may be proven by drug testing[3,4] and they are expensive. An alternative doping threat are the numerous small molecule chemicals that increase the expression of the Epo gene (*EPO*).[5] With respect to doping practices, of particular interest are cobalt (II) ions (Co^{2+}). The mechanism of the action of Co^{2+} is completely separate from that of the organic cobalt-containing vitamin, cobalamin.[6] Co^{2+} activates the hypoxia-inducible transcription factors (HIFs) that increase *EPO* expression.[7] By this way, Co^{2+} stimulates Epo production, as first observed in experimental animals in the late 1950s.[8] Co^{2+} is the reference substance for the *in vivo* calibration of rhEpo drug substance; 5 µmol Co^{2+} elicits the same erythropoiesis-stimulating activity as one International Unit (IU) of rhEpo.[9] Weißbecker[10] first noted that the oral administration of $CoCl_2$ increases reticulocytes, red blood cells (RBCs) and [Hb] in healthy men. In another investigation of healthy humans, Davies and Fields[11] showed that the daily intake of 150 mg $CoCl_2$ increases RBC numbers by about 1 Mio. per µl within 7 to 22 days, with the values returning to normal within 9 to 15 days after cessation of Co^{2+} administration.[11] From the late 1940s to the late 1970s cobalt chloride ($CoCl_2$) was applied to treat anaemic patients.[10,12] The medicine was usually given as tablets, in divided doses at meal times.

Herein, a comprehensive literature search was performed to identify potential risks to health on the intake of Co^{2+}, since suspicion has been raised that cobalt salts could be misused by athletes as an alternative to rhEpo.[6,13] First, the physiology of the uptake and excretion of Co^{2+} is summarized. Second, clinical reports are evaluated of the use of Co^{2+} as an anti-anaemia treatment. The focus is on the adverse events in association with this therapy. The article will improve the knowledge of the health danger associated with the abuse of Co^{2+} as a doping means.

Methods

Pertinent literature was searched using the following databases: MEDLINE (National Library of Medicine (NLM), Bethesda, MD, USA), Springer-Verlag database (Springer-Verlag GmbH & Co KG, Heidelberg, Germany), Thieme Verlag database (Georg Thieme Verlag, Stuttgart, Germany), Wiley online library (Hoboken, NJ, USA), XToxline (NLM, Bethesda, MD, USA)) with the keywords 'doping', 'blood doping', 'cobalt chloride', 'cobalt', 'erythropoiesis', 'Epo', 'erythropoietin', 'anaemia', 'side effects', 'improvement in performance', 'HIF' and 'treatment'. Published literature up to November 2012 was taken into account.

Uptake and excretion of inorganic cobalt

Cobalt is obtained from the diet; the normal daily intake is on average about 7.5 µg. According to studies in healthy humans, the gastrointestinal uptake amounts to 5–20% on oral intake of 1 µg to 1.2 mg $CoCl_2$.[14] The absorption of soluble cobalt is higher for females than for males.[15] Rat studies with radioactive $^{57}Co^{2+}$, added to the drinking water, indicate that Co^{2+} is stored mainly in liver, lung and kidneys.[16] The cobalt concentration in human specimen can be measured by graphite furnace atomic

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absorption spectrometry, it amounts to 0.1 - 0.5 μ/L in blood plasma.[17] Jefferson *et al.*[18] have demonstrated elevated cobalt concentrations in about 50% of high-altitude dwellers with excessive erythrocytosis. The percentage of free Co^{2+} is only 5–12% of the total in the blood plasma, with the remainder being bound to albumin.[19,20] Cobalt is eliminated predominantly in urine with a small amount excreted in bile.[21] The normal cobalt concentration in urine is <2 μ/L in non-occupationally exposed persons.[17] On $CoCl_2$ intake, the urinary cobalt concentration is higher in women (median: 109.7 nmol/mmol creatinine) than in men (38.4 nmol/mmol creatinine).[15] Following a single intravenous (IV) administration of inorganic cobalt in adult males, 40% of the cobalt was excreted during the first 24 h, 70% in one week and 80 % in one month, while 10 % was still present after one year.[14]

Clinical trials

Clinical trials on the use of Co^{2+} as an anti-anaemic agent were performed from the late 1940s to the late 1970s.[10,21] Co^{2+} salt (mostly $CoCl_2$) was usually administered as tablets and at daily doses of 25–300 mg (molar mass of $CoCl_2$ hexahydrate: 238 Da). The medicine was used for the treatment of anaemias of different etiologies, including septic infection,[12] myeloid hypoplasia,[22–25] sickle-cell disease,[26,27] rheumatoid arthritis,[28] and chronic kidney disease (CKD).[28–37] Some of the most important clinical observations are summarized in the following.

In 1949, Robinson *et al.*[12] described the primal treatment of nine septic patients who received daily oral doses of 20–60 mg of $CoCl_2$ for two to six weeks. The therapy increased RBC counts, [Hb] and haematocrit (Hct). Allegedly, there were no adverse effects, apart from an appetite loss in two patients.[12] At the same time, Weißbecker[10] described his three years' experiences, resuming that 100 mg of $CoCl_2$ taken spread over the day was effective in stimulating erythropoiesis. The treatment was recommended primarily for patients with hypochromic or infectious anaemia, but it was also proposed in the treatment of pernicious anaemia and thalassaemia minor. 'Serious' complications were not noted in any of the approximately 100 patients under study.[10] Since the intramuscular (IM) injection of $CoCl_2$ proved to be painful Weißbecker[38] suggested to use inorganic cobalt (5 mg once or twice a day) with an amino acid complex for the IM route, as this combination would ensure a slow dissociation at the site of injection. However, there were no details reported with respect to the tolerance of such formulations.

Seaman and Koler[22] treated a 40-year-old female patient suffering from anaemia due to bone marrow hypoplasia with daily oral doses of 100 mg $CoCl_2$. The number of reticulocytes and RBCs, as well as [Hb] were increased after one month, but later there was relapse of bone marrow hypoplasia.[22] In another hypoplastic patient $CoCl_2$ therapy caused an increase in the fraction of reticulocytes in the bone marrow, though not in RBC numbers or [Hb].[22] Voyce[23] reported on a patient with pure red cell aplasia (PRCA), whose [Hb] rose from <70 g/L to >120 g/L on treatment with 100 mg $CoCl_2$ twice a day (b.i.d.). In another case of PRCA haematological recovery was achieved on two months oral treatment with 50 mg $CoCl_2$ b.i.d.[24] Anaemia improvement was observed in four patients with sickle-cell anaemia treated with 300 mg $CoCl_2$ for six weeks.[26] Weinsaft and Bernstein[28] administered daily 80 mg $CoCl_2$ to eight patients with therapy-resistant anaemias of differing etiologies. RBC numbers, [Hb] and Hct increased on 2–8 months Co^{2+} treatment. However, two patients died: one 66 days after the start of Co^{2+} application due to a haemorrhage, the other after 135 days because of renal and cardiac failure. The authors did not comment on the possible relationship between death and Co^{2+} therapy.[28]

Geill[32] treated a total of 107 anaemic CKD patients with a combination of 55 mg iron and 20 mg $CoCl_2$, b.i.d. or three times a day (t.i.d.) for three months. This therapy resulted in a rise in RBC numbers and [Hb]. An 82-year-old female patient developed erythrocytosis and thrombosis of the superior mesenteric artery during the treatment period.[32] When Gardner[29] treated 17 CKD patients with daily oral doses of 50–150 mg $CoCl_2$, erythropoiesis was stimulated in most patients within one month. In six out of twelve nephrectomized patients on dialysis who received daily 25–50 mg $CoCl_2$, [Hb] increased,[36] indicating that Co^{2+} can stimulate Epo production at extra-renal sites, most likely in the liver. Kasanen *et al.*[31] treated anaemic CKD patients orally with cobalt chloride equivalent to either 5 or 15 mg cobalt per day. [Hb] increased on average at the lower dose from 79 g/L to 92 g/L and at the higher dose from 92 g/L to 107 g/L within a month. The maximum reticulocyte count (2.1–3.8% of RBCs) was measured one week after initiation of therapy.[31] Bowie and Hurley[34] treated 14 anaemic CKD patients on dialysis with 25 mg $CoCl_2$ orally for four weeks, and with 50 mg of $CoCl_2$ for another four weeks. On average, Hct increased by 23% and RBC mass by 20%. Similar effects were seen by Schleisner[30] who applied daily doses of 60 mg $CoCl_2$. Curtis *et al.*[33,35] administered 50 mg of $CoCl_2$ daily for three months to 23 CKD patients on haemodialysis. This treatment resulted in an increase in [Hb] by 10 g/L in approximately 50% of the patients. One patient died three months after completing a course of Co^{2+} therapy. Histological examination of his myocardial tissue revealed that he had developed cardiomyopathy. At post-mortem the myocardial cobalt concentration was 1.65 μg/g, some 25–80 times higher than in control samples. Curtis *et al.*[35] performed a prospective study of the cobalt concentrations in the blood of healthy controls and of haemodialysis patients after two weeks of administration of $CoCl_2$. In both groups, there were long-term increases in blood concentrations of cobalt, which returned to normal six weeks after Co^{2+} discontinuation.[35]

Unwanted effects of Co^{2+} ingestion

Several aspects of the biochemical properties of inorganic cobalt (metallic and stable salts) with respect to bio-accessibility and potential hazards have been summarized previously.[39–41] A monograph describing toxic effects of $CoCl_2$ has been compiled by the staff of the Birmingham Centre of the National Poisons Information Service of the UK.[42] The uptake of cobalt and its compounds in the human body can occur in different ways (orally, dermally, inhalative, intravenously, subcutaneously). Depending on the route of administration the toxicity of cobalt differs with respect to the site and severity of damage. In addition, the exposure time and the quantitative amount of cobalt intake are critical. On high dosing (>25 mg/day) there is danger of intolerability and organ injury.[42,43]

Acute $CoCl_2$ poisoning

Mucklow *et al.*[44] have reported on a 6-year-old boy who developed abdominal pain and vomited after taking a drink containing 2.5 g $CoCl_2$. The cobalt concentration in his blood plasma was 7.23 μmol/L (normal value <0.02 μmol/L) seven hours post

ingestion and 0.09 µmol/L one month later.[44] Jacobziner and Raybin[45] have described a worse case: A 19-month-old boy died about seven hours after he had swallowed a mouthful (~ 30 ml) of $CoCl_2$ solution. The autopsy revealed a necrotic gastric mucosa; liver, kidney and spleen were overloaded with cobalt.[45]

Gastrointestinal sickness

Weißbecker[10] first noted that the oral administration of 500 mg of $CoCl_2$ can cause gastrointestinal sickness. Schirrmacher[46] has described the impressive case of a 35-year-old woman who developed nausea, vomiting and weight loss in addition to neurological symptoms, when she was treated with 25 mg $CoCl_2$ four times a day (q.i.d.). Several authors have confirmed gastrointestinal complaints in association with the oral intake of $CoCl_2$.[26,28–31,33–36,38,46,47]

Thyroidal dysfunction

Co^{2+} inhibits thyroidal iodide uptake.[48] Thus, myxoedema and thyroid hyperplasia were relatively common side effects of Co^{2+} treatment.[49] Kriss *et al.*[27] observed thyroid gland abnormalities during Co^{2+} therapy in five patients. Among them were four children with sickle cell disease, who were treated with 30 to 100 mg of $CoCl_2$ daily for 14–30 weeks. A few weeks after cessation of therapy the goiter and the dysfunction of the thyroid gland resumed. Since the unwanted effects were clearly attributed to the Co^{2+} treatment, the authors criticized the prevalently careless use of Co^{2+}as a therapeutic means.[27]

Myocardial effects

Cardiomyopathies were observed in hard metal workers who inhaled cobalt in concentrations exceeding 100 micrograms Co/m^3 air with different cobalt exposure duration.[50] Heart failure may also result from the pharmacologic administration of Co^{2+}. Reportedly, a 17-year-old woman with CKD died from rapidly progressive dilated cardiomyopathy after nine months $CoCl_2$ therapy (25 mg b.i.d.). At necropsy the myocardial cobalt content was 8.9 µg/g (dried tissue), compared to a normal of 0.2 µg/g.[37]

The cobalt-associated 'beer drinker's cardiomyopathy' was a special syndrome.[51,52] Cobalt chloride/sulfate (1–1.5 mg per litre) was earlier added to beer to act as a foam stabilizer. The syndrome characteristics were cardiomegaly, galloping rhythm, cyanosis, low cardiac ejection, pericardial effusion and hypotension.[53] The disease occurred in people who daily consumed several litres of the Co^{2+}-added beer,[51,52] still the cobalt amounts (up to 10 mg/day) were less than the doses used in anaemia treatment.

Weißbecker[10] earlier noted an increase in systolic blood pressure in all of the patients who had undergone Co^{2+} therapy. This is in harmony with present knowledge that the treatment with rhEpo can be associated with an increase in blood pressure, although the mechanisms of this increase are not fully understood.[54,55]

Nerval and sensory effects

Schirrmacher[46] has described the instructive case of a 35-year-old woman who was treated with 100 mg of $CoCl_2$ every day because of renal anaemia. Due to neurological disease, $CoCl_2$ administration was discontinued after six months. On examination, the patient presented with a bilateral neural hearing loss, a loss of vibration sense in both legs and a lack of posterior tibial reflex, in addition to a diffuse thyroid enlargement.[46] There are other reports indicating hearing impairment on Co^{2+} therapy.[29,30,36,46] When Gardner[29] treated 17 CKD patients with daily oral doses of 50–150 mg $CoCl_2$, four patients complained about the onset of tinnitus after four to 16 weeks. One patient also presented with a reduction hearing after 12 weeks of therapy. The audiogram showed a hearing loss at frequencies >1000 Hz, which was reversible when the Co^{2+} therapy was discontinued.[29] Other investigators confirmed a hearing loss on $CoCl_2$ therapy, and its reversibility on treatment cessation.[34]

Licht *et al.*[25] have described an optic nerve atrophy of a 32-year-old patient who was treated on the basis of a pancytopenia with up to 200 mg of $CoCl_2$ daily in four treatment intervals, each lasting three to four monts, for a total of three years. Apart from nausea and vomiting, the patient developed a reduced choroideal perfusion and atrophy of the optic nerve with a visual acuity.[25]

Discussion

This paper focuses on unwanted effects associated with the therapeutic administration of Co^{2+} for stimulation of erythropoiesis. Evidence suggests that Co^{2+} may cause severe gastrointestinal, endocrine, cardiovascular, haematological, reproductive, neurological, and immunological responses.[43] The treatment with Co^{2+} was abandoned in the late 1970s. Instead, androgens were increasingly used for treatment of anemic patients,[56] until rhEpo became available approximately 10 years thereafter.

Co^{2+} activates the hypoxia-inducible transcription factors (HIFs) that enhance *EPO* expression. The HIFs are heterodimeric proteins composed of α- and ß-subunits. The C-termini of the HIF-α subunits comprise O_2-dependent degradation domains, in which proline residues are hydroxylated by means of specific HIF-α prolyl hydroxylases in the presence of O_2 ('normoxia'). Prolyl hydroxylated HIF-α binds the von Hippel-Lindau tumor suppressor protein (pVHL) in complex with an ubiquitin-protein E_3-ligase and undergoes immediate proteasomal degradation. Co^{2+} is thought to bind to HIF-α thereby preventing the interaction with pVHL and the proteasomal degradation, even under normoxic conditions.[57–59] HIF-α then translocates into the nucleus, where it couples with HIF-1ß and binds to hypoxia-response elements (HREs) in the *EPO* enhancer.

Suspicion has been raised that Co^{2+} salts could be misused by athletes as an alternative to conventional blood doping by RBC transfusion or rhEpo injection.[13] Indeed, $CoCl_2$ is readily available, inexpensive, easy to dose, and very efficient. However, the intake of inorganic cobalt bears serious risks to health. Taken together, therapeutic doses of $CoCl_2$ have ranged from 25 to 300 mg per day, usually taken as tablets or in drinks. There were only a few trials of the IV or IM administration of Co^{2+}.[38] The parenteral route of application prevents gastrointestinal unwanted effects. However, the specific toxicity of the IV administration of Co^{2+} has not been investigated, and the IM administration may be painful. The risk of the occurrence of unwanted events increases with the dose and length of treatment interval. The duration of the therapeutic administration of Co^{2+} averaged approximately 10 weeks. Finley *et al.*[43] have recently determined a chronic oral reference dose (RfD) for inorganic cobalt, employing the standard US EPA risk assessment methodology for establishing a chronic RfD, a potential point of departure dose (POD) of 0.9 mg cobalt per kg body weight (b.w.) and day, and an aggregate uncertainty factor of 30 to the POD. This approach has yielded a chronic oral RfD of

0.03 mg cobalt per kg b.w. and day, a value considered to be protective of non-cancer health effects in the general population for a lifetime of daily exposure to cobalt.[43] Thus, the oral RfD is much lower than the Co^{2+} doses used to increase RBC production in humans.

Co^{2+}, by means of stabilising HIF, can activate several hundred other genes apart from *EPO*.[60] These include genes encoding proteins that are involved in tumor growth (e.g. vascular endothelial growth factor and multidrug resistance transporter P-glycoprotein). Indeed, the administration of cobalt salt was found to promote the development of carcinomas in experimental animals.[61] Furthermore, inorganic cobalt proved to induce DNA strand breaks, DNA-protein cross-linkage, sister chromatid exchanges and formation of micronuclei in mammalian cell cultures.[62]

Some of the HIF-activated genes encode proteins which may increase physical performance independent of erythropoiesis (e.g. glycolytic enzymes, glucose transporters, angiogenic peptides). Research has been initiated to investigate effects of Co^{2+} in this regard. The administration of cobalt salt did not affect the formation of capillaries in skeletal muscle of experimental animals.[63] However, preconditioning with high-dosed $CoCl_2$ (12.5 mg/kg b.w.) protected hypoxic rats against high altitude pulmonary edema.[64] In another rat study preconditioning by $CoCl_2$ supplementation increased mitochondrial biogenesis, glucose uptake and metabolism by aerobic respiration in skeletal muscle, which increased physical performance.[65]

First attempts have been made to develop strategies for detection of cobalt salt doping in athletes. It has been proposed to use the cobalt content of RBCs as a parameter, as the $CoCl_2$ uptake by the RBCs is practically irreversible and reflects the plasma cobalt concentration.[66] Unice *et al.*[67] used a biokinetic model to estimate whole blood and urine cobalt levels resulting from oral exposure or ingestion of cobalt in amounts exceeding typical dietary intake rates. Following 10 days of cobalt supplementation at a daily rate of 0.4 to 1.0 mg, the predicted cobalt concentrations ranged from 1.7 to 10 μg/L in blood, and 20 to 120 μg/L in urine. Chronic supplementation (> 1 year) at a rate of 1.0 mg cobalt per day was predicted to result in blood levels of 5.7 to 13 μg/L, and in urine from 65 to 150 μg/L.

More detailed information on the health hazards of cobalt salt is demandable to discourage athletes from a potentially deleterious doping practice. In addition, knowledge on the pharmacokinetics of cobalt will be advantageous for anti-doping laboratories planning to establish methods for detection of cobalt in biological samples. Co^{2+} stimulates *EPO* expression by stabilising HIF-α. The '2013 Prohibited List' of the World Anti-Doping Agency (WADA) cites (S2), among others, hypoxia-inducible factor (HIF) stabilizers. Whether this would suffice to sanction athletes misusing Co^{2+} to improve performance could become a matter of legal debate.

References

[1] W. Jelkmann. Erythropoiesis stimulating agents and techniques: A challenge for doping analysts. *Curr. Med. Chem.* **2009**, *16*, 1236.
[2] W. Jelkmann, C. Lundby. Blood doping and its detection. *Blood* **2011**, *118*, 2395.
[3] C. Reichel, G. Gmeiner. Erythropoietin and analogs. *Handb. Exp. Pharmacol.* **2010**, *195*, 251.
[4] M. Okano, M. Sato, E. Kaneko, S. Kageyama. Doping control of biosimilar epoetin kappa and other recombinant erythropoietins after intravenous application. *Drug Test. Anal.* **2011**, *3*, 798.
[5] S. Beuck, W. Schanzer, M. Thevis. Hypoxia-inducible factor stabilizers and other small-molecule erythropoiesis-stimulating agents in current and preventive doping analysis. *Drug Test. Anal.* **2012**, *4*, 830.
[6] W. Jelkmann. The disparate roles of cobalt in erythropoiesis, and doping relevance. *Open J. Hematol.* **2012**, *3*, 1.
[7] F. J. Rodriguez-Jimenez, V. Moreno-Manzano. Modulation of hypoxia-inducible factors (HIF) from an integrative pharmacological perspective. *Cell. Mol. Life Sci.* **2012**, *69*, 519.
[8] E. Goldwasser, L. O. Jacobson, W. Fried, L. F. Plzak. Studies on erythropoiesis V. The effect of cobalt on the production of erythropoietin. *Blood* **1958**, *13*, 55.
[9] W. Jelkmann. Efficacy of recombinant erythropoietins: Is there unity of international units? *Nephrol. Dial. Transpl.* **2009**, *24*, 1366.
[10] L. Weißbecker. Die Kobalttherapie. *Dtsch. Med. Wochenschr.* **1950**, *75*, 116.
[11] J. E. Davis, J. P. Fields. Experimental production of polycythemia in humans by administration of cobalt chloride. *Proc. Soc. Exp. Biol. Med.* **1958**, *99*, 493.
[12] J. C. Robinson, G. W. James III, R. M. Kark. The effect of oral therapy with cobaltous chloride on the blood of patients suffering with chronic suppurative infection. *New Engl. J. Med.* **1949**, *240*, 749.
[13] G. Lippi, M. Franchini, G. C. Guidi. Blood doping by cobalt. Should we measure cobalt in athletes? *J. Occup. Med. Toxicol.* **2006**, *1*, 18.
[14] T. Smith, C. J. Edmonds, C. F. Barnaby. Absorption and retention of cobalt in man by whole-body counting. *Health Phys.* **1972**, *22*, 359.
[15] J. M. Christensen, O. M. Poulsen, M. Thomsen. A short-term cross-over study on oral administration of soluble and insoluble cobalt compounds: Sex differences in biological levels. *Int. Arch. Occup. Environ. Health* **1993**, *65*, 233.
[16] J. Edel, G. Pozzi, E. Sabbioni, R. Pietra, S. Devos. Metabolic and toxicological studies on cobalt. *Sci. Total Environ.* **1994**, *150*, 233.
[17] WHO International Agency for Research on Cancer Working Group. Cobalt in hard metals and cobalt sulfate, gallium arsenide, indium phosphide and vanadium pentoxide. *IARC Monogr. Eval. Carcinog. Risks Hum.* **2006**, *86*, 1. Available at: http://monographs.iarc.fr/ENG/Monographs/vol86/mono86.pdf Access date: [16 August 2013].
[18] J. A. Jefferson, E. Escudero, M. E. Hurtado, J. Pando, R. Tapia, E. R. Swenson, J. Prchal, G. F. Schreiner, R. B. Schoene, A. Hurtado, R. J. Johnson. Excessive erythrocytosis, chronic mountain sickness, and serum cobalt levels. *Lancet* **2002**, *359*, 407.
[19] A. K. Nandedkar, M. S. Hong, F. Friedberg. Co2+ binding by plasma albumin. *Biochem. Med.* **1974**, *9*, 177.
[20] H. M. Jansen, S. Knollema, L. V. van der Duin, A. T. Willemsen, A. Wiersma, E. J. Franssen, F. G. Russel, J. Korf, A. M. Paans. Pharmacokinetics and dosimetry of cobalt-55 and cobalt-57. *J. Nucl. Med.* **1996**, *37*, 2082.
[21] A. Taylor, V. Marks. Cobalt: A review. *J. Hum. Nutr.* **1978**, *32*, 165.
[22] A. J. Seaman, R. D. Koler. Acquired erythrocytic hypoplasia: A recovery during cobalt therapy; report of two cases with review of the literature. *Acta Haematol.* **1953**, *9*, 153.
[23] M. A. Voyce. A case of pure red-cell aplasia successfully treated with cobalt. *Brit. J. Haematol.* **1963**, *9*, 412.
[24] J. R. Fountain, M. Dales. Pure redcell aplasia successfully treated with cobalt. *Lancet* **1955**, *268*, 541.
[25] A. Licht, M. Oliver, E. A. Rachmilewitz. Optic atrophy following treatment with cobalt chloride in a patient with pancytopenia and hypercellular marrow. *Isr. J. Med. Sci.* **1972**, *8*, 61.
[26] J. Wolf, I. J. Levy. Treatment of sickle cell anemia with cobalt chloride. *AMA Arch. Intern. Med.* **1954**, *93*, 387.
[27] J. P. Kriss, W. H. Carnes, R. T. Gross. Hypothyroidism and thyroid hyperplasia in patients treated with cobalt. *J. Am. Med. Assoc.* **1955**, *157*, 117.
[28] P. P. Weinsaft, L. H. Bernstein. Cobaltous chloride in the treatment of certain refractory anemias. *Am. J. Med. Sci.* **1955**, *230*, 264.
[29] F. H. Gardner. The use of cobaltous chloride in the anemia associated with chronic renal disease. *J. Lab. Clin. Med.* **1953**, *41*, 56.
[30] P. Schleisner. Cobalt in anaemia. *Acta Med. Scand.* **1956**, *154*, 177.
[31] A. Kasanen, M. Kulonen, J. Forsstrom. Oral cobalt therapy in renal anemia. *Ann. Med. Intern. Fenn.* **1963**, *52*, 43.
[32] T. Geill. On the treatment of the nephrogenic anaemias with a combined cobalt-iron preparation. *Gerontol. Clin.* **1969**, *11*, 48.
[33] M. S. Edwards, J. R. Curtis. Use of cobaltous chloride in anaemia of maintenance hemodialysis patients. *Lancet* **1971**, *2*, 582.
[34] E. A. Bowie, P. J. Hurley. Cobalt chloride in the treatment of refractory anaemia in patients undergoing long-term haemodialysis. *Aust. N.Z. J. Med.* **1975**, *5*, 306.

[35] J. R. Curtis, G. C. Goode, J. Herrington, L. E. Urdaneta. Possible cobalt toxicity in maintenance hemodialysis patients after treatment with cobaltous chloride: A study of blood and tissue cobalt concentrations in normal subjects and patients with terminal and renal failure. *Clin. Nephrol.* **1976**, *5*, 61.
[36] J. M. Duckham, H. A. Lee. The treatment of refractory anaemia of chronic renal failure with cobalt chloride. *Q. J. Med.* **1976**, *45*, 277.
[37] I. H. Manifold, M. M. Platts, A. Kennedy. Cobalt cardiomyopathy in a patient on maintenance haemodialysis. *Brit. Med. J.* **1978**, *2*, 1609.
[38] L. Weissbecker. Neue Möglichkeiten der Kobalttherapie. *Klin. Wochenschr.* **1951**, *29*, 80.
[39] D. G. Barceloux. Cobalt. *J. Toxicol. Clin. Toxicol.* **1999**, *37*, 201.
[40] A. Oller, H. Bates. Metals in perspective: Introduction. *J. Environ. Monitor.* **2004**, *6*, 145.
[41] L. O. Simonsen, H. Harbak, P. Bennekou. Cobalt metabolism and toxicology-A brief update. *Sci. Total Environ.* **2012**, *432*, 210.
[42] S. Bradberry, M. Sabatta, J. Vale. Cobalt Chloride. Available at: www.inchem.org/documents/ukpids/ukpids/ukpid50.htm Access date: [16 August 2013].
[43] B. L. Finley, A.D. Monnot, D.J. Paustenbach, S.H. Gaffney. Derivation of a chronic oral reference dose for cobalt. *Regul. Toxicol. Pharm.* **2012**, *64*, 491.
[44] E. S. Mucklow, S. J. Griffin, H. T. Delves, B. Suchak. Cobalt poisoning in a 6-year-old. *Lancet* **1990**, *335*, 981.
[45] H. Jacobziner, H. W. Raybin. Poison control...accidental cobalt poisoning. *Arch. Pediatr.* **1961**, *78*, 200.
[46] U. O. Schirrmacher. Case of cobalt poisoning. *Brit. Med. J.* **1967**, *1*, 544.
[47] L. Berk, J. H. Burchenal, T. Wood, W.B. Castle. Oxygen saturation of sternal marrow blood with special reference to pathogenesis of polycythemia vera. *Proc. Soc. Exp. Biol. Med.* **1948**, *69*, 316.
[48] K. R. Paley, E. S. Sobel, R. S. Yalow. Effect of oral and intravenous cobaltous chloride on thyroid function. *J. Clin. Endocr. Metab.* **1958**, *18*, 850.
[49] J. C. Weaver, V. M. Kostainsek, D. N. Richards Jr. Cobalt tumor of the thyroid gland. *Calif. Med.* **1956**, *85*, 110.
[50] P. Seghizzi, F. D'Adda, D. Borleri, F. Barbic, G. Mosconi. Cobalt myocardiopathy. A critical review of literature. *Sci. Total Environ.* **1994**, *150*, 105.
[51] Y. Morin, A. Tetu, G. Mercier. Cobalt cardiomyopathy: Clinical aspects. *Brit. Heart J.* **1971**, *33*(8), 175.
[52] C. S. Alexander. Cobalt-beer cardiomyopathy. A clinical and pathologic study of twenty-eight cases. *Am. J. Med.* **1972**, *53*, 395.
[53] D. Lison. Human toxicity of cobalt-containing dust and experimental studies on the mechanism of interstitial lung disease (hard metal disease). *Crit. Rev. Toxicol.* **1996**, *26*, 585.
[54] R. Krapf, H. N. Hulter. Arterial hypertension induced by erythropoietin and erythropoiesis-stimulating agents (ESA). *Clin. J. Am. Soc. Nephrol.* **2009**, *4*, 470.
[55] W. Jelkmann, S. Elliott. Erythropoietin and the vascular wall: The controversy continues. *Nutr. Metab. Cardiovasc. Dis.* **2012** [Epub ahead of print]. PMID: 22682530.
[56] N. T. Shahidi. Androgens and erythropoiesis. *New Engl. J. Med.* **1973**, *289*, 72.
[57] K. Kanaya, T. Kamitani. pVHL-independent ubiquitination of HIF1alpha and its stabilization by cobalt ion. *Biochem. Biophys. Res. Commun.* **2003**, *306*, 750.
[58] Y. Yuan, D. Beitner-Johnson, D. E. Millhorn. Hypoxia-inducible factor 2alpha binds to cobalt in vitro. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 849.
[59] Y. Yuan, G. Hilliard, T. Ferguson, D. E. Millhorn. Cobalt inhibits the interaction between hypoxia-inducible factor-alpha and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor-alpha. *J. Biol. Chem.* **2003**, *278*, 15911.
[60] S. Nagel, N. P. Talbot, J. Mecinovic, T. G. Smith, A. M. Buchan, C. J. Schofield. Therapeutic manipulation of the HIF hydroxylases. *Antioxid. Redox Signal.* **2010**, *12*, 481.
[61] M. De Boeck, M. Kirsch-Volders, D. Lison. Cobalt and antimony: Genotoxicity and carcinogenicity. *Mutat. Res.* **2003**, *533*, 135.
[62] D. Beyersmann, A. Hartwig. The genetic toxicology of cobalt. *Toxicol. Appl. Pharmacol.* **1992**, *115*, 137.
[63] J. Suzuki. Time-course changes in VEGF expression and capillarity in the early stage of exercise training with Co treatment in rat skeletal muscles. *Acta Physiol. Scand.* **2004**, *181*, 225.
[64] D. Shukla, S. Saxena, J. Purushothaman, K. Shrivastava, M. Singh, S. Shukla, V. K. Malhotra, S. Mustoori, A. Bansal. Hypoxic preconditioning with cobalt ameliorates hypobaric hypoxia induced pulmonary edema in rat. *Eur. J. Pharmacol.* **2011**, *656*, 101.
[65] S. Saxena, D. Shukla, A. Bansal. Augmentation of aerobic respiration and mitochondrial biogenesis in skeletal muscle by hypoxia preconditioning with cobalt chloride. *Toxicol. Appl. Pharmacol.* **2012**, *264*, 324.
[66] L. O. Simonsen, A. M. Brown, H. Harbak, B. I. Kristensen, P. Bennekou. Cobalt uptake and binding in human red blood cells. *Blood Cell Mol. Dis.* **2011**, *46*, 266.
[67] K. M. Unice, A. D. Monnot, S. H. Gaffney, B. E. Tvermoes, K. A. Thuett, D. J. Paustenbach, B. L. Finley. Inorganic cobalt supplementation: Prediction of cobalt levels in whole blood and urine using a biokinetic model. *Food Chem. Toxicol.* **2012**, *50*, 2456.

STAFF ANALYSIS
DISCUSSION AND ACTION REGARDING THE REPORT ON KETOPROFEN AND THE RACING COMMISSIONERS INTERNATIONAL (RCI) RECOMMENDATION TO LOWER THE RECOMMENDED DOSAGE IN ORDER TO PREVENT RACE DAY ADMINISTRATION

Medication and Track Safety Committee Meeting
October 22, 2014

BACKGROUND

Business and Professions Code section 19580 provides that the Board shall adopt regulations to establish policies, guidelines, and penalties relating to equine medication in order to preserve and enhance the integrity of horse racing in the state. Business and Professions Code section 19581 states no substance of any kind shall be administered by any means to a horse after it has been entered to race in a horse race, unless the Board has, by regulation, specifically authorized the use of the substance and the quantity and composition thereof. Board Rule 1843, Medication, Drugs and Other Substances, provides that no horse participating in a race shall carry in its body any drug substance or its metabolites or analogues, foreign to the horse except as hereinafter expressly provided. No drug substance shall be administered to a horse which is entered to compete in a race to be run in this state except for approved and authorized drug substances as provided in these rules. The Association of Racing Commissioners International (ARCI) is the international association of the government sanctioned entities responsible for the honesty and integrity of horse and greyhound racing as well as all associated pari-mutuel wagering. ARCI sets standards used in medication policy, and drug testing laboratories.

Ketoprofen, a non-steroidal anti-inflammatory and analgesic drug, is allowed to be administered up to 24 hours prior to racing at a current regulatory threshold of 10 ng/mL of blood plasma or serum under Board Rule 1844 (c)(3), Authorized Medication. The current withdrawal guideline is 24 hours after a single intravenous dose of ketoprofen at a total dose of 2.2 mg/kg. The 10 ng/mL threshold is based on results of studies conducted in the mid-90's. Based on more modern technology using a liquid chromatographic-mass spectrometric method, a more accurate 24 hour and 48 hour threshold has been determined. April 17, 2014, ARCI adopted the 2ng/ml of ketoprofen threshold in blood plasma or serum (ARCI 011-010 Section C Paragraph 1.b)

RECOMMENDATION

This item is presented for Committee discussion and action.
The Board's Equine Medical Director is prepared to make a presentation to the Committee.

Ketoprofen

Ketoprofen is a non-steroidal anti-inflammatory and analgesic drug which is approved by the US Food and Drug Administration for use in horses as Ketofen®. Ketoprofen is allowed to be administered up to 24 horses prior to racing at a current regulatory threshold is 10 ng/mL of blood plasma or serum under CHRB 1844 (c)(3). The current withdrawal guideline is 24 hours after a single intravenous dose of ketoprofen as Ketofen at a total dose of 2.2 mg/kg.

The 10 ng/mL threshold is based results of studies of the disposition of ketoprofen in horses after intravenous administration conducted in the mid-90's. Plasma ketoprofen concentrations were determined with a high performance liquid chromatographic method with a lower limit of quantification of 10 ng/mL. Based on this single dose study, the mean time to the LOQ was d 378 minutes with a range of 248-499 minutes – these results indicated a detection time of approximately 8-10 hours.(Sams, R., *et al., Pharmacokinetics of ketoprofen after multiple intravenous doses to mares*, J. vet Pharmacol. Therap., vol. 18, pp. 108-116 (1995).) Thus, the current threshold does not reflect a 24 hour withdrawal period.

With the assistance of funding from the Kentucky EDRC, HFL Sport Science has developed and validated a liquid chromatographic-mass spectrometric method for determination of ketoprofen in plasma samples with a lower limit of quantification of 0.02 ng/mL of plasma.

Subsequently, the 24 and 48 hour administration samples from a previous RMTC administration of 2.2 mg/kg were analyzed by the new method. Based upon this new method, HFL Sport Science was able to identify a 24 hour and 48 hour threshold. This would translate into a primary (24 hour) and secondary (48 hour) threshold recommendation. Based upon the 95/95 tolerance interval calculation, the recommended thresholds are as follows:

- 24 hour (primary) threshold – 2 ng/mL of plasma or serum

The ARCI has adopted the 2ng/ml of ketoprofen threshold in blood plasma or serum (ARCI 011-010 Section C Paragraph 1.b reference http://arcicom.businesscatalyst.com/assets/arci-controlled-therapeutic-medication-schedule---version-2.1.pdf , p6)

1844 (c) Not more than one approved non-steroidal anti-inflammatory drug substance (NSAID) may be administered to a horse that is entered to race and shall be only one of the following authorized drug substances:

(1) Phenylbutazone in a dosage amount that the test sample shall contain not more than 2 micrograms of the drug substance per milliliter of blood plasma or serum.

(2) Flunixin in a dosage amount that the test sample shall contain not more than 20 nanograms of the drug substance per milliliter of blood plasma or serum.

(3) Ketoprofen in a dosage amount that the test sample shall contain not more than ~~10~~ **2** nanograms of the drug substance per milliliter of blood plasma or serum.

Non-Steroidal Anti-Inflammatory Drug (NSAID) Rules**

Controlled Therapeutic Medication	Threshold (Primary)	Withdrawal Guideline	Dosing Specifications	Reference Notes	Threshold (Secondary)
Flunixin	20 nanogram per milliliter of plasma or serum	32 hours	Single intravenous dose of flunixin as Banamine® (flunixin meglumine) at 1.1 milligram per kilogram	University of California at Davis/RMTC study	Secondary anti-stacking threshold: 3.0 nanograms per milliliter of plasma or serum (Administration 48 hours prior)
Ketoprofen	2 nanograms per milliliter of plasma or serum	24 hours	Single intravenous dose of ketoprofen as Ketofen® at 2.2 milligrams per kilogram	HFL Sport Sciences/ Kentucky Equine Drug and Research Council/RMTC study	Secondary anti-stacking threshold: 1 nanogram per milliliter of plasma or serum (Administration 48 hours prior)
Phenylbutazone	2 micrograms per milliliter of plasma or serum	24 hours	Single intravenous dose of phenylbutazone at 4.0 milligrams per kilogram	ARCI model rule	[illegible] stacking threshold: 0.3 micrograms per milliliter of plasma or serum (Administration 48-hours prior)

** Samples collected may contain one of the NSAIDs in this chart at a concentration up to the Primary Threshold. Samples may also contain another of the NSAIDs in this chart up at a concentration up to the Secondary Threshold. No more than 2 of the NSAIDs in this chart may be present in any sample.

STAFF ANALYSIS
DISCUSSION AND ACTION REGARDING THE PROPOSED AMENDMENT TO CHRB RULE 1843.3, PENALTIES FOR MEDICATION VIOLATIONS, TO PROHIBIT: 1) SUSPENDED TRAINERS FROM TRANSFERRING HORSES TO EMPLOYEES; 2) THE USE OF ANY SIGNAGE, COLORS OR IDENTIFIABLE TACK OF A SUSPENDED TRAINER DURING A SUSPENSION; AND TO CHANGE FROM 60 DAYS TO 45 DAYS, THE REQUIREMENT THAT TRAINERS SUSPENDED FOR SUCH TIME BE BANNED FROM THE ENCLOSURE AND FORFEIT ALL ASSIGNED STALL SPACE AND REMOVE FROM THE INCLOSURE ALL SIGNAGE, ADVERTISEMENTS, TRAINING-RELATED EQUIPMENT, TACK, OFFICE EQUIPMENT AND OTHER PROPERTY

Medication and Track Safety Committee Meeting
October 22, 2014

BACKGROUND

Business and Professions Code section 19580 provides that the Board shall adopt regulations to establish policies, guidelines, and penalties relating to equine medication in order to preserve and enhance the integrity of horse racing in the state. Business and Professions Code section 19581 states no substance of any kind shall be administered by any means to a horse after it has been entered to race in a horse race, unless the Board has, by regulation, specifically authorized the use of the substance and the quantity and composition thereof. Board Rule 1843, Medication, Drugs and Other Substances, provides that no horse participating in a race shall carry in its body any drug substance or its metabolites or analogues, foreign to the horse except as hereinafter expressly provided. No drug substance shall be administered to a horse which is entered to compete in a race to be run in this state except for approved and authorized drug substances as provided in these rules. Board Rule 1843.2, Classification of Drug Substances, categorizes and defines drug substances based on the Association of Racing Commissioners International (ARCI) drug classifications. Board Rule 1843.3, Penalties for Medication Violations, defines the penalties medication violations involving the substances defined and categorized in Board Rule 1843.2. 1843.3(i) prohibits a suspended licensee from transferring horses to licensed family members.

The proposed amendment to Board Rule 1843.3 would add language to subsection 1843.3(i) which would prohibit a suspended licensee from transferring horses to any other licensee employed by the suspended licensee within the previous year. This is necessary to further ensure that a licensee does not benefit financially during the period of suspension.

The proposed amendment would add section 1843.3(k) which would prohibit the use of any signage, colors, or tack identifiable as belonging to a suspended trainer, for the duration of the suspension.

The proposed amendment would change the section labeled as 1843(j)(1) to 1843(l). This section currently states, for the purpose of this regulation, licensed trainers suspended 60 days or more, or whose license is revoked, shall be banned from all inclosures under the jurisdiction of the CHRB. The proposed amendment would reduce 60 days to 45 days, requiring licensed

trainers suspended 45 days or more be banned from all inclosures under the jurisdiction of the CHRB.

RECOMMENDATION

This item is presented for Committee discussion and action.

CALIFORNIA HORSE RACING BOARD
TITLE 4. CALIFORNIA CODE OF REGULATIONS
ARTICLE 15. VETERINARY PRACTICES
PROPOSED AMENDMENT OF
RULE 1843.3. PENALTIES FOR MEDICATION VIOLATIONS.

1843.3. Penalties for Medication Violations.

(a) In reaching a decision on a penalty for a violation of Business and Professions Code section 19581, the Board, the board of stewards, the hearing officer or the administrative law judge shall consider the penalties set forth in subsections (d) and (e) of this Rule and any aggravating and mitigating circumstances. Deviation from these penalties is appropriate where the facts of the particular case warrant such a deviation, for example: there may be mitigating circumstances for which a lesser or no penalty is appropriate, and aggravating factors may increase the penalties beyond the minimum.

(b) Mitigating circumstances and aggravating factors, which must be considered, include but are not limited to:

(1) The past record of the licensee regarding violations of Business and Professions Code section 19581;

2) The potential of the drug(s) to influence a horse's racing performance;

(3) The legal availability of the drug;

(4) Whether there is reason to believe the responsible party knew of the administration of the drug or intentionally administered the drug;

(5) The steps taken by the trainer to safeguard the horse;

(6) The steps taken by an owner to safeguard against subsequent medication violations including, but not limited to, the transfer of the horse(s) to an unaffiliated trainer;

(A) For the purpose of this regulation "unaffiliated trainer" means a trainer or an assistant

trainer who is not related by blood, marriage or domestic partnership, or who is not or was never employed by the trainer from whose care such horse(s) were transferred.

(7) The probability of environmental contamination or inadvertent exposure due to human drug use or other factors;

(8) The purse of the race;

(9) Whether the drug found to be present in the official test sample was one for which the horse was receiving treatment as determined through the process described in Rule 1842 of this division;

(10) Whether there was any suspicious wagering pattern on the race;

(11) Whether the licensed trainer was acting under the advice of a licensed veterinarian.

(c) For the purpose of this regulation, the Board shall consider the classification of a drug substance as referred to in Rule 1843.2 of this division and the California Horse Racing Board (CHRB) Penalty Categories Listing By Classification, (1/08), which is hereby incorporated by reference, if a determination is made that an official test sample from a horse contained:

(1) Any drug substance, medication, metabolites or analogues thereof foreign to the horse, whose use is not expressly authorized in this division, or

(2) Any drug substance, medication or chemical authorized by this article in excess of the authorized level or other restrictions as set forth in the article.

(d) Penalties for violation of each classification level are as follows:

CATEGORY "A" PENALTIES

Penalties for violations due to the presence of a drug substance in an official test sample, which CHRB drug classification is categorized as warranting a Category A penalty are as follows:

LICENSED TRAINER:		
1st offense	**2nd LIFETIME offense**	**3rd LIFETIME offense**
◦ Minimum one - year suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a three-year suspension. **AND** ◦ Minimum fine of $10,000 or 10% of gross purse (greater of the two) absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $25,000 or 25% of purse (greater of the two). **AND** ◦ May be referred to the Board for any further action deemed necessary by the Board.	◦ Minimum two-year suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a three-year suspension. **AND** ◦ Minimum fine of $20,000 or 25% of gross purse (greater of the two) absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $50,000 or 50% of purse (greater of the two). **AND** ◦ May be referred to the Board for any further action deemed necessary by the Board.	◦ Minimum three -year suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of permanent license revocation. **AND** ◦ Minimum fine of $25,000 or 50% of gross purse (greater of the two) absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of $100,000 or 100% of purse (greater of the two). **AND** ◦ May be referred to the Board for any further action deemed necessary by the Board.
LICENSED OWNER:		
1st offense	**2nd LIFETIME offense in owner's stable**	**3rd LIFETIME offense in owner's stable**
◦ Disqualification of horse and loss of purse. **AND** ◦ Horse may be placed on the veterinarian's list for up to 90 days and must pass a Board - approved examination pursuant to Rule 1846 before becoming eligible to be entered. **AND** ◦ Be subject to drug testing at the owner's expense and be negative for prohibited drug substances as defined in Rule 1843.1.	◦ Disqualification of horse and loss of purse. **AND** ◦ Horse shall be placed on the veterinarian's list for up to 120 days and must pass a Board - approved examination pursuant to Rule 1846 before becoming eligible to be entered. **AND** ◦ Be subject to drug testing at the owner's expense and be negative for prohibited drug substances as defined in Rule 1843.1.	◦ Disqualification of horse, loss of purse and absent mitigating circumstances, minimum fine of $10,000. The presence of aggravating factors could be used to impose a maximum fine of $50,000. **AND** ◦ Horse shall be placed on the veterinarian's list for up to 180 days and must pass a Board-approved examination pursuant to Rule 1846 before becoming eligible to be entered. **AND** ◦ Be subject to drug testing at the owner's expense and be negative for prohibited drug substances as defined in Rule 1843.1. **AND** ◦ Referral to the Board with a recommendation of a suspension of owners license for a minimum of 90 days.

CATEGORY "B" PENALTIES

Penalties for violations due to the presence of a drug substance in an official test sample, which CHRB drug classification is categorized as warranting a Category B penalty are as follows:

LICENSED TRAINER:		
1st offense	**2nd offense (two years)**	**3rd offense (five years)**
◦ Minimum 30 -day suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a 60-day suspension. **AND/OR** ◦ Minimum fine of $500 absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $10,000.	◦ Minimum 60-day suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a 180-day suspension. **AND/OR** ◦ Minimum fine of $1,000 absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $20,000.	◦ Minimum 90-day suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a one-year suspension. **AND/OR** ◦ Minimum fine of $2,500 absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $50,000 or 10% of purse (greater of the two). **AND** ◦ May be referred to the Board for any further action deemed necessary by the Board.
LICENSED OWNER:		
1st offense	**2nd offense in stable (two years)**	**3rd offense in stable (five years)**
◦ Disqualification of horse and loss of purse. **AND** ◦ Horse must pass a Board-approved examination pursuant to Rule 1846 before becoming eligible to be entered. **AND** ◦ Be subject to drug testing at the owner's expense and be negative for prohibited drug substances as defined in Rule 1843.1.	◦ Disqualification of horse and loss of purse. **AND** ◦ Horse must pass a Board-approved examination pursuant to Rule 1846 before becoming eligible to be entered. **AND** ◦ Be subject to drug testing at the owner's expense and be negative for prohibited drug substances as defined in Rule 1843.1.	◦ Disqualification of horse, loss of purse and absent mitigating circumstances minimum fine of $5,000. The presence of aggravating factors could be used to impose a maximum fine of $20,000. **AND** ◦ Horse shall be placed on the veterinarian's list for up to 45 days and must pass a Board-approved examination pursuant to Rule 1846 before becoming eligible to be entered. **AND** ◦ Be subject to drug testing at the owner's expense and be negative for prohibited drug substances as defined in Rule 1843.1.

CATEGORY "B" PENALTIES FOR RULE 1843.6 TOTAL CARBON DIOXIDE (TCO_2) TESTING

Penalties for violations due to exceeding permitted levels of TCO_2 as defined in Rule 1843.6 are as set forth below. All concentrations are for measurements in serum or plasma.

LICENSED TRAINER:		
1st offense TCO_2 (> 37.0mml/l-<39mml/l)	**2nd offense TCO_2 (> 37.0mml/l-<39mml/l)**	**3rd offense TCO_2 (> 37.0mml/l-<39mml/l)**
◦ Up to a 30-day suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a—60-day suspension. **AND/OR** ◦ Minimum fine of $1,500 absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $5,000.	◦ Minimum 60-day suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a 120-day suspension. **AND/OR** ◦ Minimum fine of $2,500 absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $10,000.	◦ Minimum 90-day suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a 180-day suspension. **AND/OR** ◦ Minimum fine of $5,000 absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $15,000.
LICENSED OWNER:		
1st offense TCO_2 (> 37.0mml/l-<39mml/l)	**2nd offense TCO_2 (> 37.0mml/l-<39mml/l)**	**3rd offense TCO_2 (> 37.0mml/l-<39mml/l)**
◦ Disqualification of horse and loss of purse.	◦ Disqualification of horse and loss of purse.	◦ Disqualification of horse, loss of purse and in the absence of mitigating circumstances, $2,500 fine.
LICENSED TRAINER:		
1st offense TCO_2 (≥ 39.0mml/l)	**2nd offense TCO_2 (≥ 39.0mml/l)**	**3rd offense TCO_2 (≥ 39.0mml/l)**
◦ Minimum 30-day suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a—60-day suspension. **AND/OR** ◦ Minimum fine of $2,500 absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $10,000.	◦ Minimum 60-day suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a 180-day suspension. **AND/OR** ◦ Minimum fine of $5,000 absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $15,000.	◦ Minimum 90-day suspension absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum of a 365-day suspension. **AND/OR** ◦ Minimum fine of $10,000 absent mitigating circumstances. The presence of aggravating factors could be used to impose a maximum fine of $25,000.
LICENSED OWNER:		
1st offense TCO_2 (≥ 39.0mml/l)	**2nd offense TCO_2 (≥ 39.0mml/l)**	**3rd offense TCO_2 (≥ 39.0mml/l)**
◦ Disqualification of horse and loss of purse.	◦ Disqualification of horse and loss of purse.	◦ Disqualification of horse, loss of purse and a fine ranging from a minimum of $5,000, up to a maximum of $20,000.

CATEGORY "C" PENALTIES

Penalties for violations due to the presence of a drug substance in an official test sample, which CHRB drug classification is categorized as warranting a Category C penalty and for the presence of more than one non-steroidal anti-inflammatory (NSAID) in a plasma/serum sample, as defined in Rule 1844 of this division, and furosemide as defined in Rule 1845 of this division in an official test sample are as set forth below. All concentrations are for measurements in serum or plasma.

LICENSED TRAINER:		
1st offense	**2nd offense (365-day period)**	**3rd offense (365-day period)**
◦ Minimum fine of $500 to a maximum fine of $1,000 absent mitigating circumstances.	◦ Minimum fine of $1,000 to a maximum fine of $2,500, and up to a 15 - day suspension absent mitigating circumstances.	◦ Minimum fine of $2,500 and up to a 30 - day suspension absent mitigating circumstances.

CATEGORY "C" PENALTIES FOR RULE 1844, AUTHORIZED MEDICATION (C) (1), (2), (3)

Penalties for violations due to overages for permitted non-steroidal anti-inflammatory drug substances (NSAIDs) as defined in Rule 1844 (c) (1), (2) and (3) of this division. All concentrations are for measurements in serum or plasma.

The official veterinarian shall consult with the treating veterinarian in all violations of 1844 (c). With permission of the official veterinarian the trainer may elect to pay the minimum fine in lieu of a stewards' hearing. If the trainer has not had an 1844 (c) violation within the previous three years, the official veterinarian or the board of stewards may issue a warning in lieu of a fine for violations of 1844 (c)(1), phenylbutazone, provided the reported level is below 5.1 mcg/ml.

LICENSED TRAINER:	Phenylbutazone (5.1-<10.0mcg/ml) Flunixin (20 100 ng/ml) Ketoprofen (11-49 ng/ml)	Phenylbutazone (5.1-<10.0mcg/ml) Flunixin (20 100 ng/ml) Ketoprofen (11-49 ng/ml)
1st offense	2nd offense (365-day period)	3rd offense (365-day period)
◦ Minimum fine of $500 to a maximum fine of $1,000.	◦ Minimum fine of $1,000 to a maximum fine of $2,500.	◦ Minimum fine of $2,500 to a maximum fine of $5,000.
LICENSED OWNER:	**Phenylbutazone (5.1-<10.0mcg/ml) Flunixin (20 100 ng/ml) Ketoprofen (11-49 ng/ml)**	**Phenylbutazone (5.1-<10.0mcg/ml) Flunixin (20 100 ng/ml) Ketoprofen (11-49 ng/ml)**
1st offense	2nd offense (365-day period)	3rd offense (365-day period)
No penalty administered.	No penalty administered.	No penalty administered.
LICENSED TRAINER:	**Phenylbutazone (≥ 10.0 mcg/ml) Flunixin (≥ 100 ng/ml) Ketoprofen (≥ 50 ng/ml)**	**Phenylbutazone (≥ 10.0 mcg/ml) Flunixin (≥ 100 ng/ml) Ketoprofen (≥ 50 ng/ml)**
1st offense	2nd offense (365-day period)	3rd offense (365-day period)
◦ Minimum fine of $1,000 to a maximum fine of $2,500.	◦ Minimum fine of $2,500 to a maximum fine of $5,000.	◦ Minimum fine of $5,000 to a maximum fine of $10,000.
LICENSED OWNER:	**Phenylbutazone (≥ 10.0 mcg/ml) Flunixin (≥ 100 ng/ml) Ketoprofen (≥ 50 ng/ml)**	**Phenylbutazone (≥ 10.0 mcg/ml) Flunixin (≥ 100 ng/ml) Ketoprofen (≥ 50 ng/ml)**
1st offense	2nd offense (365-day period)	3rd offense (365-day period)
◦ Horse must pass Board-approved examination pursuant to Rule 1846 before being eligible to run.	◦ Disqualification of horse and loss of purse. If same horse, placed on veterinarian's list for up to 45-days, must pass Board-approved examination pursuant to Rule 1846 before being eligible to run.	◦ Disqualification of horse and loss of purse. Minimum $5,000 fine. If same horse, placed on veterinarian's list for 60 days, must pass Board-approved examination pursuant to Rule 1846 before being eligible to run.

(e) Violations due to the presence of a drug substance in an official test sample, which CHRB drug classification is categorized as warranting a Category "D" penalty, may result in a written warning to the licensed trainer and owner. A Category "D" penalty for a first offense may result in a written warning or fine that will remain on the licensee's record for a period of two years. After the two year period, if the licensee has had no further violations of CHRB Rule 1843, the Category "D" penalty will be expunged from the licensee's record for penalty purposes.

CATEGORY "D" PENALTIES

1ST offense (365 day period)	2nd offense (365 day period)	3rd offense (365 day period)
Minimum of an official written warning to a maximum fine of $250.	Minimum of a $250 fine to a maximum fine of $500.	Minimum of a $500 fine to a maximum fine of $750.

CATEGORY "D" PENALTIES FOR RULE 1844(c)(1) VIOLATIONS

Phenylbutazone 2.1ug/ml to 5.0 ug/ml		
1st offense (365 day period)	2nd offense (365 day period)	3rd offense (365 day period)
Minimum of an official written warning to a maximum fine of $250.	Minimum of a $250 fine to a maximum fine of $500.	Minimum of a $500 fine to a maximum fine of $750.

(f) Any drug or its metabolite or analogue thereof found to be present in an official test sample that is not classified in Rule 1843.2 of this division shall be classified as a Class 1 substance and a Category "A" penalty until classified by the Board.

(g) The administration of a drug substance to a race horse must be documented by the treating veterinarian through the process described in Rule 1842 of this division.

(h) Any licensee found to be responsible for the administration of any drug substance resulting in a positive test may be subject to the same penalties set forth for the licensed trainer and his presence may be required at any and all hearings relative to the case.

(1) Any veterinarian found to be involved in the administration of any drug substance resulting in a positive test in Penalty Category "A" shall be referred to the California Veterinary Medical Board (CVMB) for consideration of further disciplinary action.

(2) Any veterinarian found to be involved in the administration of any drug substance resulting in a positive test in Penalty Category "B" or "C" may be referred to the CVMB for consideration of further disciplinary action upon the recommendation of the Equine Medical Director, the board of stewards or hearing officers.

(i) A licensee who is suspended, or whose license is revoked, because of a medication violation, is not able to benefit financially during the period of suspension or revocation. This includes, but is not limited to, ensuring that horses are not transferred to licensed family members or to any other licensee who has been an employee of the suspended licensee within the previous year.

(j) For the purpose of this regulation "licensed family members" means any person who holds an occupational license issued by the CHRB and who is related to the suspended licensee, or the licensee whose license is revoked, by blood, or by marriage or domestic partnership, or who is related by blood to the spouse or domestic partner of such licensee.

(k) For the purpose of this regulation, the use of any signage, colors, or tack identifiable as belonging to a suspended licensed trainer is prohibited for the duration of the suspension.

~~(1)~~ (l) For the purpose of this regulation, licensed trainers suspended ~~60~~ 45 days or more, or whose license is revoked, shall be banned from all inclosures under the jurisdiction of the CHRB. In addition, during the period of suspension, or revocation, such trainer shall forfeit all assigned stall space and shall remove from the inclosures all signage, advertisements, training-related equipment, tack, office equipment, and any other property.

Authority: Sections 19440, 19461 and 19580, Business and Professions Code.

Reference: Sections 19461, 19580, 19581 and 19582, Business and Professions Code. Section 11425.50, Government Code.

STAFF ANALYSIS
DISCUSSION AND ACTION REGARDING THE PROPOSED AMENDMENT TO CHRB RULE 1844, AUTHORIZED MEDICATION, TO ADD ISOFLUPRODONE AND ITS SPECIFIED AUTHORIZED LEVEL TO THE LIST OF CALIFORNIA'S AUTHORIZED MEDICATION

Medication and Track Safety Committee Meeting
October 22, 2014

BACKGROUND

Business and Professions Code section 19440 provides that the Board shall have all powers necessary and proper to enable it to carry out fully and effectually the purposes of this chapter. Responsibilities of the Board shall include adopting rules and regulations for the protection of the public and the control of horse racing and pari-mutuel wagering. Business and Professions Code section 19562 states the Board may prescribe rules, regulations and conditions under which all horse races with wagering on their results shall be conducted in California. Business and Professions Code section 19580 requires the Board to adopt regulations to establish policies, guidelines, and penalties relating to equine medication to preserve and enhance the integrity of horse racing in California. Business and Professions Code section 19581 provides that no substance of any kind shall be administered by any means to a horse after it has been entered to race, unless the Board has, by regulation, specifically authorized the use of the substance and the quantity and composition thereof. Board Rule 1844, Authorized Medication, names drug substances and medications authorized by the Board that may be administered to safeguard the health of the horse entered to race. The rule lists the drug substances that may be found in official test samples and the level at which such drugs may occur. The Association of Racing Commissioners International (ARCI) is the international association of the government sanctioned entities responsible for the honesty and integrity of horse and greyhound racing as well as all associated pari-mutuel wagering. ARCI sets standards used in medication policy, and drug testing laboratories.

The proposed amendment to Board Rule 1844 would add isofluprodone, in an amount that does not exceed 100 picograms per milliliter, to the list of drug substances that a blood serum or plasma sample may contain. Isoflupredone acetate is a long acting corticosteroid that can be used for the treatment of allergic, musculoskeletal, and inflammatory processes in the horse. Isoflupredone acetate can be administered via intra-articular, intravenous, and intra-muscular/subcutaneous routes. On April 17, 2014, ARCI adopted the 100pg/ml isoflupredone threshold in blood plasma or serum (ARCI 011-010 Section C Paragraph 1.b).

RECOMMENDATION

This item is presented for Committee discussion and action.
The Board's Equine Medical Director is prepared to make a presentation to the Committee.

Isoflupredone

Isoflupredone acetate is a long acting corticosteroid that can be used for the treatment of allergic, musculoskeletal, and inflammatory processes in the horse. It can be administered via intra-articular, intravenous, and intra-muscular/subcutaneous routes. It is marketed by Zoetis under the trade name Predef 2X. The glucocorticoid activity of isoflupredone acetate is approximately 10X that of prednisolone.

RMTC in conjunction with the EDRC and Rood and Riddle Equine Hospital performed two separate administration studies. The first was completed at the University of Florida. For that study, exercised horses were administered the following doses of isoflupredone acetate via the following routes:

Route of Administration	Power (n)	Dose
Intramuscular	6	20 mg total dose
Intra-articular	6	20 mg total dose
Intravenous	6	20 mg total dose

Subsequently, at the request of the AAEP, the RMTC performed a second administration study on fit but not racing horses with the purpose of adding an additional route of administration. (See ICRAV Abstract)

Blood samples were collected at time zero as well as 2, 4, 24, 48, 72, 96, 120, 144, 168, and 336 hours. The samples for both administration studies were analyzed at HFLSS with the use of EDRC funding. Based upon a 95/95 Threshold Interval assessment of the 168 hour data, RMTC recommended a threshold of 100 pg/mL based upon the subcutaneous (10 mg) and intra-articular (20 mg) doses. The 100 picogram per milliliter thresholds in blood plasma or serum was adopted by the ARCI (ARCI 011-010 Section C Paragraph 1.b reference http://arcicom.businesscatalyst.com/assets/arci-controlled-therapeutic-medication-schedule---version-2.1.pdf , p3)

CHRB 1844 Authorized Medication

(f) Official blood test samples may contain the following drug substances, their metabolites and analogs, in an amount that does not exceed the specified levels in serum or plasma:

(16) Isofluprodone; 100 picograms per milliliter

Controlled Therapeutic Medication	Threshold	Withdrawal Guideline	Dosing Specifications	Reference Notes	Note
Dimethyl sulfoxide (DMSO)	10 micrograms per milliliter of plasma or serum	48 hours	Intravenous	ARCI model rule	Applicable analyte is DMSO in plasma or serum
Firocoxib	20 nanograms per milliliter of plasma or serum	14 days	Oral administration of firocoxib as EQUIOXX oral paste at a daily dose of 0.1 milligram per kilogram for four days	RMTC study	Applicable analyte is firocoxib in plasma or serum
Furosemide	100 nanogram per milliliter of plasma or serum	4 hours	Single Intravenous dose of furosemide up to 500 milligram	ARCI model rule	Must also have urine specific gravity < 1.010 for a violation.
Glycopyrrolate	3 picograms per milliliter plasma or serum	48 hours	Single intravenous dose of 1 milligram of glycopyrrolate as Glycopyrrolate Injection, USP (American Regent product # 0517-4601-25)	RMTC study; *Journal of Veterinary Pharmacology and Therapeutics* doi: 10.1111/j.1365-2885.2011.01272.x	Applicable analyte is glycopyrrolate in plasma or serum
Isoflupredone	100 picograms per milliliter of plasma or serum	7 days	10 milligrams total dose subcutaneous or 20 milligrams total dose in one articular space	RMTC Study	
Lidocaine	20 picograms per milliliter of total 30H-lidocaine in plasma	72 hours	200 milligrams of lidocaine as its hydrochloride salt administered subcutaneously	European Horseracing Scientific Liaison Committee data; Iowa State University study.	Applies to total major hydroxylated metabolite (i.e., includes conjugates)

ARCI (ARCI 011-010 Section C Paragraph 1.b)

ICRAV Abstract

Development of a plasma threshold and withdrawal guidance for isoflupredone administered intra-articularly and subcutaneously.

D. Benson,[i] R. Arthur;[ii] L. Bramlage;[iii] P. Colahan;[iv] R. Sams;[v] M. Scollay[vi]

Purpose and Relevance

The use of corticosteroids for reducing inflammation is common in race horses. Previously, RMTC conducted administration studies investigating the pharmacokinetics of methylprednisolone, prednisolone, triamcinolone acetonide, dexamethasone, and betamethasone to set regulatory thresholds and provide withdrawal guidance in the US. This project examined isoflupredone acetate administered intra-articularly and subcutaneously at doses used by race track practitioners.

Methods

Intra-articular (IA) and subcutaneous (SQ) administrations of isoflupredone acetate as Predef 2X were completed in Thoroughbred, Quarter Horse, and Warmblood horses aged 2 to 18 years. After aseptic preparation of the site, 8 horses were administered 20 mg in the left metacarpophalangeal joint (IA) and 6 horses were administered 10 mg SQ over the left front proximal suspensory ligament. Blood samples were collected before and 2, 4, 24, 48, 72, 96, 120, 144, 168, and 336 hours post administration.

Samples were analyzed by LCMS/MS using a stable-isotope labeled analogue of isoflupredone as an internal standard. The calibration curve was linear and the LLQ of the validated method was 10 pg/mL and the LOD was approximately 5 pg/mL. Accuracy and precision of the method were acceptable.

Results

At 7 days after the IA administration, the average (SD) concentration of isoflupredone was 10.75 (6.12) pg/mL of plasma whereas 7 days after SQ administration, it was 17.99 (6.17) pg/mL of plasma. Isofluprodone was below 100pg/ml in 8/8 samples at 72 hours after a single IA administration of 20mg and in 6/6 SQ samples at 96 hours after SQ administration of 10mg. The 95/95 tolerance intervals at 7 days were 91.20 pg/mL and 59.28 pg/mL of plasma, respectively after IA and SQ administration. (see figures 1 & 2). No isoflupredone was detected at 14 days.

Conclusions

The results of this study have demonstrated that IA and SQ administration of isoflupredone as the acetate ester in aqueous suspension can be regulated via plasma analysis. Based upon the data obtained and the application of a 95/95 tolerance interval calculation, the RMTC-recommended threshold for isoflupredone regulation in the US is 100 pg/mL of plasma or serum.

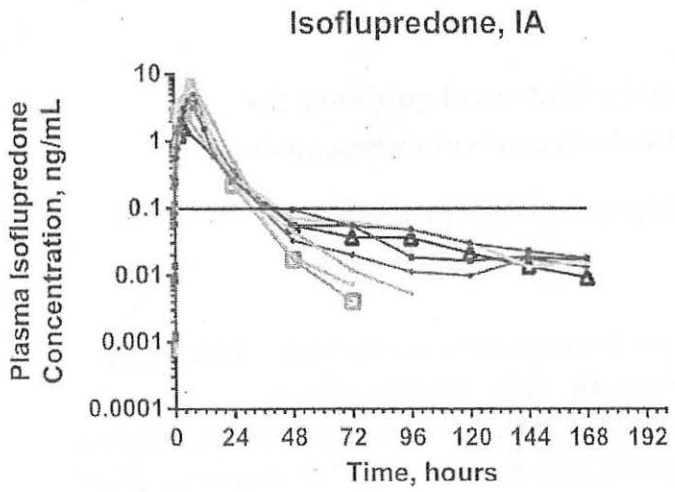

Figure 1. 2 0 mg isofluprodone IA left metacarpophalangeal joint

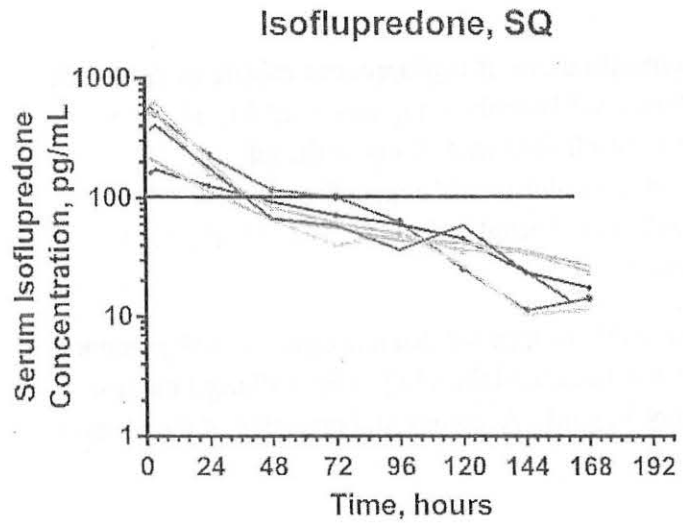

Figure 2. 10 mg isofluprodone SQ over the left front proximal suspensory ligament

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CALIFORNIA HORSE RACING BOARD
TITLE 4. CALIFORNIA CODE OF REGULATIONS
ARTICLE 15. VETERINARY PRACTICES
PROPOSED AMENDMENT OF
RULE 1844. AUTHORIZED MEDICATION

1844. Authorized Medication.

Consistent with the intent of these rules, drug substances and medications authorized by the Board for use may be administered to safeguard the health of the horse entered to race provided that:

(a) No person shall administer a drug substance to any horse entered to race except upon authorization of the official veterinarian in conformance with these rules.

(b) No drug substance, other than authorized bleeder medication, shall be administered to a horse entered to race within 24 hours of the race in which entered.

(c) Not more than one approved non-steroidal anti-inflammatory drug substance (NSAID) may be administered to a horse that is entered to race and shall be only one of the following authorized drug substances:

(1) Phenylbutazone in a dosage amount that the test sample shall contain not more than 2 micrograms of the drug substance per milliliter of blood plasma or serum.

(2) Flunixin in a dosage amount that the test sample shall contain not more than 20 nanograms of the drug substance per milliliter of blood plasma or serum.

(3) Ketoprofen in a dosage amount that the test sample shall contain not more than 10 nanograms of the drug substance per milliliter of blood plasma or serum.

(4) Metabolites or analogues of approved NSAIDs may be present in post race test samples.

(d) If the official chemist reports that a blood test sample contains an authorized NSAID in excess of the limit for that drug substance under this rule, the official veterinarian shall, in conjunction with the veterinarian who administered or prescribed the authorized drug substance, establish a dosage amount or time of administration of the drug substance that will comply with the limits under this rule; or the official veterinarian may, if in his/her judgment no such reduced dosage amount or amendment to time of administration will result in a test sample level within the limits of this rule, withdraw authorization for the use of any one NSAID.

(e) Official urine test samples may contain one of the following drug substances, their metabolites and analogs, in an amount that does not exceed the specified levels:

(1) Acepromazine; 10 nanograms per milliliter

(2) Mepivacaine; 10 nanograms per milliliter

(3) Albuterol; 1 nanograms per milliliter

(4) Procaine; 25 nanograms per milliliter

(5) Salicylates; 750 micrograms per milliliter

(6) Clenbuterol; 140 picograms per milliliter

(7) Omeprazole; 1 nanogram per milliliter

(8) Nandrolone; 1 nanograms per milliliter for geldings, fillies and mares; 45 nanograms for males other than geldings.

(9) Boldenone; 15 nanograms per milliliter in males other than geldings.

(10) Testosterone; 20 nanograms per milliliter in geldings.

(A) Testosterone at any level in males other than geldings is not a violation of this regulation.

(11) Testosterone; 55 nanograms per milliliter in fillies or mares.

(12) Butorphanol 300 nanograms per milliliter

(f) Official blood test samples may contain the following drug substances, their metabolites and analogs, in an amount that does not exceed the specified levels in serum or plasma:

(1) Bethamethasone; 10 picograms per milliliter

(2) Dantrolene; 100 picograms per milliliter

(3) Detomidine; 1 nanogram per milliliter

(4) Dexamethasone; 5 picograms per milliliter

(5) Diclofenac; 5 nanograms per milliliter

(6) Dimethylsulfoxide (DMSO); 10 micrograms per milliliter

(7) Firocoxib; 20 nanograms per milliliter

(8) Lidocaine; 20 picograms per milliliter

(9) Methocarbamol; 1 nanogram per milliliter

(10) Methylprednisolone; 100 picograms per milliliter

(11) Glycopyrrolate; 3 picograms per milliliter

(12) Prednisolone; 1 nanogram per milliliter

(13) Triamcinolone Acetonide; 100 picograms per milliliter

(14) Xylazine; 10 picograms per milliliter of serum or plasma

(15) Butorphanol; 2 nanograms per milliliter

(16) Isofluprodone; 100 picograms per milliliter

(g) Official blood test samples shall not contain any of the drug substances, or their metabolites or analogs listed in subsection (e)-(1)(12).

(h) Procaine, following administration of procaine penicillin, is an authorized medication provided:

(1) Official blood test samples shall not contain any procaine, or its metabolites or analogs in excess of 25 nanograms per milliliter.

(2) all procaine penicillin administrations have been reported pursuant to Rule 1842 of this division,

(3) procaine penicillin was not administered after entry to race,

(4) the horse was under surveillance for a minimum of six hours prior to racing.

(i) All expenses related to surveillance and testing for procaine under subsection (h) of this regulation shall be paid by the owner of the horse.

Authority: Sections 19440 and 19562, Business and Professions Code.

Reference: Sections 19580 and 19581, Business and Professions Code.

CALIFORNIA HORSE RACING BOARD

OCTOBER 22, 2014

MEDICATION AND TRACK SAFETY COMMITTEE MEETING

There is no package material for Item 6

STAFF ANALYSIS
DISCUSSION AND ACTION REGARDING THE REPORT ON THE INTERNATIONAL FEDERATION OF HORSERACING AUTHORITIES (IFHA) RECOMMENDED POLICIES ON MEDICAL RECORDS AND OUT OF COMPETITION TESTING

Medication and Track Safety Committee Meeting
October 22, 2014

BACKGROUND

Business and Professions Code section 19580 provides that the Board shall adopt regulations to establish policies, guidelines, and penalties relating to equine medication in order to preserve and enhance the integrity of horse racing in the state. The International Federation of Horseracing Authorities (IFHA), founded in 1993, is an association of horseracing authorities of the United States, France, Great Britain, and Ireland. The IFHA mission includes the coordination of the rules of its member-countries regarding breeding, racing and wagering. The International Agreement on Breeding, Racing and Wagering (IABRW) is a voluntary agreement designed for the guidance of recognized racing and breeding authorities.

The IABRW defines medication practices for horses in training and out-of-competition testing. The IABRW also provides a general list of prohibited substances.

RECOMMENDATION

This item is presented for Committee discussion and action.
The Board's Equine Medical Director is prepared to make a presentation to the Committee.

Overview of IFHA Article 6D – HORSES in TRAINING

Definition of Treatment:

- The administration of any substance (including any medication) to a horse
- The administration or application of any physical procedure or therapy to horse intended to have an effect.

Principles:

- All treatments are the responsibility of the trainer and must be administered under veterinary supervision.
- Every treatment must be administered in the best health and welfare interests of the horse.

Provisions:

- The trainer must obtain veterinary advice
- The trainer is responsible for creating and maintaining full and accurate records of all treatments given to a horse, including all veterinary procedures performed and all medications administered.
- The trainer must comply with mandatory horse rest periods for specific drugs or treatments
- Horses that are unable to be trained due to injury or illness must be taken out of training and given appropriate veterinary treatment and/or rest. All treatments must be administered in the best interests of the horse

IFHA Article 6E – OUT-OF-COMPETITION TESTING

To ensure fair competition, transparency, welfare and sound breeding, Racing Authorities will at their discretion carry out testing for prohibited substances at any time in the career of any horse, from the commencement of training to final retirement from racing.
To this effect:

1. Trainers must notify their domestic racing jurisdiction the names of horses in training with them and specify where relevant the exact location of such horses.

2. When a racehorse is out of training at any time in its career from the commencement of training to final retirement from racing, the owner(s) must readily be able to inform the domestic Racing Authority of the exact location of the horse.

3. If full traceability of any racehorse, whether in training or out of training, cannot be established at any time in its racing career, such horse will only be permitted to be entered in a race after a period of six (6) months in training with a duly licensed trainer.

4. The following prohibited substances, including other substances with a similar chemical structure or similar biological effect(s), are not to be administered to racehorses at any time in their career:-

   4.1 *Non-approved substances*
   Any substance not addressed by any of the subsequent classes of substances, and which has no current approval by any government regulatory authority for veterinary use, or any substance not universally recognised by veterinary regulatory authorities as valid veterinary therapeutic treatment.
   4.2 *Anabolic agents*
   (a) anabolic androgenic steroids,
   (b) other anabolic agents, including but not limited to selective androgen receptor modulators (SARMs),
   (c) beta-2 agonists, unless the substance is prescribed by a veterinarian as a bronchodilator at the appropriate dose,
   4.3 *Peptide hormones, growth factors and related substances*
   (a) erythropoiesis-stimulating agents, including but not limited to erythropoietin (EPO), epoetin alfa, epoetin beta, darbepoetin alfa, and methoxy polyethylene glycol-epoetin beta, peginesatide, hypoxia inducible factor (HIF)-1 stabilisers,
   (b) growth hormones and growth hormone releasing factors, insulin-like growth factor-1 (IGF-1), and other growth factors,

(c) synthetic proteins and peptides and synthetic analogues of endogenous proteins and peptides not registered for medical or veterinary use,
4.4 *Hormones and metabolic modulators*
(a) aromatase inhibitors,
(b) selective estrogen receptor modulators (SERMS) and other anti-estrogenic substances,
(c) agents modifying myostatin function, including but not limited to myostatin inhibitors,
(d) insulins
(e) peroxisome proliferator activated receptor δ (PPARδ) agonists, including but not limited to GW 1516,
(f) AMPK activators, including but not limited to AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside)

5. 5. Therapeutic use of substances specified in point 4 above may only be exceptionally applied in the following circumstances:

When the Racing Authority has decided to offer the facility for such exceptional use for therapeutic purposes and where no other reasonable therapeutic alternative exists.

a) The specified prohibited substance being exceptionally used therapeutically must be prescribed by a veterinarian for the sole purpose of treating an existing illness or injury, and the details of the diagnosis, substance and administration protocol must be recorded and supplied by the trainer to the Racing Authority. If the horse is not under the direct control of a trainer at any time in its career from the commencement of training to final retirement from racing, the owner is responsible for this notification to the Racing Authority. This system must be supervised by the Racing Authority's veterinarian(s).

b) A horse shall be ineligible to race until a minimum of six (6) months has elapsed after the administration of any of the substances specified in point four (4) above, and the Racing Authority must test to ensure that a horse treated therapeutically with any of these substances is free from the presence of such substances before racing.

c) A Racing Authority must record, within the details it holds of the horse in question, information which it has received on the administration to that horse of such substances under exceptional use for therapeutic purposes. This information must be included when providing details on the horse to a Horseracing Authority or Stud Book Authority in any country to which the horse travels (including within Racing Clearance Notifications), including in the case of permanent export of the horse.

d) The number of exceptional uses for therapeutic purposes and the details of the substances involved shall be notified to and reviewed by the International Federation annually.

STAFF ANALYSIS
DISCUSSION AND ACTION REGARDING THE REPORT ON THE UNIVERSITY OF CALIFORNIA, DAVIS'S CARDIOLOGY PROJECT, SCHEDULED TO COMMENCE AFTER THE CONCLUSION OF THE 2014 BREEDER'S CUP

Medication and Track Safety Committee Meeting
October 22, 2014

BACKGROUND

Business and Professions Code section 19580 provides that the Board shall adopt regulations to establish policies, guidelines, and penalties relating to equine medication in order to preserve and enhance the integrity of horse racing in the state. Dr. Joshua Stern, DVM, PhD, DACVIM, is an Assistant Professor at the UC Davis School of Veterinary Medicine, whose research is primarily focused on the study of inherited heart disease.

Dr. Stern is the principal investigator in a clinical trial that will evaluate the incidence of subclinical heart dysfunction within a thoroughbred horse population, changes that confer risk for sudden cardiac death, and identify an impact on race season performance in order to help design best screening practices. Identification of horses prior to symptomatic heart disease may reduce the risk of sudden cardiac death, a problem of increased frequency in thoroughbreds.

RECOMMENDATION

This item is presented for Committee discussion and action.
The Board's Equine Medical Director is prepared to make a presentation to the Committee.

Identification of asymptomatic heart disease in racing Thoroughbreds and impact on performance and risk for sudden cardiac death.

Investigators:
Joshua A Stern, DVM, PhD, ACVIM (Cardiology) (614) 390-1516; jstern@ucdavis.edu
Anita Varga, DVM, MS, ACVIM (Large Animal Internal Medicine)

Background of the Problem: Subtle evidence of heart disease may be clinically in apparent in high level athletes until evaluated after extreme exercise. Changes in blood parameters that indicate heart disease, cardiac arrhythmias and heart function as seen by cardiac ultrasound may all be indicative of subtle but significant heart dysfunction after work. This concept, termed exercise-induced cardiac fatigue has been documented in performance horses but never evaluated in racing thoroughbreds as a possible explanation for poor performance and even the incidence of sudden cardiac death.

Hypothesis: The investigators hypothesize that subclinical heart disease exists in racing Thoroughbreds and may explain poor racing performance and increase risk for sudden death. Furthermore, identification of this condition in racing Thoroughbreds may impact health screening recommendations and be used to support testing protocols to reduce the risk of sudden cardiac death.

Study Objectives:

- Characterize mechanical heart function, electrical heart function and biochemical markers of heart dysfunction prior to and immediately after high level racing in 30 Thoroughbred horses.

- Identify the incidence of subclinical heart dysfunction in this racing population, changes that confer risk for sudden cardiac death, and identify an impact on race season performance in order to help design best screening practices.

Overview of Experimental Approach: Thirty racing Thoroughbred horses from the Santa Anita Park race track will be evaluated by functional cardiac ultrasound (echocardiography), blood parameters that can indicate heart disease (cardiac troponin I), electrolyte parameters which may alter heart rhythm, and for the presence of heart rhythm disturbances by electrocardiogram. This investigation will be performed at the race site, one day prior to racing and immediately after racing (within 30-60 minutes). Changes from the pre to post race evaluations will be quantified and analyzed for significant differences. Blood values that indicate heart disease will be further evaluated 4hrs, and 24 hrs after racing as their pattern of release within the body is described in phases that make serial measurements critical to their interpretation. Results will be correlated to each individual horses racing performance for the season. Findings of significant markers of heart disease post-race will be reported as evidence of exercise induced cardiac fatigue. The most informative testing results will be used to derive recommendations for additional screening tests that may aid in evaluating horse performance, risk for heart disease and ultimately sudden cardiac death in the racing population.

Anticipated Benefits to the Equine Industry: Similar to human beings, Identification of a subset of racing Thoroughbreds with post-exercise identified heart dysfunction or injury would represent a group of equine athletes at the highest risk for sudden cardiac death. Identification of these horses prior to symptomatic heart disease may reduce the risk of sudden cardiac death in racing horses (a problem of increased frequency in Thoroughbreds). The findings will aid in development of best screening practices to identify at risk horses and help trainers, veterinarians and equine enthusiasts understand the role that subclinical heart disease may play in race performance.

Current Veterinary Clinical Trials

EXERCISE-INDUCED CARDIAC FATIGUE IN RACING THOROUGHBREDS

Background

- Subtle evidence of heart disease may be clinically in apparent in high-level athletes until evaluated after extreme exercise. Changes in blood parameters that indicate heart disease, cardiac arrhythmias and heart function as seen by cardiac ultrasound may all be indicative of subtle but significant heart dysfunction after work. This concept, termed exercise-induced cardiac fatigue has been documented in performance horses but never evaluated in racing thoroughbreds as a possible explanation for poor performance and even the incidence of sudden cardiac death. Therefore, we are evaluating the occurrence of post-exercise heart dysfunction or injury in Thoroughbreds to identify the incidence of subclinical heart dysfunction in this racing population, changes that confer risk for sudden cardiac death, and identify an impact on race season perforamance in order to help design best screening practices.

Participation Requirements

- Racing Thoroughbreds from the Santa Anita Park race track

Procedures

- A board certified cardiologist will evaluate your horse and perform an ultrasound and electrocardiogram 24 to 48 hours prior to racing and then 30 to 60 minutes after the race (NOTE: The echocardiography does not require sedation).
- We will also take a blood sample from the jugular vein (neck) of your horse at 4 different time points (24 hours prior to racing, 30 to 60 min, 4 and 24 hours post race) to test for specific blood parameters that may indicate heart disease (cardiac troponin I) and electrolyte parameters, which may alter heart rhythm.
- We will also follow up on the racing performance of your horse over the racing season.

Owner Responsibilities

- If you allow your horse to participate in this study, you will be responsible for allowing us to perform the evaluations in a timely fashion and it would be appreciated if the trainer or owner could hold the horse during the study.

Benefits

- There is no charge for you to allow your horse to participate in this clinical trial, as all costs associated with the study will be paid by the sponsor/department.
- Sudden cardiac death in racing horses is an ever-increasing problem among Thoroughbreds. Identification of heart conditions in these horses prior to symptomatic heart disease may reduce this risk. The findings from this trial will hopefully aid in development of best screening practices to identify at-risk horses and help trainers, veterinarians and equine enthusiasts understand the role that subclinical heart disease may play in race performance.

PRINCIPAL INVESTIGATOR

Dr. Josh Stern

CONTACT INFORMATION

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www.vetmed.ucdavis.edu/clinicaltrials/

UCDAVIS VETERINARY MEDICINE

UCDAVIS
VETERINARY MEDICINE

Protocol Number:

Owner Informed Consent Form

Title of clinical trial: Identification and impact of exercise-induced cardiac fatigue in racing Thoroughbreds: A pre and post race investigation into presence of exercise-induced systolic dysfunction, cardiac dysrhythmias and cardiac biomarker derangements

Investigator(s): Joshua A Stern, DVM, PhD, ACVIM (Cardiology), phone number 530-752-2475, Department: Medicine and Epidemiology, e-mail: jstern@ucdavis.edu
Anita Varga, DVM, MS, DACVIM (LAIM), phone number 530-752-0290, Department: Medicine and Epidemiology, e-mail: avarga@ucdavis.edu

Why is my horse being invited to take part in this clinical trial?
We invite your horse to take part in this clinical trial because we are evaluating the occurrence of post-exercise heart dysfunction or injury in Thoroughbreds. Identification of heart conditions in these horses prior to symptomatic heart disease may reduce the risk of sudden cardiac death in racing horses (a problem of increased frequency in Thoroughbreds).

Why is this clinical trial being done?
The findings of this study will aid in the development of the best screening practices to identify at risk horses and help trainers, veterinarians and equine enthusiasts understand the role that subclinical heart disease that may play in race performance.

If I choose to enroll my horse in this clinical trial, what will happen to my horse?
If you agree to let your horse participate in this study, the following will happen:

- A board certified cardiologist will evaluate your horse and an ultrasound of its heart and an electrocardiogram will be performed 24 to 48 hours prior to racing. A second echocardiographic and electrocardiographic examination will be performed 30 to 60 minutes after the race.
- The echocardiography will be performed in standing, non-sedated horses. We will apply some gel to the chest area underneath the elbow to be able to see the heart of the horses with a probe. The echocardiographic evaluation will take approximately 20 to 30 min per study. At the same time, we will place the electrodes for the electrocardiogram onto your horse's skin.
- We will also take 12 ml blood from the jugular vein (neck) of your horse at 4 different time points (24 hours prior to racing, 30 to 60 min, 4 and 24 hours post race). All blood samples will be tested for specific blood parameters that may indicate heart disease (cardiac troponin I) and electrolyte parameters, which may alter heart rhythm. Each blood collection will take approximately 3 minutes and can be performed at stall side.
- We will also follow up on the racing performance of your horse over the racing season. The results of this comprehensive pre and post race cardiovascular evaluation will be correlated to each individual horses racing performance for the season.
- The study will be performed at the Santa Anita Race track and horses can be evaluated at the stall side. No drugs will be given to your horse in this study and all data will stay confidential and the identity of the horse will not be shared to third parties.

What happens if I do not want to enroll my horse in this clinical trial?
Participation in any clinical trial is voluntary. If you decide not to participate in the study, your choice will not affect your horse's future medical care.

UCDAVIS VETERINARY MEDICINE

Protocol Number:

What happens if I choose to enroll my horse, but I change my mind later?
You can remove your horse from the study at any time and it will not be affect the medical care of your horse.Our study is purely observational in nature and does not intervene with racing performance. Please note that we will not remove any data from the trial database that has already been collected if you choose to remove your horse from the study.

If I choose to enroll my horse what are my responsibilities?
If you allow your horse to participate in this study, you will be responsible for allowing us to perform the evaluations in a timely fashion and it would be appreciated if the trainer or owner could hold the horse during the study.

Will being in this trial help my horse or other horses in any way?
We cannot promise any benefits to your horse or other animals from your taking part in this clinical trial; however, possible benefits include that we can evaluate risk factors for cardiac disease in equine athletes and might be able to correlate findings of the study with increased incidence of cardiac injury. This may help to determine future horse performance and health. No financial compensation is provided to study participants.

Could this trial hurt my horse?
The echocardiography and electrocardiography are non-invasive procedures which will not lead to any discomfort of your horse.

An experienced veterinarian will take the blood from your horse and the procedure will only lead to minor discomfort and will not produce any significant pain response.

What happens to the information collected for the clinical trial?
All client and animal details, and information obtained from the study will be considered confidential and will be used for research purposes. We will limit the use and/or disclosure of your information or that of your horse to people who have a need to review this information. All data and remaining samples will be stored in the principal investigators laboratory. All data and specimens obtained are considered property of the University of California and may be used in future research investigations. The specimens could lead to discoveries or inventions that may be of value to the University of California or to other organizations. Under state law, you do not have any right to money or other compensation stemming from products that may be developed from the specimens.

We intend to publish the results of this research. However, we will keep your name, the name of your horse, and other identifying information confidential.

Can I be removed from the research without my OK?
The investigators reserve the right to remove any participant from the study at any time.

What about cost?
There is no charge for you to allow your horse to participate in this clinical trial. All costs associated with the study will be paid by the sponsor/department.

Who can I talk to if I have questions?
If you have questions, concerns, complaints, or think the study has negatively affected your horse please contact the investigator (Joshua Stern; jstern@ucdavis.edu; 530-752-2475). This research has

UCDAVIS
VETERINARY MEDICINE

Protocol Number:

been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Clinical Trials Review Board (CTRB). You may talk to 530-752-2364 or iacuc-staff@ucdavis.edu if you cannot reach the investigator.

By signing below I agree to permit my horse

_____(insert name)

to participate in this clinical study and undergo the procedures described to me above.

By signing below, I understand the statements in this informed consent document and that a signed and dated copy of the consent form will be given to me.

_____ _____

Signature of Owner Date

_____

Printed Name of Owner

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent