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MEDICATION, DOPING AND THE LEVEL PLAYING FIELD

Moderator/Speaker:
Dr. Scot Waterman, Executive Director, Racing Medication & Testing Consortium

Speakers:
Dr. Rick Arthur, Equine Medical Director, California Horse Racing Board
Dr. Lawrence Soma, Professor, Veterinary Medicine, University of Pennsylvania
Dr. Scott Stanley, Associate Professor, University of California, Davis

MS. WENDY DAVIS: We're ready to get started. First of all, I'd like to thank the American Quarter Horse Association for sponsorship of this panel. Certainly without our sponsors we couldn't bring the Symposium to you, and we do appreciate everything they do for us.

This afternoon we're starting out with "Medication, Doping and the Level Playing Field." I think this will be an interesting panel. We have four doctors up here but I know they will be able to keep it at a level I can understand and the rest of us can understand out there.

Today leading us off will be Dr. Scot Waterman. He will be speaking and moderating this panel. I'm pleased to say that Scot is a graduate of the Race Track Industry Program. He is currently executive director of the Racing Medication and Testing Consortium. He has been involved with medication and drug he testing since he left us as a student at the Race Track Program. So he will be our moderator for the afternoon with this esteemed panel.

Without further ado I want to remind you of one thing, and I was just talking about the panelists about this, they are up here in bright light and you see them kind of squinting. It is a bright light of here, but the reason all these lights are on is because we are taping all these panel session. We have DVDs available of all the panel sessions and they are wonderful quality. If you would like to see one there's one running outside of the registration area. If there are panel sessions you attended and you would like copies of, please let us know. If there are some you missed because we had concurrent sessions going, this is a great way to take it all

in. We have order forms available now or you can e-mail us when you get home if you find there's something you would like a copy of. I apologize for the bright lights, Scot says it's kind of like being interrogated, I don't know how he knows that, but anyway I will now turn it over to our panelists and thanks very much Scot for leading us off.

DR. SCOT WATERMAN: Thanks Wendy. I didn't sign the release form so on the DVD can you get one of those green spots to blank out my face?

Thanks to everybody for attending. This is obviously the garden spot of the Symposium agenda being on Thursday afternoon. Getaway day, so I'm surprised to see this many people in the audience, and I truly appreciate your attendance, particularly for those of us that are fortunate enough to go back to the Midwest or East Coast where it's probably about 20 degrees. In my hometown, they had snow this morning. So giving up the beautiful 70-degree weather out there to sit inside and listen to us talk about medication issues, I appreciate it.

The lineup, Dr. Scott Stanley will be doing lead off, Dr. Larry Soma will be going second. Dr. Rick Arthur is third and I will be in the clean-up spot. Meaning I will clean up whatever time is left.

Without further ado we have kind of an action-packed agenda here, I will turn the microphone over to Dr. Scott Stanley who will be talking about a study that was done last year on flunixin. Dr. Stanley earned his bachelor's degree at the University of Kentucky in animal science and a PhD in toxicology and equine pharmacology. He has more than 70 peer-reviewed papers published in scientific journals.

Dr. Stanley is a recognized leader in the field of equine drug testing, his other achievements include managing the Maddy Equine Analytical Chemistry Laboratory at the University of California Davis in the School of Veterinary Medicine where he served as associate professor of clinical diagnostic equine analytical chemistry for more than nine years. Wow, that's a long one.

Scott, take it away.

DR. SCOTT STANLEY: Thank you, Scot, for that kind introduction, and I want to thank the Race Track Industry Program for inviting me to talk on the work we did on flunixin. And all of you for coming today. As Scot said, it's a good turnout for Thursday afternoon. What my presentation is going to be discussing is the work we did on flunixin following the implementation in California of their rule from the RMTC.

I don't sing, I don't dance, so this is pretty much all you get — here we go — I was in trouble without the slides.

The issue here is we're trying to establish threshold and have therapeutic medication be monitored so practitioners and folks at the racetrack can use them

appropriately, provide withdrawal times and not run into a post-race finding.

So the title of this work is on therapeutic medications for thoroughbreds in California, we're looking at the clearance after intravenous administration of flunixin, which most of you know is Banamine, in exercised horses.

My co-investigators, Dr. Knych, Dr. Sams, Dr. Arthur and myself worked together on this project. What the efforts of our lab are focused for is not only the area of drug detection and identification and confirmation, we do a lot of work on the pharmacokinetics and clearance of therapeutic medications and also some prohibited substances. We also have been working recently on proteomics detection in erythropoietin and growth hormone.

And we are a new facility at U.C. Davis. It's only about six years old. So it accommodates us with some new analytical tools and these tools here, in addition to that, what we've done is we've incorporated the use of exercised horses into our research, and I'll show you the work that was done on our horses as well as the work and study that was done at the racetrack in Southern California on thoroughbreds that were in training.

The mass spectrometers I show here are just to demonstrate that we have some really nice technology and we are an accredited laboratory.

What the genesis of this project was from the model rules put forward by the RMTC in 2005. California was quick to adopt those model rules, and those include the new regulations or new permitted medication use of furosemide allowing horses to have a certain specific gravity level and plasma level of furosemide when they race. In addition to that they had, model rules for non-steroidal anti-inflammatories. The phenylbutazone rule was consistent with the California rule for the last number of years for five micrograms per mil.

In addition to that they had a ketoprophen concentration, California permitted ketoprophen for a number of years. The level was slightly lower than what we had, but we had little trouble in transitioning. You notice in each one of these cases the recommendation is for a dose that will be equally used without problem equivalent to a therapeutic single dose application from the manufacturer's recommendation.

Flunixin was the last one that we have on the list that was approved. The plasma level put forward on flunixin was 20 nanograms per mil. It was quite a reduction from what we had in California previously.

All that sounded well and good. California was quick to adopt those. They brought them on in late May of 2005. But we did run into a problem. We ran into a problem because of this rule. This is the rule we changed in 2005. It decreased the level of flunixin and ketoprophen. Flunixin was frequently used. Banamine is often used by practitioners on the backside, and it was administered to a number of horses per the recommended threshold or dose recommendation through the

RMTC.

Well, that was fine except for the fact that once we instituted that we had a 60-day phase-in period. During that 60-day phase-in period we had 70 flunixin positives that we reported back, which was more that we anticipated, and we talked to some practitioners that had used the recommended 1.1 milligram per kilogram dose IV 24 hours.

So we undertook a study to investigate why it happened in California and whether the threshold was something we needed to change for California or whether we can institute that. We also wanted to investigate a secondary plasma level for trainers that want to use or train a horse on Banamine, withdraw at 48 hours and still clear in a post-race test.

Flunixin is a non-steroidal anti-inflammatory, it's mostly used for inflammatory issues, horses that come up a little bit sore after they race. It's been approved by the FDA for a number of years and there is not only the Banamine product but other generic formulations of that are available for horsemen. And it was previously prohibited by most of the jurisdictions but was allowed in California for a number of years.

The regulatory limits for that range from 20 to 1,000 nanograms per mil depending on the jurisdiction that did authorize that. It's a fairly small molecule, it's rapidly absorbed, well distributed throughout the body, has a short half-life, highly protein bound, which means we can easily identify the bound versus unbound fraction in the plasma.

What we chose to do is administer it at the 1.1 milligram per kilogram label dose in a single administration, collect samples periodically through the 48-hour period so we could identify that. The reason that flunixin is of concern and regulators have investigated that is because of this data here. You can see on the top curve it's actually phenylbutazone, the bottom is flunixin.

This represents a fairly standard therapeutic dose, you can see the flunixin is clearing more rapidly than the phenylbutzone. The phenylbutazone levels in plasma are well detectible out to 24 hours. But flunixin was not detectible by many jurisdictions after about 12 hours, until recent technology. The other concern about flunixin is the drug is eliminated about 12 hours but it still has some analgesic properties.

This is a carpus inflammation model they used to evaluate the efficacy for flunixin. What you are seeing here is the horse was given flunixin, and its ability or stride length increased over the time when the therapeutic concentrations were the highest and came back to normal around 24 hours. The regulators were concerned it might be abused for pain-killing properties in that 24-hour window

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In addition to that the data of pharmacokinetics and pharmacodynamics of flunixin will show this one milligram per kilogram dose here provides good analgesic

or stride length out to 16 to 20 hours. Doubling that dose only provides it slightly further out to about 24 hours. This is important as well. A half dose provided very little analgesic property, very little improvement in stride length.

The data available was quite sparse for 500 milligram IV dose. The label claim and what's done by the racetrack are not always consistent. The label claim is 1.1 million gram per kilogram. At the racetrack we found 500 milligrams is the standard dose per horse regardless of size. We wanted to investigate what they were doing at the racetrack.

The research we did was based on the 500-milligram dose. This is the only published data on 500 milligrams and it was only done on four horses. This data here shows the oral absorption and elimination of plasma concentrations of flunixin, and you can see here the oral dose could be very problematic out to past 24 to 48 hours on these horses. Again, we wanted to investigate the recommended RMTC IV dose of 500 milligrams. In our phased research we used six 3-year-old thoroughbreds that we had in training on the treadmill. We inserted catheters in right and left jugular vein, one to administer the drug and one to draw samples.

We took samples periodically out through 48 hours. Simultaneously with that, Dr. Arthur, in conjunction with our research, took 12 thoroughbred horses in California ranging in ages from two to five and gave 500 milligrams IV to those horses. Because they were in training, they were racehorses, we could not insert the catheters to get the same blood time points so we got the six-, 24- and 48-hour time points on those horses.

What we found is after we harvested the samples, we collected them, we did pharmacokinetic analysis on the six research horses that we did. And used a non-compartmental analysis to determine the bioequivalence of that particular drug. What we found was it was consistent with published data, meaning pharmacokinetics is the way the drug is eliminated from the horse. We wanted to make sure the 500-milligram dose that we gave was consistent with published data. And we were able to demonstrate or verify the traditional studies were consistent with the data we received. In addition to that we wanted to plot a time course of the multiple horses that we used, and this is the time course from the single 500-milligram dose IV.

What we have are the earlier time points we were able to obtain from all 18 horses, 24-hour concentrations and a 48-hour concentration. We did a comparison with the laboratory at Ohio State of those concentrations that we had and we found in almost every case we had comparable concentrations in the initial analysis of the 18 horses. From that data we were able to discern that flunixin in the plasma following 24 hours had an average concentration of 9.3 nanograms. We actually found the standard deviation was high, at 6.2 nanograms per mil. What we found in that data, two of the horses that were dosed had concentrations at 20 nanograms per mil. It told us the concentration was not an adequate threshold for us to use in California because we ourselves were able to give the drug as 500 milligrams and resulted in two hours horses being violations of the 18.

So we did a population analysis statistical application, we targeted 99 percent of the population and found that the range for those horses would be between zero and 48 nanograms per mil.

We wanted to get the most out of this, so we did a separate statistical analysis as well. The separate statistical analysis was based on a program where 20 horses were used. It was a more sophisticated data analysis. I won't bore you with the details, but basically we're looking at 99 percent confidence interval for 99 percentile of those distributed time points.

What we found from the analysis of the horses just used in Southern California, Santa Anita and Hollywood Park, ended up a plasma concentration threshold of about 47.4.

And the 31 horses that we have done in total, we had a 40.1 nanograms per mil concentration when the statistical application was applied to the data that was generated at U.C. Davis.

What that told us was we needed a slightly higher threshold than had been put forward by the RMTC for California. The data that I wanted show you here, again, is repeated from earlier, but the critical argument was, what if somebody gives a smaller dose of the drug inside of that 24 hours. Wouldn't it be problematic? Based on the pharmacokinetics and pharmacodynamics data, you can see here half doses, almost no change in the therapeutic stride length of the horses induced for inflammatory carpus model. So you don't get good efficacy in therapeutic applications unless you give those doses.

For the nay-sayers out there that say somebody can always give a smaller dose, they can and they will be capable of doing that, but are they doing any pharmacological change? It seems unlikely from this data here.

To compare that, to explain possibly why we got a different result with our exercised horses and research horses at U.C. Davis, I showed the information done from three different model approaches. European model, sedentary horse model and our racehorse or exercise horse model. The way those are set up, the European model is based on literature, not on current information or current study. And pulls from that a plasma effective dose and uses a very high safety factor.

And from that data in Europe, they actually predict a plasma threshold at 1.5 parts per billion, or nanograms per mil, which is quite a bit away from a therapeutic application of 500 milligrams at 24 hours. Their recommend is a 5-day — 4-day withdrawal period for flunixin. So if we used that we wouldn't know how many inadvertent positives we would run into. In addition to that a sedentary horse model used for most of the data that was generated at the 20 nanogram per mil level, only used a limited number of horses, the elimination of the drug appears to be quite different in exercise versus non-exercised horses. The threshold predicted was 20 nanograms per mil, and we feel we would have a number of inadvertent

positives if we continued with that threshold.

Our final project or model was the racehorse model. The use of 20 horses is recommended, we have 31 in total now. We use a validated analytical method. We use a measure of uncertainty calculations, and ultimately we established, for putting forward in California, threshold concentration of 50 nanograms per mil for flunixin. We feel that one will suit California appropriately, result in no inadvertent positives and effectively regulate the flunixin in California.

The reason I believe I was asked to go over this was to show some folks that may not know it how complex it is to through the process of establishing a therapeutic level for therapeutic medication. It's not a simple process, it takes a lot of laboratory and analytical efforts.

And if it wasn't for the people I listed here as acknowledgement, Dick Mandella was very instrumental. He even allowed us to use a horse called Rock Hard Ten on this project. So obviously he felt it was important to get established, as well as the people at the University of California Davis that I work with.

So that's a quick overview of what it takes to establish a therapeutic plasma level for medication that's permitted to be used.

DR. WATERMAN: I think what we'll do since we have a fair number of presentations, if you've got a burning question in your mind, write it down and we'll do questions once all the presentations are over with. Thank you very much, Scott.

Dr. Larry Soma is our next speaker. Dr. Soma's primary areas of research are clinical pharmacology, exercise physiology and anesthesiology. Dr. Soma is a senior investigator for the Pennsylvania Racing Commission's Research Contract.

Dr. Soma works with the Association of Racing Commissioners International in the field of drug testing standards and practices. Since 1991, Dr. Soma participated on a drug classification subcommittee. He also worked in a testing integrity partnership quality assurance program since 1995. Dr. Soma will be talking about the new method developed by the Pennsylvania Equine Toxicology and Research Lab for erythropoietin.

Dr. Soma.

DR. LAWRENCE SOMA: Thank you very much. I'm reporting on the work of a number of colleagues that you see — oops, I did it — a number of colleagues you see here. Dr. Guan, Dr. Birks, Dr. Uboh, Dr. Chin—this is one of our students that did some summer work—Dr. Mitchell, and I'm at the tail end doing some reporting.

What I'd like to talk about is EPO, erythropoietin and protein-based drugs. So these are drugs, which we'll be confronted with more often, and it behooves us to learn how to tackle some of these newer compounds.

What I'm going to show you first is the difference that we're dealing with, small versus large molecules. This is one of a drug found in black ice, it's an erythroid, and this is oxyglobin. This is a — naked red blood cells. This is a compound manufactured from the red blood cells of the bovine, they strip off the outer coating and it will carry oxygen and has been used to carry oxygen when given to a human, dogs or the horse. It was supposed to be or is hopefully a blood substitute. I want you to look at the molecular weight, this is 65,000 Daltons. This is EPO, you can compare EPO with dexamethasone, which you all know. This is a little bit smaller, but still 3,400 Daltons. It's a very large molecule. It's a very complex protein. It's a very complex protein, and I'll go through the method that has be to be employed to break the molecule up, identify the specific peptides.

Let's just briefly go over what a protein or protein-based drug is. Proteins are the body functional machinery, and made up according to your DNA, have a DNA blueprint. The protein carries out the function of this blueprint.

All naturally occurring proteins are made up of approximately 20 amino acids. And proteins, you can almost consider them the regulatory proteins of bodily function.

So if I have a disease process or a body function is being conducted now, a protein is being secreted and that protein is doing its function. In a disease situation, proteins can be a biomarker for a specific disease or metabolic disorder. For example, if we had a biomarker for laminitis and we could identify it by a marker before the laminitis occurred, we could really help a lot of horses. Unfortunately, at the current time they believe there are some materials being released that go to the hoof that produce the disorder but they have not been identified. That would be a biomarker.

A deficiency can result, a deficiency in a protein can result in a metabolic disorder. If you are deficient in a specific protein you may have a metabolic disorder. And now protein-based drugs are substitutes for that particular specific protein.

So recombinant drugs are replicates of a naturally occurring protein that's been made in a jar basically.

And the prime example of this is recombinant human EPO and the second generation compound is darbepoetin, and I will show the difference between these two compounds.

Forms of EPO. This is the protein base, this is the protein base, and erythropoietin is a glycoprotein. That is, you have a protein base and you add some sugars to it. And the EPO has three of these sugars and salic acid here. The only difference between EPO and darbepoetin is the addition of these two more sugar compounds or glycoprotein. So this is a five-link glycoprotein and this is a three-linked glycoprotein, that's the only basic difference between the two.

Now, the additional carbohydrates do a number of things.

Number one, it makes the drug long acting. The half-life of EPO is six to eight hours, where the half-life of darbepoetin is three times that. What I mean by the half-life, if you have a specific concentration, half is gone in one hour. The additional carbohydrates create this longer half-life. They remain in the bloodstream for a longer period of time and the half-life. Darbepoetin is three times the half-life of EPO, and the consequence with darbepoetin is in the plasma longer it has a—acts for a longer period of time so you have to give fewer doses, but we have the opportunity to find it because it lasts in plasma a longer period of time

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Now, the primary effect of EPO, it's a regulator of mammalian red blood cells, whether human dog or cat or horse, it regulates the production of red blood cells. As we speak here, I am probably creating more naturally occurring EPO because I'm at a higher altitude and the stimulus is the reduction in oxygen tension not only in the plasma but also in tissues. You go from sea level to higher ground, higher altitude, you will begin to secrete EPO, it's a naturally occurring process.

Now, EPO is produced in the kidney, the bulk is produced in the kidney, some in the liver, goes to the bone marrow and in the bone marrow it begins to stimulate red blood cell production.

Or, you can simply take the recombinant human product and inject it, it will go to the bone marrow and stimulate red blood cell production.

Man versus horse, in the humans, for a normal athlete, not diseased, you can see the change in the red blood cell and hematocrit rise, and the athlete that wishes to cheat, they monitor the hematocrit closely. The hematocrit is basically how thick the relationship between the red blood cell plasma. They follow it and when they get to 55 they stop. In the human anti-doping, if your hematocrit is over 55, you are suspect for the administration of EPO. There's a lot of other differences. The human produces immature red blood cells you can see floating around and produces an enzyme they can detect.

In the horse none of this happens. It's difficult to discern an increase in hematocrit. The horse does not produce any immature red blood cells and the enzyme is not found in the equine. If you give EPO to the horse you can't tell the difference by looking at any physiological parameters that we know of, by looking at hematocrit or red blood cell count. The horse is unique, it has a huge spleen and sequesters a lot of red blood cells in the spleen.

Uses, it has legitimate uses in man dogs and cats. It's treated in humans, if you had renal disease and don't produce EPO they give you the recombinant. It's been used in dogs and cats, also because they do renal transplants and cats and dogs do have renal disease and it's been used effectively in those cases.

As far as I know there's no known medical use for EPO in the horse. I

haven't seen a renal transplant in a horse recently, and cancer treatment in a horse is very rare. EPO has been misused, as you know. It's a performance-enhancing drug used by athletes. It's been banned by all sports, all organizations, and EPO administration can be very dangerous to healthy humans, and deaths have been reported in the horse. And I will discuss that these last two aspects later in the discussion.

The mechanism for deaths in the horse and human are different. Or apparently different.

Remember what the EPO molecule looks like, you will have a quiz at the end of the session, and see if we can compare hemoglobin with EPO.

Here we go.

This is the amino acid sequence of EPO, all right? And you can see that this is the top one, human EPO, and the bottom is naturally occurring equine EPO, and they are approximately 80 percent the same. Approximately 20 percent difference between the human EPO and the equine EPO. The sugars or carbohydrates are attached here, here, and here. How do we—if you look at right here in the beginning, you can see there's differences in the amino acids in this area, as compared to here, that the horse is starting out with APPR and this is PPPR. I want you to focus here, here and here.

And later we'll show you chromatograms where we can distinguish that in the equine EPO we can see and identify those specific peptides. Again, this is the sequence, the top is the human EPO, the bottom is — next one is darbepoetin and blue is equine.

What you're going to see next is trypsin digestion. Instead of taking this, what most chemists do, take the small molecules, put in some kind of solvent and extract it, go through a column. We have to remove it from plasma, and I'll discuss it later. Then break the molecule apart and try to identify the pieces that are different between the human EPO and the equine EPO or any other protein, human X protein versus equine X protein. That's the basic background and how to distinguish one of these protein compounds from another one.

Now, what trypsin does, it's predictable on where it breaks up the molecules. It breaks it up at the R and K location. Here's an R location, this is a K, you can anticipate that the molecules are broken up in these specific locations, each one has approximately 161 amino acids and you can see general similarity between these two proteins.

What we're trying to do is find the dissimilar peptides to distinguish one protein from another. We happen to be using trypsin here, but there are other compounds that will break the molecule and different locations and you might have to go through by trial and error to determine which ones will give you the most reliable separation of proteins.

This is a chromatogram, I'm showing this, they don't even let me touch this equipment. But I can recognize this is a chromatogram. Who I'm showing is this section here — and this section here, if you go back I had these sections identified in the two sequences, this is the human EPO. Simply because we identified this peptide and this peptide we've been able to chromatograph these two peptides. Now, there's a peptide right here — I have a fat finger. There's a peptide right here at T11, which you can see most of the time. There's a couple here, but the reason they are not being used, one is superimposed on the other, and over here we have a T4 segment and a T14 segment.

So there are at least two you can identify all the time and possibly a third one you can identify most of the time. And the difference is usually the concentration and how the protein breaks up. That basically is the basic way that you try to identify or separate and say this protein is human, or dog, or horse.

In this case this happens to be darbepoetin. As far as the two compounds right now, we have not been able to distinguish the difference between the two because they are identical except for the two additional sugars.

The way we get it out of plasma, I'll go through it quickly: EPO antibodies are linked to magnetic beads. The beads are incubated with equine plasma for 24 hours. The beads are washed, the darbepoetin is exchanged with a buffer and you—this is—we do an exchange and the final is to trypsinize and digest the protein and put it on the liquid chromatography LCMS system and we use the most sensitive instrument for the test. An LTQ linear LCMS. Remember this is a foreign protein and foreign proteins produce antibodies in an animal. I guess we're just about done. Five minutes, okay.

Remember, recombinant EPO is not a horse protein, it's human. Actions of foreign proteins when you inject them into another animal is the production of antibodies against that protein.

If you inject recombinant EPO in the equine, if you inject in dog or cat, long-term, the red blood cell counts starts to decrease and the animal becomes anemic. When they use it therapeutically in small animals, dogs and cats, they give it for two or three weeks in a row, then reduce or stop the administration until the red blood cell count begins to come back and then start the administration. If they had cat EPO or dog, they would not have to do that.

There's been reports of deaths in horses, a group from Ohio State, and the common denominator is the trainer had admitted he had given multiple doses of EPO in horses. It doesn't occur in every horse, but it seems in high doses it suppresses the release of naturally occurring EPO and produces anemia of the equine. That seems to be the mechanism.

Antibodies can be measured in a horse, and I'll show you a graph of our method for measuring antibodies where you can see a consistent increase in

antibodies following multiple administrations of EPO.

So this is a double sandwich technique that we have, and you can see this is the baseline, this is dose one, and a week later, you can see the antibodies beginning to rise. This is dose five, dose eight, which is the last dose, rises more. And then stabilize. We went to 50 days and the antibody level was still high. We don't have this horse on campus anymore so we can't check if the antibody levels have decreased.

But there have been certainly horses out there where they measure antibody levels for a year. It's the same as you being vaccinated for you flu. You get a vaccine, you get a reaction, you get antibodies against the flu vaccine. You would hope that the antibodies would remain at least in your body for the flu season. Apparently the antibodies are not dropping as fast as we thought they were dropping.

This is, we measured antibodies in several equine populations and there are 338 thoroughbreds. And we had 11 animals with a titer above 1-2. What that means is dilute the plasma one to two and you can still find evidence of antibodies. That's about two percent. We had 19 percent in standardbreds, we went to a local farm, this happens to be an area, his wife has ponies for riding therapy, we sampled 50 horses. These horses had never seen the racetrack, never seen EPO, the closest they were was about six miles from a racetrack. This is the baseline. Zero antibodies in the non-racing animals. We have 16 retired thoroughbreds on campus, two had antibodies, and we never administered antibodies in the horses. We injected three horses so far and all of these three horses developed antibodies. So they do develop antibodies, whether they are detrimental to that horse is probably dependent on how often and how frequent, I think. I think that's the mechanism. Because the trainers complained about horses crashing, but as far as bona fide deaths, the report from the group of Ohio State is indicating multiple doses of EPO can be detrimental to the horse.

Okay. Just, this is drug administration of 400,000 units. Eight thousand units and 16,000 units and these are the times we can find it or, this is ELISA indicating that the EPO is there. This portion, 48 and 24 hours, is as far out as we can confirm it using our technique. As I said, one of the attributes of darbepoetin, you only have to give it once a week. Human therapy, once a week to 10 days, and we were able to confirm that is 25 milligrams of darbepoetin, we can confirm it to 144 hours. We have not done a lot of the administrations. I think we're just starting to get a handle on protein-based drugs and this is our first, our second protein-based drug we have been able to break apart identify and confirm.

In the near future we will have a handle on how to approach these protein-based compounds. Thank you very much.

DR. WATERMAN: Thank you, Larry.

Next speaker is Dr. Rick Arthur. Dr. Arthur is the newly minted equine medical director for the California Horse Racing Board, and Dr. Arthur has been an

active participant on the Racing Medication and Testing Consortium board pretty much from day one. Rick is going to be talking about the RMTC deliberations on the regulation of anabolic steroids.

DR. RICK ARTHUR: First of all, I'd like to congratulate the researchers at Pennsylvania and Dr. Uboh and Dr. Soma for getting this work on EPO moving along. We're going to actually start out-of-competition testing in California, which is the only way we're going to be able to identify these horses in the future.

Moving on to anabolic steroids, we're the only major racing jurisdiction in the world that permits anabolic steroids. And the RMTC has looked at this issue for well over a year. It's been in discussion from the very beginning of our organization, and we're making the recommendation to the racing industry that the stakeholders sit down develop a consensus that anabolic steroids should not be permitted in horse racing. We've looked at this problem very carefully and we are absolutely confident it can be done, done effectively, efficiently and it's well overdue.

Certainly all of us should recognize that Congressman Whitfield's bill that wants to give the jockeys all the money also has a provision in it that prohibits anabolic steroids in horse racing. Even though that bill is not going anywhere, I don't think we need somebody to push us along to take the leadership to get this done. This may move along even without any further participation, I know the pharmacology group that deals with the RCI is going to make the recommendation that all anabolic steroids with the exception of testosterone, nandrolone, boldenone and stanozolol be classified as RCI class three drugs.

The ones that are listed there are the ones most commonly used, either FDA-approved or have been in common use for a long period of time. And this can be done as early as the RCI wants to move on this, and hopefully as early as April 2007. It just takes a side issue off the table.

Testosterone, nandrolone, boldenone and stanozolol would ultimately be reclassified sometime at agreed-upon time in the future. Hopefully no later than 2008; conceivably it could be done sometime in 2007. If the industry can get together and agree upon this.

Withdrawal time recommendations can be established for testosterone, boldenone, stanozolol, nandrolone by either direct research or a voluntary pre-entry anabolic steroid test, basically allowing the horseman to help us develop withdrawal times, and this would be a fairly reasonable and inexpensive way to do this particular program. We're probably looking at withdrawal times around 60-plus days for all but stanozolol, which would probably be a little bit shorter—has to do with the chemical structure of the drugs. LCMS capability will be necessary to effectively and efficiently regulate anabolic steroids.

Urine is the preferred testing medium, but the ability to test in the blood has already been demonstrated and can be expected to improve in the future. That information or ability to do that transferred to other laboratories. The laboratory

costs of regulating anabolic steroids in states using LCMS will be negligible. And frankly, if you are not using LCMS you are not on the A team and you should really think about making sure your lab has the technology to do this, and this may be a way to help improve the quality of labs around the country.

Testosterone, nandrolone, and boldenone, are endogenous to intact males. But threshold levels for nandrolone and boldenone have been established for intact males internationally because they have been regulating anabolic steroids for decades now. Testosterone in intact males is not regulated and there's some physiological and pharmacological reasons why this is not necessary

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One change would have to take place, and the samples would have to be differentiated as intact males, geldings, and fillies or females, because geldings and females do not have nandrolone and boldenone naturally in their systems.

The ability to test for anabolic steroids in the blood provides the opportunity to take the technology and principle to sales. We certainly have a mechanism within horse racing to regulate drugs. They really don't in many jurisdictions where there's racing, but our feeling is that the marketplace will eventually demand this and the sales companies will handle this on their own.

The reality of it is, horse racing has the most stringent drug testing program in any professional sport with one exception, and that exception is anabolic steroids. I don't think—the only reason we have ducked this issue so far, the public doesn't know that anabolic steroids are used so commonly in horse racing, and they are commonly used.

And I think it is about time where the industry gets together shows leadership and gets this done, and it can be done very quickly and very effectively, and the RMTC is recommending the industry do this.

So anyway, hopefully we can move forward with this and bring American racing on the same level as Europe, Japan, south Asia, and the other major racing jurisdictions in the world which have taken this step a long time ago. Thank you.

DR. WATERMAN: I feel no need to introduce myself. If you want to know about my background, turn to page 74 of the speaker bio and you can read it for yourself. Basically, we can get the right PowerPoint presentation up there.

I'm Dr. Scot Waterman, I'm executive director of the Racing Medication and Testing Consortium and I'm going to talk about one of our main projects in 2007 working on developing withdrawal times, uniform withdrawal times that can be disseminated out to the racing jurisdictions to build a system somewhat comparable to what they have in Canada currently.

We sort of looked at this two different ways. One path takes more time than the other. I'll briefly go over where our projects are taking us in 2007. The first path is the effort towards uniformity.

Essentially, we're working with a list of 47 therapeutic medications that were identified as most essential by a committee of practicing veterinarians that we put together back in the early days of the Consortium. What we're focusing on is trying to determine concentrations of drug in blood that will support a particular withdrawal time. This may or may not be possible. And unfortunately, a lot of the existing scientific literature doesn't give us much help. Primarily because most of these studies were done a long time ago when the technology wasn't as good as we have now and the focus was on urine rather than blood. So we don't have the ability to pick through the scientific archives and come up with a number. And certainly I think Dr. Stanley's presentation shows you how you can make a wrong decision based on a small sample size. That's the other issue, a lot of the existing academic work is based on four-, five-, six-, seven-horse studies.

What we have done is taken the list of 47 medications and prioritized them. It would be too difficult to try to deal with all of them at once, and frankly, we don't have enough money to deal with all of them at once. Our idea was to try to prioritize them based on the number of positives that were called around the country; the more positives, the more problematic that medication was for jurisdictions to deal with it and therefore it was a higher priority for us to try to develop some uniformity.

We also use surveys actually one done by the University of Arizona that asked practitioners what were the most important drugs to their practice. We try to combine those two bits of data to determine what drugs we would prioritize as the first subset of drugs we wanted to deal with. And you can see on the slide: Lidocaine, mepivacaine, glycopyrrolate, pyrilamine, acepromazine, detomidine, methocarbonol, butorphenol were the drugs that made the first cut.

University of Pennsylvania administered each one of those drugs at a label dose, or practitioner's dose if a label dose was not available, to a single horse at the University of Pennsylvania. Samples were collected over a period of time and those samples were sent to two laboratories. Each drug was sent to two different laboratories, and that means each one of the laboratories on the slide received two different drugs. The work is being done by the University of Pennsylvania, Iowa State, University of Florida and University of California Davis.

The pilot study, which is what we're calling the small horse administration study, is complete and what we are hoping is that the data and what we learned about the drug in terms of concentrations in blood will actually help us establish a much larger-scale study using 20 horses that we can do in a very directed fashion. Rather than starting off without knowing anything about the drugs with a 20 horse study and potentially having to repeat the process all the over again if we didn't find out what we needed to know.

That was the idea behind doing the pilot study first. Now we move on too much larger population studies. We will be using the 20-horse groups wherever we can find them basically, academic institutions, the University of Florida, for

instance, has a 20-horse stable, but we are talking about Kentucky Equine Drug Council, part of the Kentucky Horse Racing Authority to establish a 20- to 25-horse herd in the State of Kentucky that will be kept in race training to try to mimic the racehorse environment as much as possible and do the administration studies on that population trying to remove at least one variable we oftentimes face in the published studies.

The second path that we've taken, obviously you look at that and the amount of work that went into Dr. Stanley's project, you can get some sense that these things don't happen quickly. Research is sometimes slow moving and there's very little you can do about that. There's so many things you are trying to control to make a research project publishable you can't make it move any faster than it wants to. The idea was, while we're working on the research aspect of the project we would try to start collecting withdrawal time information from around the country. Be it commissioned, sanctioned withdrawal time given out by commissions to horsemen and veterinarians, down to even getting anecdotal information from practicing veterinarians. Our goal was to collect as much information from as many jurisdictions as possible and put it up on our Web site so it could be searchable.

And just to get an idea of how the Web site is going to work, the first page you'll have to enter a name a license type, and what state you are primarily licensed in. Click on the "I agree," and the next page is "hold harmless" information. Essentially it's saying we're giving the information to you but if it results in a positive test, it's not our fault. We based this language sort of loosely on the hold harmless language in the Canadian guidebook. They have similar language in place, basically stating this is information only and that we suggest in individual states they still contact the commission or the state vet in order to verify this information is still accurate.

One of the things we face, at least until we are uniform, is testing methodologies can change. So we want to ensure that people are not using this information as gospel, and we're actually making them agree to this language before they can access the site with the withdrawal times.

Basically, this is how the Web site will work. This is an actual example, you can search by jurisdiction and medication. This example is State of Kentucky, acepromazine, you hit the submit request button. Acepromazine, there's no specified dose or route in Kentucky but the withdrawal time is suggested at five days for this drug. Again, it's not uniformity, it's not perfect, but we felt like there were situations, particularly this summer there was a case of a horse that shipped up to run in a stakes race in Illinois that tested positive for isoxoprene. Post-fact they learned Illinois recommends a three-week withdrawal time and were coming from a state where the withdrawal time was much less than that. Those are the types of situations we feel we can avoid just by collecting this information and making it accessible to stakeholders.

Time lines, the Web site database will go live on January 1, 2007. And it's

www.rmtcnet.com. We will probably be only 70 percent of the way there. As you can imagine, it's tough to find withdrawal times for the State of Montana, for instance. I don't think we'll have all the states in there but the major racing jurisdictions are in that database.

The large-scale administrations studies, 20-horse studies will hopefully begin early in 2007. And, then again, we'll sort of deal with them the same way we have done the model rules. We'll do it in phases. As we get a scientific on the drugs we'll insert them in model rules and go back and pick off the next eight drugs. That's the plan at this point.

I think that's it. It is. We're a little bit over, I think the next panel starts at 2:45. There's time for two or three questions if we have got them.

Stan?

MR. STAN BERGSTEIN: The study of two weeks ago suggests that long-term use of EPO in humans for treatment of anemia may lead to increased heart risks. Brings to mind there's a number of deaths to horses now that are being checked on necropsy, there's two cases—is there any study being done or any value in having studies that relate to that possible risk of heart disease through blood sludging or whatever?

DR. SOMA: The blood sludging or the increasing viscosity or increase in hematocrit reported in humans does not or doesn't seem to occur in horses. The horse, for example, if a human gets above 55, the hematocrit above 55, they are in danger of having a heart attack, stroke or other serious deficiency disorder.

When a bicyclist gets above 55 and gets dehydrated there's a possibility he could have a stroke or heart attack. Remember, the horse runs at a hematocrit of 60 to 65. And that at 60 and 65 the blood viscosity, how thick the blood is, is different than in humans. They can move or function at 60 to 65, which is not the same as the human. The horse and the human are totally different athletes and many times people confuse that. They say, we give this to a human, it's going to act the same way in the equine. It's not true. You're right, as I pointed it out before, the deaths in humans and the equine athlete occur, but they are totally different mechanisms.

A VOICE: This goes back to the Banamine study. When you start at that point in time are you aiming for a goal. If you withdraw in five days out and come up with a 50 nanogram reading it would be the threshold, with 50 nanograms meet the withdrawal time. If you test for 40 nanograms you would not be charged with a positive. Are they not coordinated? Do you understand what I'm saying?

DR. STANLEY: I think I do, and my understanding is if you're below the plasma threshold concentration it would not be a positive based on the research that establishes that threshold as being the pharmacologically active concentration. For a therapeutic drug, the idea for a threshold is to get to a plasma concentration that

would result in no pharmacological advantage to the horse based on the route of administration and data available. Anything below that would be an inadvertent finding of no performance advantage to the horse.

A VOICE: When you get to the penalties and everything in different states, as you know in Illinois we had a mepivacaine positive a couple years ago where it came back in Canada the withdrawal time was 48 hours and Illinois was more like four days to get to the level. And it came back there was really no threshold, it just showed up, period. One of things they are looking to do, if the threshold is established and they fall below the threshold, not automatically taking purses away and stuff. Is there any thought on that in your studies?

DR. WATERMAN: What the threshold does, if you go through the steps we're going through in terms of doing the multi-horse studies, what the threshold does is give you confidence that below that concentration a horse is not getting an advantage. It's not something pursued at all. That's it. It doesn't even go down the road of a positive test.

The flip side of the argument is also there, if somebody is above that threshold you can be assured they did not follow the recommended guidelines and therefore the punishment could be maybe aggravated, a little more severe. So I think it gives confidence on either side of that number to go one way or the other with the penalty.

A VOICE: Dr. Stanley, one last question on the Banamine. The original threshold level was 20. Because enough of the horses were testing higher than 20, it was changed to 50. How do you establish a confidence that 50 is not affecting the performance of the horse? You knew 20 wasn't, how do you draw the line? What if enough of the horses had been at 70, how do you distinguish that level?

DR. STANLEY: It's done in conjunction with the veterinarians and the research they established. What they targeted was a single 500-milligram dose IV of Banamine 24 hours before the performance. Along with the pharmacokinetics and pharmacodynamics data that I showed, horses with an inflamed joint were treated with that and they showed improvement for 16 to 18 hours after the therapeutic application. Twenty-four hours later they were back to normal. It's looking at both sets of data, the veterinary medical professionals, the indication of how the medication is used, what it's used for, in addition to the plasma concentrations we're detecting in the sample, and put it together.

In a case like flunixin it's a little easier because we know what the dose and recommended route of administration is, we just need to know what the concentration expected after that treatment is going to be. In our case, we found in order to put statistical relevance to it we needed to use a level a little higher than the 20 nanograms per mil. There's not a huge difference between the 20 and 50, but it allows us to have confidence that we won't have an inadvertent positive by someone who followed our recommendations for route and administration concentration.

A VOICE: For us layman, what's a normal Banamine five hours after you gave Banamine, what would be your nanograms? Is 50 an insignificant number compared to 1,000 or 2000.

DR. STANLEY: Between 1,000 and 500 would be the concentration of plasma flunixin after about five or six hours. So it would be somewhere on the order of 10 times to 20 times higher than the threshold.

DR. ARTHUR: Part of the goal of this was not only setting a threshold level. I mean, to use this safety factor involved in the 50 nanogram the trainers are still going to average around 10 nanograms if they administer it properly. We also need to have a threshold where we can prevent the race-day administration of Banamine, which has been a problem in horse racing.

If you look at the 50-nanogram level and effective dose and all the different scenarios from the information we provided we think you can very effectively prevent that. Your chances of getting a positive over 50 if you try to treat a horse on race day, and I don't remember what it was it was something like cc or two six hours before the race, you'd still have a 50-50 chance of getting a high Banamine. So that essentially prevents the administration of Banamine on race day, it's not worth the risk and there's no reward for a dose that low. And the data shows that. I don't have it in front of us. We're very confident that we can prevent the race-day administration of Banamine.

DR. WATERMAN: I think we need to leave it there to give the next panel a chance to set up. I'd like to thank all of the speakers today.

Hopefully, we kept you interested.

(Applause)